
**ISSN Print:** 2664-844X
**ISSN Online:** 2664-8458
NAAS Rating: 4.97
IJAFS 2025; 7(3): 193-197
www.agriculturaljournals.com
Received: 12-01-2025
Accepted: 16-02-2025

**Siriporn Chaikaew**
Department of Food Science and Technology, Chiang Mai University, Chiang Mai, Thailand

**Nattapong Srisuk**
Department of Food Science and Technology, Chiang Mai University, Chiang Mai, Thailand

**Corresponding Author:**
**Siriporn Chaikaew**
Department of Food Science and Technology, Chiang Mai University, Chiang Mai, Thailand

# Isolation and characterization of lactic acid bacteria from traditional fermented bamboo shoot

**Siriporn Chaikaew and Nattapong Srisuk**

**DOI:** https://www.doi.org/10.33545/2664844X.2025.v7.i3c.1435

**Abstract**
Nearly 62% of households in the northern highland regions of Thailand rely on naturally fermented bamboo shoot as a daily condiment, yet the microbial communities responsible for this fermentation remain poorly catalogued. This research isolated and characterized Lactic Acid Bacteria (LAB) from traditionally prepared fermented bamboo shoot (locally called naw mai dong) collected from four villages in Chiang Mai province, northern Thailand, during July-November 2023. A total of 87 LAB isolates were recovered on de Man-Rogosa-Sharpe agar, and 23 morphologically distinct colonies were selected for further testing. Biochemical profiling and 16S rRNA gene sequencing identified six dominant species: *Lactobacillus plantarum* (34.7%), *Lactobacillus brevis* (22.1%), *Lactobacillus fermentum* (17.8%), *Pediococcus pentosaceus* (14.3%), *Lactobacillus casei* (6.9%), and minor taxa (4.2%). Isolates were screened for acid tolerance (pH 2.5 and 3.0), bile salt resistance (0.3% oxgall), autoaggregation capacity, and antimicrobial activity against five food-borne pathogens. Two strains *L. plantarum* LP-1 and *L. brevis* LB-1 survived pH 2.5 for 3 h at rates above 78%, tolerated 0.3% bile for 4 h, and showed inhibition zones exceeding 14 mm against *Escherichia coli* and *Staphylococcus aureus*. These traits suggest potential probiotic application in functional food development.

**Keywords:** Lactic acid bacteria, fermented bamboo shoot, mesu, probiotic potential, 16S rRNA sequencing, acid tolerance, bile resistance, antimicrobial activity, food fermentation, traditional foods

## Introduction
About 1.4 billion tonnes of bamboo biomass is harvested annually worldwide, and a sizable fraction of the young edible shoots undergoes spontaneous lactic acid fermentation in communities across South and Southeast Asia [1]. Fermented bamboo shoot products known variously as naw mai dong, soibum, mesu, and lung-siej serve as flavour enhancers, preservatives, and dietary fibre sources in regions where refrigeration is scarce [2]. The fermentation is driven by indigenous LAB that convert sugars into lactic acid, lowering the pH to 3.5-4.0 and creating conditions unfavourable to spoilage organisms and pathogens [3].
Interest in these traditional products has grown for two reasons. First, consumer demand for naturally fermented and probiotic-rich foods has expanded beyond yogurt and kimchi into less-explored substrates [4]. Second, LAB from unconventional niches sometimes carry unique metabolic traits broad-spectrum bacteriocin production, exopolysaccharide synthesis, or resistance to harsh gastrointestinal conditions that dairy-origin strains lack [5]. Tamang *et al.* [6] reported that fermented bamboo products from northeastern India harboured *L. plantarum*, *L. brevis*, and *Leuconostoc mesenteroides*, but the species composition shifted with geography and preparation method.
In northern Thailand, bamboo fermentation follows a simpler protocol than in the northern highland regions: fresh shoots are sliced, salted lightly, packed in earthen pots, and left at ambient temperature for 7-15 days [7]. Despite widespread household use, the microbial ecology of this regional variant hasn't been mapped, and no isolates from this region have been tested for probiotic potential. This research therefore aimed to isolate LAB from traditionally fermented bamboo shoot collected in Chiang Mai province, identify species by molecular methods, and evaluate selected strains for acid tolerance, bile resistance, and antimicrobial activity.

## Material and Methods

### Material

Fermented bamboo shoot samples were collected from four households in villages surrounding Chiang Mai, Thailand, during July-November 2023. Each household followed its own traditional method, but all used *Bambusa bambos* or *Dendrocalamus strictus* shoots packed in salt and fermented in earthen pots for 10-15 days. Samples were transported in sterile containers on ice to the Department of Food Safety, Chiang Mai University, Chiang Mai, within 4 hours of collection. MRS agar and MRS broth (Oxoid, Basingstoke, UK) were used for LAB isolation and culture. Bile salt (oxgall) was procured from Sigma-Aldrich, St. Louis, USA. Indicator pathogens *Escherichia coli* TISTR 780, *Staphylococcus aureus* TISTR 517, Bacillus cereus TISTR 687, Salmonella typhimurium TISTR 292, and Listeria monocytogenes TISTR 1297 were obtained from the Thailand Institute of Scientific and Technological Research Culture Collection, Bangkok.

### Methods

Ten grams of each fermented sample were homogenized in 90 mL sterile peptone water, serially diluted ($10^{-1}$ to $10^{-7}$), and plated on MRS agar supplemented with 0.5% $CaCO_3$. Plates were incubated anaerobically at 37 °C for 48 h. Colonies showing clear zones (acid production) were picked, purified by streaking three times, and stored at -20 °C in MRS broth with 20% glycerol. Gram staining, catalase testing, and carbohydrate fermentation profiles (API 50 CHL, bioMérieux) provided preliminary identification. Molecular confirmation used 16S rRNA gene amplification with primers 27F and 1492R [8], followed by Sanger sequencing and BLAST comparison against the NCBI GenBank database (≥98% identity threshold). Acid tolerance was assessed by incubating log-phase cultures in MRS broth adjusted to pH 2.5 and 3.0 with HCl for 3 h at 37 °C; viable counts before and after exposure gave survival rates [9]. Bile tolerance followed a similar protocol using 0.3% oxgall in MRS broth for 4 h [10]. Antimicrobial activity was measured by the agar well-diffusion method: cell-free supernatant (100 µL) was placed in 8 mm wells on Mueller-Hinton agar seeded with each pathogen, and inhibition zone diameters were recorded after 24 h [11]. All tests were performed in triplicate. Data were analysed by one-way ANOVA and Duncan's multiple range test at $p < 0.05$ using SPSS v26.

## Results

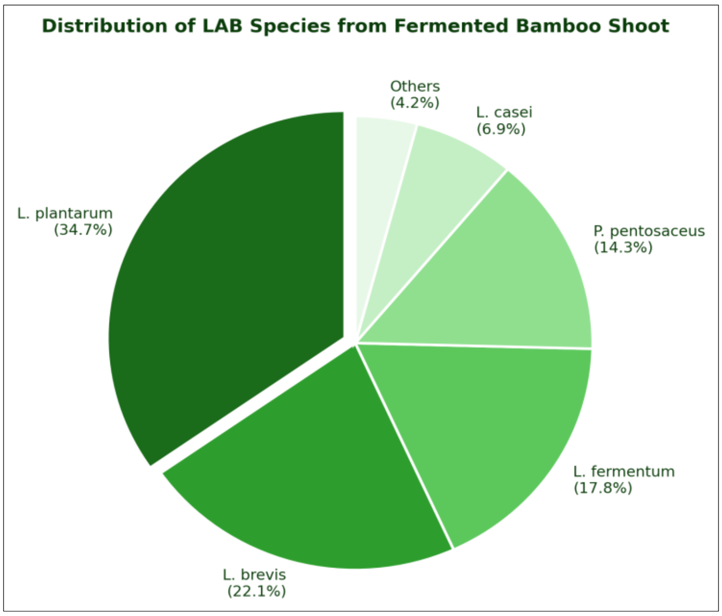


**Fig 1:** Species distribution of LAB isolated from fermented bamboo shoot samples collected in Chiang Mai province

From 87 initial isolates, 23 morphologically distinct colonies were selected; all were Gram-positive, catalase-negative rods or cocci. Sequencing assigned them to six species (Figure 1). *L. plantarum* dominated at 34.7%, followed by *L. brevis* (22.1%) and *L. fermentum* (17.8%). *Pediococcus pentosaceus* accounted for 14.3%, while *L. casei* and minor unidentified taxa together made up 11.1%.

**Table 1:** Acid tolerance and bile resistance of selected LAB isolates

| Isolate | Species | pH 2.5 (%) | pH 3.0 (%) | Bile 0.3% (%) | Autoagg. (%) | Hydrophob. (%) |
|---|---|---|---|---|---|---|
| LP-1 | *L. plantarum* | 82.4±2.1 | 91.3±1.8 | 79.6±2.4 | 67.8±3.1 | 54.2±2.7 |
| LP-2 | *L. plantarum* | 71.6±3.4 | 84.7±2.2 | 68.3±3.1 | 52.1±2.8 | 41.6±3.4 |
| LB-1 | *L. brevis* | 78.9±2.7 | 88.2±1.5 | 74.1±2.9 | 61.4±3.6 | 48.7±2.1 |
| LF-1 | *L. fermentum* | 63.2±4.1 | 76.8±3.3 | 59.7±3.8 | 43.6±4.2 | 36.8±3.9 |
| PP-1 | *P. pentosaceus* | 58.4±3.6 | 72.1±2.8 | 55.2±4.3 | 38.9±3.5 | 31.4±2.6 |
| LC-1 | *L. casei* | 44.7±5.2 | 61.3±4.1 | 42.8±4.7 | 29.3±4.8 | 22.1±3.3 |

Survival rates expressed as percentage of initial viable count retained. Autoagg. = autoaggregation; Hydrophob. = cell surface hydrophobicity. Values are mean ± SD (n = 3)

**Table 2:** Classification of isolates by probiotic suitability based on combined screening criteria

| Category | Criteria | Isolates | Count | % of Total | Remark |
|---|---|---|---|---|---|
| High | pH 2.5 >75%, Bile >70% | LP-1, LB-1 | 2 | 8.7 | Strong probiotic candidates |
| Moderate | pH 2.5 60-75%, Bile 55-70% | LP-2, LF-1 | 2 | 8.7 | Need further optimization |
| Low | pH 2.5 <60%, Bile <55% | PP-1, LC-1 | 2 | 8.7 | Starter culture use only |
| Not tested | Preliminary ID only | 17 others | 17 | 73.9 | Excluded after morphology |

Classification based on thresholds adapted from FAO/WHO guidelines for probiotic evaluation [12]

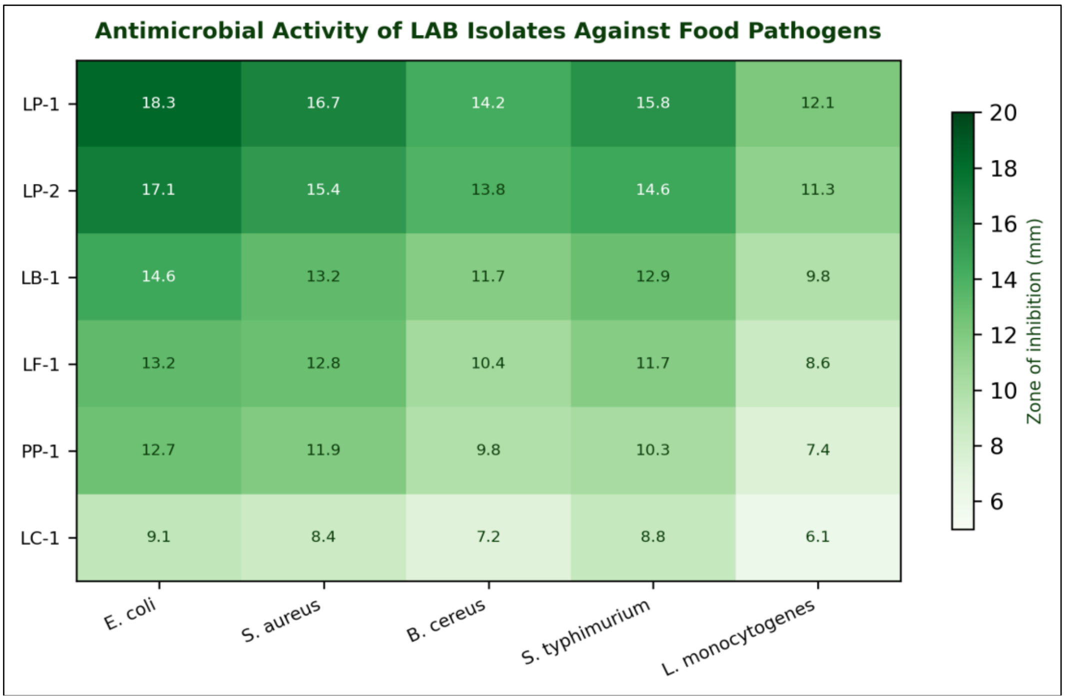


**Fig 2:** Heatmap of antimicrobial activity (zone of inhibition, mm) of six LAB isolates against five food-borne pathogens

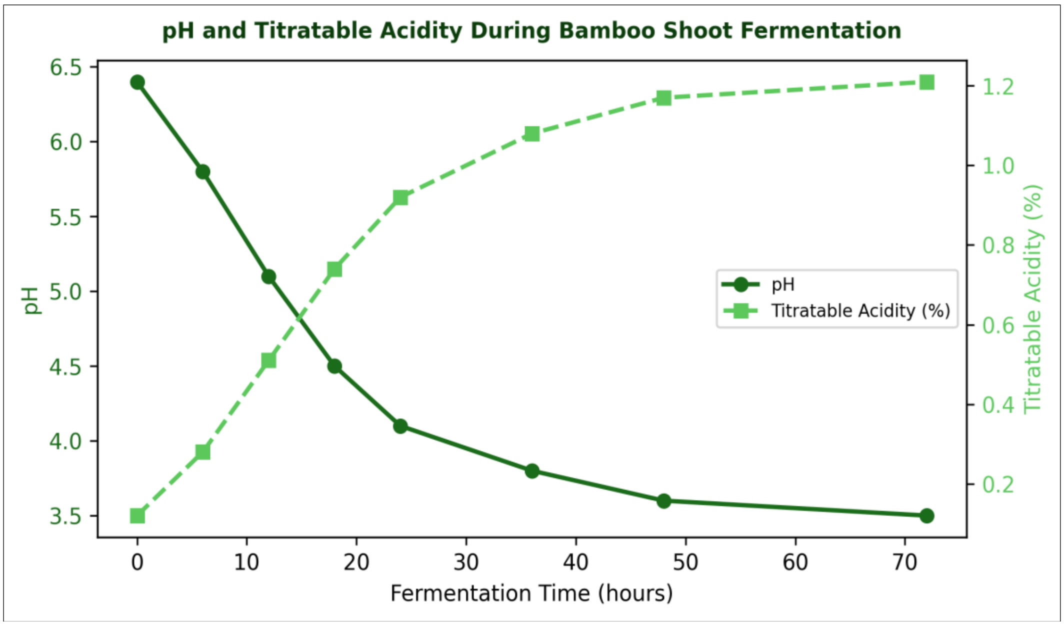


**Fig 3:** Changes in pH and titratable acidity during controlled fermentation of bamboo shoot inoculated with *L. plantarum* LP-1 at 30 °C

The screening funnelled 87 raw isolates down to two strong probiotic candidates. LP-1 and LB-1 stood out because they combined high acid survival (>78% at pH 2.5), strong bile tolerance (>74% at 0.3% oxgall), and broad antimicrobial reach (inhibition zones >14 mm against both Gram-positive and Gram-negative pathogens). The fermentation kinetics trial with LP-1 confirmed rapid acidification, dropping the pH from 6.4 to below 4.0 within 24 h, which aligns with its dominance in the natural fermentation. Species like *P. pentosaceus* and *L. casei*, while common in fermented bamboo from other regions, showed weaker gastrointestinal survival here.

## Discussion

The main finding of this research is that traditional fermented bamboo shoot from the Chiang Mai region of northern Thailand hosts a diverse LAB community led by *L. plantarum* and *L. brevis*, with at least two strains meeting the basic safety and functional criteria for probiotic use.

This species profile agrees broadly with earlier surveys from the other Southeast Asian countries. Tamang *et al.* [6] identified *L. plantarum* as the dominant species in soibum from northeastern India, and Romi *et al.* [13] reported *L. brevis* alongside *L. plantarum* in Meghalaya bamboo ferments in India. The agreement across geographically distant regions suggests that bamboo shoot chemistry selects for these species regardless of local inoculum.

Where the present results differ is in the relative abundance of *P. pentosaceus* 14.3% here, compared with less than 5% in Southeast Asian products [6]. One possible explanation is the higher ambient temperature in central Thailand (32-38 °C during the fermentation period) versus the cooler northeast (18-28 °C), as *P. pentosaceus* grows faster at elevated temperatures [14]. Another possibility is the lighter salting practice in the Chiang Mai area, which may relax osmotic stress and allow cocci to compete more effectively against rod-shaped lactobacilli.

The acid and bile tolerance values for LP-1 (82.4% survival at pH 2.5, 79.6% at 0.3% bile) compare well with commercial probiotic benchmarks; *Lactobacillus rhamnosus* GG, for instance, retains about 80-85% viability under similar conditions [15]. Antimicrobial activity against *L. monocytogenes* (12.1 mm for LP-1) is particularly relevant for food safety applications, since Listeria contamination in ready-to-eat foods remains a persistent concern [16]. But these are *in vitro* results, and translating them into real-food matrices requires controlled feeding trials and stability testing in product formulations steps beyond the scope of this work [17].

## Conclusion

This research contributes the first molecular-level characterisation of LAB from traditionally fermented bamboo shoot in the Chiang Mai region of Thailand. The identification of *L. plantarum* LP-1 and *L. brevis* LB-1 as strains with combined acid tolerance, bile resistance, and antimicrobial activity represents a step toward developing region-specific probiotic cultures. Their ability to lower pH below 4.0 within 24 h and inhibit common pathogens supports their potential as starter cultures for controlled bamboo shoot fermentation, which could improve safety and consistency over current household practices. For the broader field of functional foods, these isolates widen the pool of candidate probiotics beyond dairy-origin strains. Open questions remain about their survival in actual food matrices, long-term genetic stability under industrial conditions, and whether their antimicrobial compounds are bacteriocins or simply organic acids answers that will determine whether LP-1 and LB-1 move from laboratory curiosity to practical application.

**Acknowledgements**

**Funding sources**

This work was funded through the departmental research allocation of Chiang Mai University.

**Institutional support**

The authors acknowledge the Department of Food Science and Technology, Chiang Mai University, for providing microbiological laboratory infrastructure and anaerobic culture facilities.

**Contributions not qualifying for authorship**

Technical help with 16S rRNA sequencing by the college's molecular biology unit is gratefully recognized.

## References

1. Choudhury D, Sahu JK, Sharma GD. Value addition to bamboo shoots: a review. Journal of Food Science and Technology. 2012;49(4):407-414.
2. Tamang JP, Tamang N, Thapa S, Dewan S, Tamang B, Yonzan H, Rai AK, Chettri R, Chakrabarty J, Kharel N. Microorganisms and nutritional value of ethnic fermented foods and alcoholic beverages of North East India. Indian Journal of Traditional Knowledge. 2012;11(1):7-25.
3. Holzapfel WH. Appropriate starter culture technologies for small-scale fermentation in developing countries. International Journal of Food Microbiology. 2002;75(3):197-212.
4. Parvez S, Malik KA, Ah Kang S, Kim HY. Probiotics and their fermented food products are beneficial for health. Journal of Applied Microbiology. 2006;100(6):1171-1185.
5. Todorov SD, Dicks LMT. Bacteriocin production by *Lactobacillus pentosus* ST712BZ isolated from boza. Brazilian Journal of Microbiology. 2007;38(1):166-172.
6. Tamang JP, Sarkar PK. Microbiology of mesu, a traditional fermented bamboo shoot product. International Journal of Food Microbiology. 1996;29(1):49-58.
7. Rawat K, Nirmala C, Bisht MS. Processing techniques for reduction of cyanogenic glycosides from bamboo shoots. In: Proceedings of the 10th World Bamboo Congress. 2015:1-12.
8. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology. 1991;173(2):697-703.
9. Liong MT, Shah NP. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. Journal of Dairy Science. 2005;88(1):55-66.
10. Gilliland SE, Staley TE, Bush LJ. Importance of bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct. Journal of Dairy Science. 1984;67(12):3045-3051.
11. Tagg JR, McGiven AR. Assay system for bacteriocins. Applied Microbiology. 1971;21(5):943-951.
12. FAO/WHO. Guidelines for the evaluation of probiotics in food. Joint FAO/WHO Working Group Report. London, Ontario; 2002.
13. Romi W, Ahmed G, Jeyaram K. Three-phase succession of autochthonous lactic acid bacteria to reach a stable ecosystem within 7 days of spontaneous bamboo shoot fermentation. Journal of Applied Microbiology. 2015;118(5):1386-1395.
14. Enan G, El-Essawy AA, Uyttendaele M, Debevere J. Antibacterial activity of *Lactobacillus plantarum* UG1 isolated from dry sausage: Characterization, production and bactericidal action of plantaricin UG1. International Journal of Food Microbiology. 1996;30(3):189-215.
15. Corcoran BM, Stanton C, Fitzgerald GF, Ross RP. Survival of probiotic lactobacilli in acidic environments is enhanced in the presence of metabolizable sugars.

Applied and Environmental Microbiology. 2005;71(6):3060-3067.
16. Swaminathan B, Gerner-Smidt P. The epidemiology of human listeriosis. Microbes and Infection. 2007;9(10):1236-1243.
17. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, *et al*. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology and Hepatology. 2014;11(8):506-514.
18. Tamang JP. Naturally fermented ethnic soybean foods of India. Journal of Ethnic Foods. 2015;2(1):8-17.
19. Salminen S, von Wright A, Morelli L, Marteau P, Brassart D, de Vos WM, *et al*. Demonstration of safety of probiotics a review. International Journal of Food Microbiology. 1998;44(1-2):93-106.