

Acidification and Nutritional Changes During Controlled Fermentation of *Bambusa spinosa* Roxb. Shoots Using Indigenous Lactic Acid Bacteria

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ABSTRACT

This study evaluated two indigenous lactic acid bacteria isolates as starter cultures for controlled fermentation of *Bambusa spinosa* Roxb. shoots. BSISO1 and BSISO14, recovered from fresh *B. spinosa* Roxb. shoots, were selected based on colony morphology and Gram stain reaction. Based on 16S rRNA gene sequencing, the isolates were putatively identified as *Lactiplantibacillus plantarum* BSISO1 and *Lactiplantibacillus pentosus* BSISO14. Three fermentation treatments were established: BSISO1-inoculated, BSISO14-inoculated, and uninoculated spontaneous fermentation. The inoculated treatments received 3% (v/v) starter culture and were fermented for 14 days. The pH and total titratable acidity (TTA) were monitored every two days, while fat, reducing sugar, protein, and crude fiber were measured at the end of fermentation. Treatment, fermentation day, and their interaction significantly affected acidification. BSISO1 produced the lowest overall pH and highest overall TTA, followed by BSISO14, while spontaneous fermentation showed the weakest acidification. Fermentation markedly reduced reducing sugar and crude fiber relative to fresh shoots, whereas fat remained relatively stable. BSISO14 showed numerically higher protein and crude fiber values than spontaneous fermentation. These results indicate preliminary potential that BSISO1 and BSISO14 can support controlled fermentation of *B. spinosa* shoots, with BSISO1 showing stronger acidification performance. Further microbial, metabolomic, sensory, safety, and shelf-life evaluations are required before practical starter-culture application.

Keywords: Bamboo shoot fermentation, indigenous starter culture, kawayang tinik, *Lactiplantibacillus plantarum*, *Lactiplantibacillus pentosus*

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INTRODUCTION

Plant-based fermented foods are increasingly investigated as alternatives to dairy-based probiotic products, particularly for lactose-intolerant individuals, vegan consumers, and populations with limited access to dairy products. Fermentation can improve food preservation, modify sensory characteristics, and promote the transformation of nutrients and phytochemicals (Xiao et al., 2024). Bamboo shoots are promising substrates for value-added fermented food development because they are rich in dietary fiber, contain moderate protein, and are naturally low in fat (Ma et al., 2024; Behera and Balaji, 2021).

Fermented bamboo shoot products are traditionally consumed in several Asian countries, including “soibum” in India, “suan sun” in China, “nor mai dong” in Thailand, and sour bamboo shoot products in Laos and Vietnam. Lactic acid bacteria (LAB) play an important role in these products by converting fermentable carbohydrates into organic acids. The resulting acidification contributes to preservation, flavor development, substrate transformation, and the establishment of conditions that may restrict undesirable microorganisms (Li et al., 2023; Xu et al., 2023).

Controlled inoculation with selected starter cultures may provide greater consistency than spontaneous fermentation, which depends on naturally occurring and potentially variable microbial populations. Indigenous LAB originating from bamboo shoots may be suitable starter-culture candidates because they may already be adapted to the physicochemical characteristics and carbohydrate composition of the plant substrate (Yan et al., 2025; Zhao et al., 2023; Pontonio et al., 2018). Nevertheless, favorable in vitro characteristics do not necessarily indicate effective technological performance. Selected isolates must therefore be applied directly to the bamboo shoot matrix to determine their capacity to promote acidification and influence product composition.

Previous studies have demonstrated the potential of selected LAB for bamboo shoot fermentation. Chen et al. (2024) evaluated *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* for producing GABA-enriched fermented bamboo shoots. In contrast, Zhao et al. (2021) developed a fermented beverage combining red jujube fruits and bamboo shoots using *L. plantarum*. Bothoulath et al. (2023) investigated *L. plantarum* BBS13 as a starter culture for Lao fermented bamboo shoots. These studies provide a basis for LAB-assisted fermentation, although the

technological performance of individual isolates must be evaluated directly in the intended substrate and under the proposed fermentation conditions.

In the Philippines, *Bambusa spinosa*, also reported as *B. blumeana*, is commonly known as kawayang tinik, and its young shoots are consumed as “labong”. Philippine surveys have documented its importance as an edible bamboo species and characterized the nutritional properties of its shoots (Caasi-Lit et al., 2010a, 2010b). Local research has also addressed bamboo shoot kimchi processing (Pascual and Aluag, 2024). Given the susceptibility of fresh bamboo shoots to postharvest deterioration, processing approaches that support timely utilization are relevant to bamboo food development (Ma et al., 2024). Controlled fermentation could provide an additional route to diversify locally processed labong products, with potential applications in food preservation, reducing postharvest losses, and small-scale food processing. Nevertheless, the fermentation performance of indigenous LAB recovered from Philippine *B. spinosa* remains insufficiently characterized. Specifically, evidence is needed on whether selected local isolates produce different acidification profiles and nutritional outcomes from spontaneous fermentation when applied to their source substrate.

To address this gap, the present study aimed to: (1) characterize selected LAB isolates recovered from *B. spinosa* shoots and putatively identify them using 16S rRNA gene sequencing; (2) determine the effects of BSISO1 inoculation, BSISO14 inoculation, and spontaneous fermentation on pH and total titratable acidity (TTA) over 14 days; and (3) descriptively compare the protein, crude fiber, reducing sugar, and fat contents of fresh *B. spinosa* shoots and shoots fermented under each treatment. This study directly compares these locally recovered isolates in their source bamboo species, providing evidence of their suitability as starter-culture candidates for fermented “labong” development.

MATERIALS AND METHODS

Isolation and gram characterization of indigenous lactic acid bacteria

The study employed descriptive and experimental research designs. One young and intact *B. spinosa* shoot was purposively collected from each of Rosario, Agoo, and Pugo, La Union, Philippines. The samples were placed in sterile polyethylene bags and transported to the laboratory on the same day in an insulated container maintained at 4–8 °C. In the laboratory, surface sterilization and isolation followed the procedure of Chavez et al. (2024); the outer surfaces were treated with 80% ethanol for 5 minutes and 3% hydrogen peroxide for 20 minutes, followed by three rinses with sterile distilled water. Prepared tissue portions from the three shoots were combined and mixed to form a single composite sample for LAB isolation. The sampling approach was intended for exploratory recovery and evaluation of LAB associated with the collected bamboo shoots. Twenty-five grams of prepared shoot tissue were enriched in 225 mL of buffered peptone water at 37 °C for 48 hours, then homogenized for 3 minutes. The enriched samples were serially diluted up to 10^{-5} , and 100 µL aliquots from the 10^{-2} to 10^{-5} dilutions were spread-plated on de Man, Rogosa, and Sharpe agar supplemented with calcium carbonate, sorbic acid, and bromocresol purple. The plates were incubated at 37 °C for 48–72 hours. Colonies showing clear zones or color changes indicative of acid production were selected and characterized based on shape, pigmentation, opacity, and size. Selected colonies were purified through three successive subcultures on modified MRS agar using the streak-plate method. Pure cultures were then subjected

to Gram staining, and Gram-positive isolates were retained as presumptive indigenous lactic acid bacterial isolates.

Molecular identification of selected lactic acid bacteria isolates

The selected two isolates were submitted to MacroGen, Inc. (Seoul, South Korea) through Kinovett Scientific (Diliman, Quezon City) for 16S rRNA gene sequencing. Genomic DNA was extracted from pure cultures using a resin-based boiling method, and the nearly full-length 16S rRNA gene was amplified using the universal primers 27F and 1492R under standard PCR conditions. The resulting amplicons were subjected to bidirectional Sanger sequencing using primers 785F and 907R, BigDye™ Terminator v3.1 chemistry, and a 3730XL automated DNA sequencing system. The generated sequences were compared with reference sequences in the National Center for Biotechnology Information database to determine their closest taxonomic matches, and species-level assignments were interpreted as putative because 16S rRNA gene sequencing may not fully resolve closely related species.

Phylogenetic reconstruction

An independent phylogenetic analysis was performed using the MacroGen-generated 16S rRNA gene consensus sequences of BSISO1 and BSISO14, comprising 1,468 and 1,465 nucleotides, respectively. Eleven *Lactiplantibacillus* reference sequences represented in the original MacroGen trees were retrieved from NCBI, and *Lacticaseibacillus casei* ATCC 393 (NR_041893.1) was included as the outgroup. The combined dataset comprised 14 sequences.

Multiple sequence alignment was performed using MUSCLE version 5.3 with default alignment parameters and the nucleotide option (Edgar, 2022). Alignment columns containing gaps or ambiguous nucleotides in any sequence were excluded by complete deletion, retaining 1,431 positions from the initial 1,551 aligned columns. Identical retained sequences were represented once during phylogenetic inference, yielding six unique sequence groups.

The phylogeny was reconstructed using the maximum-likelihood method in IQ-TREE version 2.4.0 (Minh et al., 2020). ModelFinder selected the Kimura two-parameter model (K2P) with uniform rates across sites, based on the Bayesian information criterion (Kalyaanamoorthy et al., 2017). Branch support was evaluated using 1,000 standard nonparametric bootstrap replicates, with a fixed random seed of 20261012. The tree was rooted using the designated outgroup. Identical sequences were restored as zero-length polytomies for presentation, without assigning bootstrap support to relationships within these groups. Branch lengths were expressed as estimated substitutions per site.

Bamboo shoots fermentation

Sample preparation

Controlled fermentation of bamboo shoots followed the protocol of Chen et al. (2024). The selected LAB isolates, BSISO1 and BSISO14, were cultured separately in MRS broth at 37 °C for 24 h. Cells were harvested by centrifugation at $5,000 \times g$ for 10 min at 4 °C, washed twice with sterile phosphate-buffered saline (PBS; pH 7.4), and resuspended in PBS. The starter suspensions were standardized to an optical density of 0.1 at 600 nm ($OD_{600} = 0.1$) using a spectrophotometer. Although the starter suspensions were standardized to $OD_{600} = 0.1$, their viable cell concentrations were not determined. Consequently,

the inoculum dose could not be expressed reliably in CFU/mL.

Fresh bamboo shoots were sourced from three local collection sites (Rosario, Ago, and Pugo, La Union) and prepared by thoroughly washing them with sterile distilled water, removing the basal root portions, peeling off the outer husk, and cutting the tender portions into uniform strips to ensure consistency in fermentation.

For the fermentation trial, prepared bamboo shoot strips from the three collection sites were pooled and mixed to form a single composite substrate batch. Three treatments were established: BSISO1 inoculation, BSISO14 inoculation, and spontaneous fermentation without bacterial inoculation. The

composite material was distributed among nine sterilized glass jars, with three fermentation jars per treatment. Each jar contained 100 g of bamboo shoot strips and 200 mL of cooled boiled water. Each inoculated jar received 6 mL of the corresponding starter suspension, equivalent to 3% (v/v) of the initial liquid volume. Each jar was prepared and maintained as a separate experimental unit, while measurements such as pH and TTA collected over time represented repeated observations within that unit. All jars were sealed under aseptic conditions and incubated at 30–37 °C for 14 days. Table 1 summarizes the fermentation setup. All jars were sealed tightly under aseptic conditions and were incubated at 30–37 °C for fourteen (14) days.

Table 1. Fermentation matrix for *Bambusa spinosa* shoots under inoculated and spontaneous treatments.

Treatment	Substrate and liquid ratio	Inoculum concentration	Number of replicates	Incubation conditions
BSISO1	100 g bamboo shoot strips + 200 mL cooled boiled water (1:2, w:v)	3% (v/v) or 6ml/200ml	3	Sealed jars, 30–37 °C
BSISO14	100 g bamboo shoot strips + 200 mL cooled boiled water (1:2, w:v)	3% (v/v) or 6ml/200ml	3	Sealed jars, 30–37 °C
Spontaneous fermentation	100 g bamboo shoot strips + 200 mL cooled boiled water (1:2, w:v)	0%	3	Sealed jars, 30–37 °C

Monitoring of pH and total titratable acidity (TTA)

During fermentation, samples were collected at two-day intervals to monitor changes in acidity. For each measurement, 5 g of fermented bamboo shoot material was homogenized with 10 mL of sterile distilled water to extract soluble metabolites. The homogenates were then centrifuged at $6,010 \times g$ for 5 minutes, after which the supernatant was carefully collected for analysis. The supernatant pH was measured using a pH meter. Total titratable acidity (TTA) was determined following the method described by Zhao et al. (2021). Briefly, 10 mL aliquots of the supernatant were titrated with standardized 0.1 N NaOH solution using phenolphthalein as an indicator until a faint pink endpoint persisted. TTA values were expressed as grams of lactic acid equivalent per 100 g of sample (g/100 g), since lactic acid is the primary organic acid produced during lactic fermentation and serves as the standard reference.

Determination of nutritional quality

Following the 14-day fermentation period, the protein, total reducing sugar, fat, and crude fiber contents of the fermented bamboo shoots were determined. Nutrient analyses followed standard AOAC methods to ensure accuracy and comparability with international benchmarks. Representative samples from each treatment group were submitted to FAST Laboratories in Angeles City, Pampanga, for proximate and nutrient composition testing. Although the fermentation treatments were established in triplicate, nutritional analyses were conducted on only one representative sample per treatment ($n=1$). The resulting measurements were therefore presented as preliminary descriptive data, and no inferential statistical comparisons were performed. These measurements describe the nutritional composition of the analyzed samples but do not establish statistically supported treatment effects. The resulting data provided a scientific basis for evaluating how probiotic fermentation influences the nutritional quality of bamboo shoots and supports the development of a functional food product with enhanced value.

Statistical analysis

pH and total titratable acidity (TTA) data were expressed as mean $\pm$ standard deviation (SD) from three independent fermentation jars per treatment. The fermentation jar served as the experimental unit, and repeated measurements from the same jar were accounted for in the analyses. Statistical analyses were performed using IBM® SPSS® (Statistical Package for the Social Sciences), with significance set at $p < .05$. The pH was analyzed using a linear mixed model across eight sampling days (days 0, 2, 4, 6, 8, 10, 12, and 14). Fermentation treatment (BSISO1, BSISO14, and spontaneous fermentation), sampling day, and their interaction were included as fixed effects, with repeated observations within fermentation jars accounted for in the model. Pairwise comparisons were used to examine treatment differences within each sampling day and differences among fermentation days.

TTA was analyzed using a mixed-design repeated-measures ANOVA across seven sampling days (days 2, 4, 6, 8, 10, 12, and 14). Treatment was the between-subjects factor, and sampling day was the within-subjects factor. Sphericity was assessed using Mauchly's test, and sphericity-assumed results were reported. Treatment comparisons used Tukey's honestly significant difference test, while fermentation-day comparisons used Bonferroni-adjusted pairwise comparisons. Superscript letters identified statistically comparable means for the comparisons specified in each table. Nutritional measurements were presented descriptively and were not subjected to inferential statistical analysis.

RESULTS

Morphological and gram-stain characterization of *bambusa spinosa* shoot-derived LAB isolates

Twenty-five distinct colonies isolated from *B. spinosa* were selected from modified MRS agar based on distinct cultural characteristics and the presence of clear zones or color changes indicative of acid production. The isolates showed variation in

colony shape, pigmentation, opacity, and size. Most colonies were circular and opaque, with pigmentation ranging from white and yellow-white to yellow and green, while colony diameters ranged from 1.0 to 4.5 mm. BSISO1 and BSISO14, subsequently selected

for controlled fermentation, both formed circular, yellow-white, opaque colonies approximately 2.5 mm in diameter and exhibited Gram-positive bacillary morphology. Table 2 summarizes the morphological and Gram stain characteristics of the isolates.

Table 2. Morphological characteristics and gram stain reaction of selected isolates.

Characteristics	BSISO1	BSISO14
Colony morphology in MRS agar	Circular, Glistening, Entire Margin, Convex Colony, Opaque and 2.5 mm in size	Circular, Glistening, Entire Margin, Convex Colony, Opaque and 2.5 mm in size
Colony color in MRS agar	Yellow- white	Yellow-white
Gram reaction	Gram positive, Bacilli	Gram positive, Bacilli

The two isolates showed similar colony characteristics and cellular morphology under the conditions examined. Figure 1 shows representative images of their colonies and Gram-stained cells. BSISO1 and BSISO14 were chosen from the 25 presumptive LAB isolates for exploratory molecular identification and

fermentation evaluation, without predefined comparative selection criteria. These characteristics supported their preliminary classification as presumptive LAB, although molecular analysis was required for taxonomic identification.

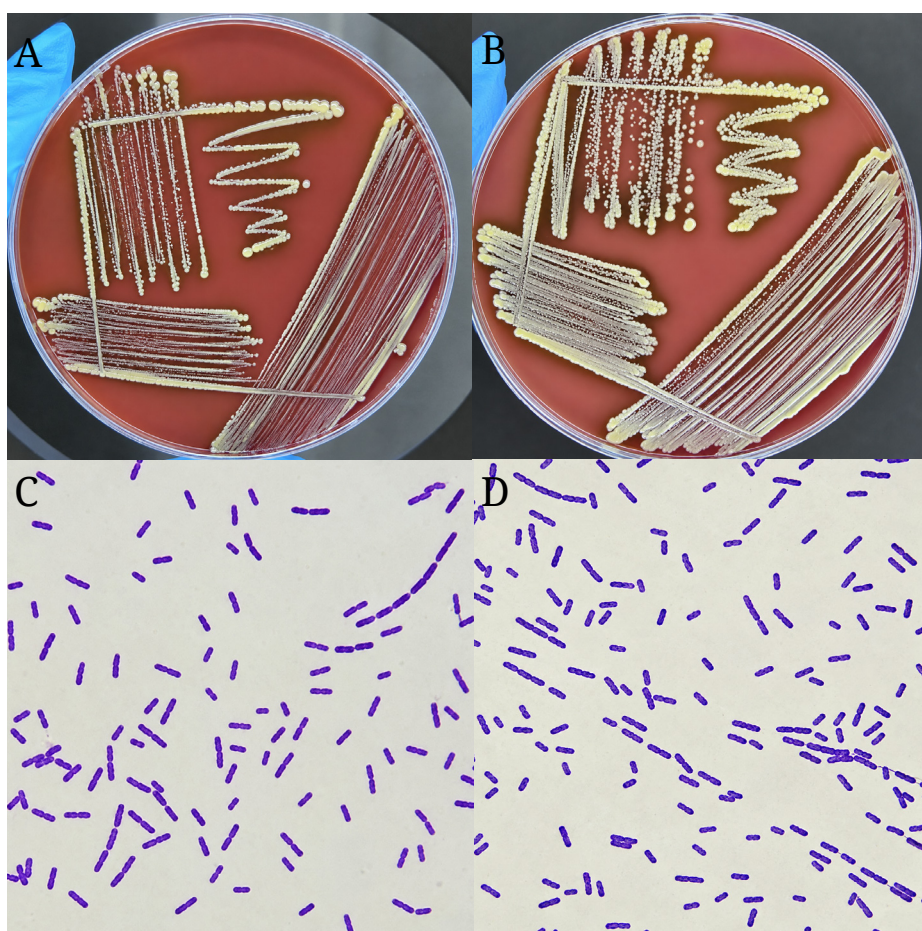


Figure 1. Original, unedited photographs of isolates BSISO1 (A) and BSISO14 (B) Growth in MRS Agar. Cell morphology of BSISO1 (C) and BSISO14 (D) Magnified 1000 $\times$.

Molecular identification of selected LAB strains using 16S rRNA gene sequencing

The two selected LAB isolates were subjected to 16S rRNA gene sequencing for molecular identification through Macrogen Inc. Sequence matching confirmed that both isolates belonged to the genus *Lactiplantibacillus*. As shown in Table 3, isolate BSISO1 had its closest match to *L. plantarum* (accession number NR_104

573.1), with a bit score of 2732, an E-value of 0.0, 97% query coverage, and 99.79% sequence identity, corresponding to 1486 matches out of 1489 aligned bases. Similarly, BSISO14 showed its closest sequence match to *L. pentosus* (NR_029133.1), with a bit score of 2739, an E-value of 0.0, 98% query coverage, and 99.86% sequence identity, corresponding to 1488 matches out of 1490 aligned bases.

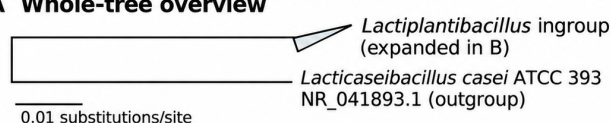
Table 3. Sequence-based molecular identification of selected isolates using 16S rRNA gene analysis.

Isolate	Accession No.	Closest taxonomic match	Length	Coverage	E-value	Pct.(%) identity	Sequence accession No. of isolate
BSISO1	NR_104573.1	<i>Lactiplantibacillus plantarum</i>	1527	97	0.0	99.79	PZ898212
BSISO14	NR_029133.1	<i>Lactiplantibacillus pentosus</i>	1519	98	0.0	99.86	PZ901799

The independently reconstructed maximum-likelihood phylogeny also placed both isolates with reference sequences from the *L. plantarum* group (Figure 2). BSISO1 and BSISO14 had identical nucleotide sequences across the 1,431 alignment positions retained for phylogenetic reconstruction and formed an unresolved group with matching *L. plantarum* and *L. pentosus* reference sequences. The study sequences were deposited in

GenBank under accession numbers PZ898212 and PZ901799 for BSISO1 and BSISO14, respectively. Together, these findings support the affiliation of both isolates with the *L. plantarum* group. At the same time, their species-level assignments remain provisional because the 16S rRNA gene's limited discriminatory power within this group requires additional molecular characterization for confirmation.

A Whole-tree overview



B Ingroup phylogram

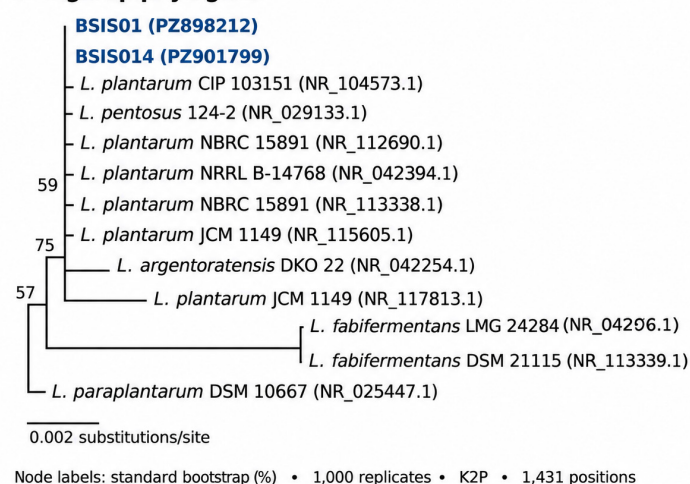


Figure 2. Maximum-likelihood phylogeny of BSISO1 and BSISO14 based on 16S rRNA gene sequences. (A) Rooted whole-tree overview with the *Lactiplantibacillus* ingroup collapsed. (B) Expanded ingroup phylogram. The analysis used 1,431 alignment positions, the K2P model, and 1,000 standard bootstrap replicates; node labels are bootstrap percentages. *Lacticaseibacillus casei* ATCC 393 (NR_041893.1) served as the outgroup. Scale bars indicate substitutions per site and differ between panels. Study isolates are shown in blue.

Changes in pH and total titratable acidity during fermentation

As shown in Figure 3, the pH of all treatments declined markedly from Day 0 to Day 2, indicating rapid initial acidifica-

tion during fermentation. Specifically, the pH values started at 6.34 ± 0.03 for BSISO1, 6.39 ± 0.03 for BSISO14, and 6.39 ± 0.04 for spontaneous fermentation, then dropped to 4.35 ± 0.09 , 4.45 ± 0.12 , and 4.34 ± 0.05 , respectively, by Day 2.

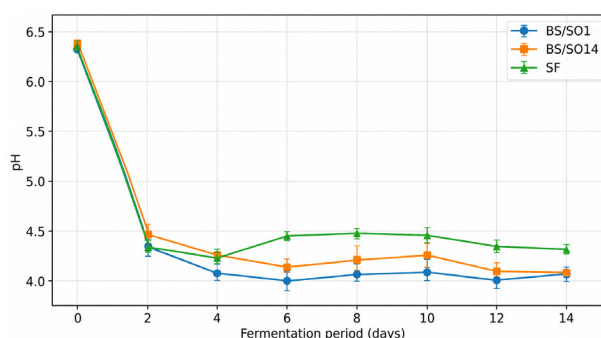


Figure 3. Changes in pH of *Bambusa spinosa* shoots under BSISO1 inoculation, BSISO14 inoculation, and spontaneous fermentation. Points represent the mean of three fermentation jars per treatment at each sampling time ($n = 3$). Error bars represent $\pm$ one standard deviation (SD).

After this initial decrease, pH values remained acidic throughout the rest of the fermentation period. Notably, BSISO1 generally showed the lowest pH values from Day 4 onward, reaching 4.05 ± 0.04 on Day 4 and 3.99 ± 0.09 on Day 6, while BSISO14 ranged from 4.26 ± 0.04 to 4.01 ± 0.07 over the same period. In contrast, spontaneous fermentation maintained relatively higher pH values on Days 6 and 8, at 4.47 ± 0.02 and 4.49 ± 0.02 , respectively. By the end of fermentation, the pH values were 4.47 ± 0.07 for BSISO1, 4.36 ± 0.06 for BSISO14, and 4.33 ± 0.04 for spontaneous fermentation.

For total titratable acidity, the opposite overall pattern was observed, with values increasing as fermentation progressed. Figure 4 shows that at Day 2, TTA was highest in BSISO1 (1.27 ± 0.06 g/100g), followed by BSISO14 (0.77 ± 0.21 g/100g) and spontaneous fermentation (0.57 ± 0.12 g/100g). TTA continued to rise in all treatments through Day 8, with the highest values at 3.03 ± 0.06 g/100g for BSISO1, 2.30 ± 0.20 g/100g for BSISO14, and 1.97 ± 0.06 g/100g for spontaneous fermentation. As shown in Figure 4, after Day 8, TTA values slightly declined or stabilized, ending at 2.50 ± 0.10 g/100g for BSISO1, 2.30 ± 0.20 g/100g for BSISO14, and 2.00 ± 0.26 g/100g for spontaneous fermentation on Day 14.

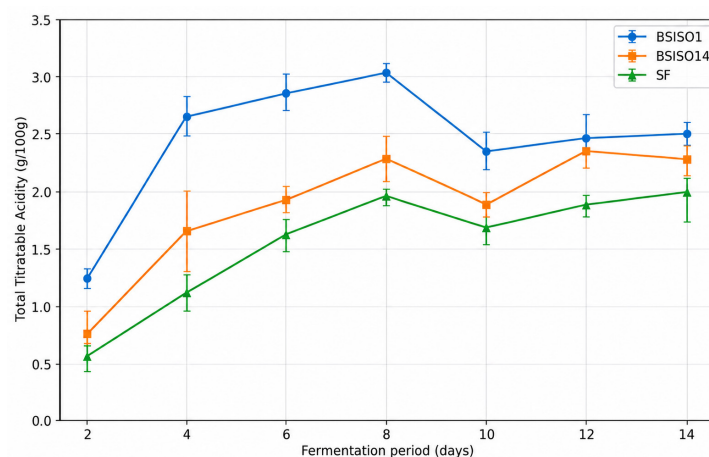


Figure 4. Changes in total titratable acidity (TTA; g/100 g) of *Bambusa spinosa* shoots under BSISO1 inoculation, BSISO14 inoculation, and spontaneous fermentation. Points represent the mean of three fermentation jars per treatment at each sampling time ($n = 3$). Error bars represent $\pm$ one standard deviation (SD).

The repeated-measures analysis showed that pH and TTA changed significantly across the fermentation period and differed among the BSISO1, BSISO14, and spontaneous fermentation treatments. The significant treatment $\times$ day interaction further indicated that the acidification pattern over time depended on the fermentation treatment. All treatments showed a marked decrease in pH from Day 0 to Day 2, indicating that acid production began early in the fermentation process.

Nutritional composition of fermented *Bambusa spinosa* shoots

The nutritional composition of fermented *B. spinosa* shoots was evaluated for fat, reducing sugar, and protein contents

(g/100 g), as well as crude fiber (%). As shown in Table 4, differences were observed among the fresh sample, the BSISO1- and BSISO14-inoculated treatments, and the spontaneous fermentation treatment. Because these data were analyzed descriptively, the observed differences should not be interpreted as statistically significant treatment effects.

Fat content varied only slightly across treatments. BSISO1 had the highest value (0.23 g/100 g), followed by BSISO14 (0.20 g/100 g), while spontaneous fermentation and the fresh sample had the lowest fat content (0.19 g/100 g). These small numerical differences suggest that fat content remained relatively stable across fermentation treatments.

Table 4. Comparison of fat, reducing sugar, protein, and crude fiber in fermented and fresh *Bambusa spinosa* shoots.

Treatment	Nutritional composition			
	Fat (g/100g)	Reducing sugar(g/100g)	Protein (g/100g)	Crude fiber (%)
BSISO1	0.23	0.116	1.06	2.09
BSISO14	0.2	0.111	1.16	2.19
Spontaneous fermentation	0.19	0.211	0.834	1.61
Fresh bamboo shoot	0.19	1.48	1.18	3.61

For reducing sugar, the fresh bamboo shoot recorded the highest value (1.48 g/100 g), while all fermented treatments showed markedly lower levels. Among these, BSISO14 exhibited the lowest reducing sugar content (0.111 g/100 g), followed by BSISO1 (0.116 g/100 g) and spontaneous fermentation (0.211 g/100 g), indicating a substantial decrease after fermentation. The lower reducing sugar values in the fermented samples indicate substantial sugar utilization during fermentation, with the greatest apparent reductions occurring in the inoculated treatments.

Regarding protein content, the fresh bamboo shoot showed the highest value (1.18 g/100 g), closely followed by BSISO14 (1.16 g/100 g) and BSISO1 (1.06 g/100 g). Spontaneous fermentation yielded the lowest protein content (0.834 g/100 g), suggesting improved protein retention in inoculated fermentations compared with spontaneous fermentation. Descriptively, the inoculated treatments maintained protein contents closer to that of the fresh sample than the spontaneous fermentation treatment.

For crude fiber, the fresh bamboo shoot had the highest value (3.61%), whereas all fermented samples had lower contents. Among the fermented treatments, BSISO14 recorded the highest crude fiber level (2.19%), followed by BSISO1 (2.09%), while spontaneous fermentation showed the lowest (1.61%). Thus, crude fiber was lower after fermentation, although the inoculated treatments remained numerically higher than spontaneous fermentation.

DISCUSSION

Morphological and gram-stain characterization of *Bambusa spinosa* shoot-derived LAB isolates

The isolates recovered from fresh *B. spinosa* shoots exhibited variation in colony color, shape, opacity, and size, indicating phenotypic diversity among the cultivable acid-producing bacteria. Their colony characteristics generally matched those reported for lactic acid bacteria, which commonly form circular, white to yellowish colonies measuring approximately 1–5 mm and may appear opaque or semi-translucent (Chaudhry et al., 2024). Clear zones around colonies on calcium carbonate-supplemented MRS agar also indicated acid production, a characteristic frequently used in the preliminary selection of presumptive LAB (Pava et al., 2024).

Gram staining showed that all selected isolates were Gram-positive, with bacillary forms predominating over coccoid forms. This Gram-positive reaction is consistent with the thick peptidoglycan cell wall typically associated with LAB. The predominance of rods was also comparable with plant- and bamboo-associated LAB commonly reported in previous studies, including members of *Lactiplantibacillus* and *Levilactobacillus* (Chen et al., 2024; Jin et al., 2023). Thus, the combined colony morphology and Gram-stain results supported the preliminary classification of the recovered bacteria as presumptive LAB, while molecular identification remained necessary for more reliable taxonomic assignment.

Taxonomic affiliation and phylogenetic placement of selected LAB isolates

The placement of BSISO1 and BSISO14 within the *L. plantarum* group is consistent with the occurrence of related LAB in bamboo shoot-derived and other plant-based fermented products. For instance, Kanpiengjai et al. (2022) recovered *L. plantarum*, *Limosilactobacillus fermentum*, and *Pediococcus acidilactici* from “naw-mai-dong”, a traditional fermented bamboo shoot product in Thailand. Similarly, Romi et al. (2015) reported *L. plantarum* and *Levilactobacillus brevis* during the later stages of “soidon” fermentation in Northeast India. These findings provide ecological context for recovering related isolates from *B. spinosa* shoots. Beyond bamboo shoots, Thierry et al. (2023) identified 93 LAB isolates in a study of 75 homemade fermented vegetable samples, with the combined *L. pentosus* or *L. plantarum* group among the most frequently identified taxa.

The reconstructed phylogeny further supports this group-level affiliation, placing BSISO1 and BSISO14 alongside matching *L. plantarum* and *L. pentosus* reference sequences (Figure 2). Their unresolved placement is consistent with the high conservation of the 16S rRNA gene among members of this group. Huang et al. (2021) reported 16S rRNA gene sequence identities of 96.7–100% among the examined type strains, whereas the *mutL* gene provided greater species discrimination. This comparison helps explain why the present phylogenetic analysis supports their taxonomic affiliation without separating the two proposed species.

Considered alongside their measured acidification responses, the recovery of BSISO1 and BSISO14 supports further investigation of *B. spinosa*-derived LAB as indigenous starter-culture candidates. Retaining their individual isolate designations also allows discussion of their fermentation performance independently of species confirmation. Nevertheless, their respective assignments as *L. plantarum* and *L. pentosus* remain provisional pending confirmation using a more discriminatory marker, such as *mutL*, or whole-genome comparisons with reference type strains using average nucleotide identity and digital DNA–DNA hybridization (Huang et al., 2021; Kingkaew et al., 2024).

Changes in pH and total titratable acidity during fermentation

pH and total titratable acidity (TTA) are among the most important indicators for monitoring fermentation because they reflect acidification progress and the development of a fermentation environment that favors lactic acid bacteria while suppressing less acid-tolerant and potentially undesirable microorganisms (Zhang et al., 2025). In bamboo shoot fermentation, organic acid accumulation is associated with decreasing pH and increasing acidity, and these pH–TTA gradients are considered major drivers of microbial succession and product stability (Wu et al., 2025). Accordingly, these measurements were used in the present study to characterize acidification during fermentation. Conversely, microbial succession, suppression of undesirable microorganisms, and product stability were not directly evaluated; therefore, the observed acidification patterns alone do not establish these outcomes.

Fermentation of *B. spinosa* shoots showed the expected inverse relationship between pH and total titratable acidity, with the rapid early-stage decline in pH accompanied by a corresponding increase in acidity (Giri et al., 2018). This pattern is characteristic of lactic fermentation, where fermentative microorganisms, particularly lactic acid bacteria, convert available carbohydrates into organic acids that acidify the substrate (Giri et al., 2018; Tang et al., 2017). Chen et al. (2024) reported a similar early pH decline, followed by a subsequent increase and stabilization. In the present study, BSISO1 reached pH 3.99 on Day 6 before increasing to 4.47 on Day 14, whereas Chen et al. reported a final pH of 3.79 for their *L. plantarum* R1 treatment. BSISO1 reached its highest TTA of 3.03 g/100 g on Day 8, compared with the R1 peak of 1.23 g/100 g on Day 10. These observations indicate differences in the timing and magnitude of acidification, although differences in strains and fermentation conditions limit direct comparisons of starter-culture performance. Previous bamboo shoot fermentation research has reported acidification, changes in viable microbial counts, and reduced detection of Enterobacteriaceae in inoculated treatments (Botthoulath et al., 2024). These findings provide context for discussing acidification’s potential role in preservation, although the present fermentation trial did not evaluate microbial dominance, suppression of undesirable microorganisms, or preservation performance.

The early decline in pH and increase in TTA indicate acidification during the first two days of fermentation. Similar early acidification patterns are common in bamboo shoot fermentations, where lactic acid bacteria become dominant and drive preservation through acid production (Xu et al., 2023). Furthermore, lactic acid bacteria often dominate, constituting over 90% of the microbial community by the end of the process, and help stabilize the fermentation environment (Xu et al., 2025). This shift in microbial composition is primarily driven by the accumulation of lactic and acetic acids, which exert selective pressure against non-acid-tolerant species like Enterobacteriaceae (Zhang et al., 2025). The microbial community

changes and organic-acid profiles reported in previous studies provide context for these acidification patterns. Whether similar changes occurred here is unknown, because microbial succession and individual organic-acid concentrations were not measured in this fermentation trial.

The BSISO1-inoculated treatment generally showed stronger acidification during mid-fermentation, as reflected by lower pH and higher TTA values on several sampling days. BSISO1 was provisionally identified as *L. plantarum*. The acidification response is consistent with fermentation-associated acid production, but substrate utilization, individual organic-acid concentrations, and BSISO1's contribution relative to resident microorganisms were not directly determined. Previous studies have evaluated the technological performance of particular *L. plantarum* strains in bamboo-related fermentation systems. Their findings provide a basis for investigating BSISO1 as a starter-culture candidate. This agrees with the known fermentative ability of *L. plantarum*, which is widely recognized as an efficient acid-producing lactic acid bacterium in plant-based fermentations (Dulf et al., 2022). *L. plantarum* has strong evidence for both probiotic functionality and starter-culture potential in bamboo shoot systems. In “Nor mai som” Lao fermented bamboo shoots, *L. plantarum* BBS13 functioned as an effective starter culture: inoculation lowered pH from above 4.6 to about 3.0, increased lactic acid, made Enterobacteriaceae undetectable in inoculated treatments, reduced cyanide by 62.09% to 90.31% by day 10, and shortened fermentation from 15 days to 5 days (Botthoulath et al., 2023). Additionally, Zhao et al. (2021) evaluated *L. plantarum* fermentation of a beverage containing red jujube fruits and bamboo shoots, reporting acidification, viable cell counts, and compositional changes in that particular matrix. These published findings illustrate the technological potential of particular strains in specific substrates.

The BSISO14-inoculated treatment also showed acidification, with a response generally intermediate between the BSISO1-inoculated treatment and spontaneous fermentation. BSISO14 was provisionally identified as *L. pentosus*. These observations describe the acidification performance of the inoculated treatment under the tested conditions. In comparison, this indicates that *L. pentosus* likewise functioned as an effective fermentative lactic acid bacterium. Still, its acidifying activity under the present conditions appeared slightly less pronounced than that of *L. plantarum*. Although *L. pentosus* is less frequently highlighted than *L. plantarum* in bamboo-shoot papers, its identification in this study is still important. Beyond bamboo shoots, *L. pentosus* is well documented in plant-derived fermentations as a species with both probiotic value and starter-culture utility. López-García et al. (2023) investigated the transcriptomic response of *L. pentosus* LPG1 during Spanish-style green table olive fermentation. In a separate study of natural Gemlik-type black olives, Alan (2024) reported acidification and chemical changes following inoculation with selected *L. plantarum* and *L. pentosus* starter cultures. In addition, *L. pentosus* 68-1 showed strong tolerance to simulated gastrointestinal juices, excellent adhesion-related properties, antibacterial and antifungal activity, and no hemolytic effect, supporting its candidacy as a probiotic strain (Xue et al., 2024). The technological and in vitro functional properties reported for other strains provide context for further evaluation of BSISO14. Still, these properties cannot be attributed to BSISO14 solely from its provisional species identification or the acidification observed in the present fermentation trial.

The observed acidification responses support further evaluation of BSISO1 and BSISO14 as starter-culture candidates for controlled fermentation of *B. spinosa* shoots, with the BSISO1-inoculated treatment showing the stronger response under

the tested conditions. The findings do not establish strain persistence, microbial dominance, preservation performance, or product safety.

Nutritional composition of fermented *Bambusa spinosa* shoots

Numerical differences in protein, crude fiber, reducing sugar, and fat contents among the analyzed samples provide preliminary information on the nutritional composition of *B. spinosa* shoots across treatments. The lower reducing sugar contents in the fermented samples are consistent with possible carbohydrate utilization during fermentation. In a related bamboo-containing food system, Zhao et al. (2021) reported decreased sucrose content after fermenting a red jujube-bamboo shoot beverage with *L. plantarum*, illustrating fermentation-associated changes in carbohydrate composition. These observations provide a basis for further investigation of carbohydrate changes during controlled *B. spinosa* fermentation. Because one representative sample was analyzed per treatment, replicated nutritional analyses and direct monitoring of sugar utilization would help confirm these descriptive findings and clarify the contributions of BSISO1 and BSISO14.

Furthermore, the lower crude fiber contents measured in the fermented samples may reflect partial modification or solubilization of structural carbohydrates during fermentation. Research on other plant substrates provides context for investigating these processes. For example, Xia et al. (2024) reported reductions in indigestible fiber fractions and improved in vitro dry matter digestibility in mixed silage treated with LAB combined with added cellulase, illustrating how microbial fermentation and enzyme supplementation can influence fiber composition. Garrido-Galand et al. (2021) also reviewed the broader nutritional and technological effects of fermentation on cereal and legume flours, providing additional context for investigating compositional changes in plant-based substrates. In the present study, the crude fiber measurements provide preliminary information on the composition of fermented *B. spinosa* shoots and identify structural carbohydrate changes as a useful area for further investigation. Replicated analyses of specific fiber fractions and enzyme activities are needed to confirm the potential contributions of solubilization and enzymatic hydrolysis.

The BSISO14 sample had higher measured protein and crude fiber contents than the spontaneous fermentation sample, providing preliminary insight into compositional differences between treatments. Previous studies offer context for investigating how selected microorganisms and substrate characteristics influence fermentation outcomes. Khiabani et al. (2024) recovered 61 LAB strains from 46 flower samples, representing 24 species across nine genera. Of these isolates, 40 exhibited slimy or ropy phenotypes on sucrose-containing agar, indicating differences in polysaccharide-associated characteristics. Testing in almond, oat, and soy milk showed acidification in all three substrates, whereas texture development occurred only in soy milk. These findings illustrate the relevance of evaluating selected cultures directly in the intended plant substrate. More directly related to nutritional composition, Munghang et al. (2025) fermented a formulation containing melon seeds, yellow yam, banana, and apple using individual LAB starters at approximately 10^6 CFU/g for 24 h at 30 °C. In the freeze-dried formulations, protein contents were $12.3 \pm 0.5\%$ with *L. plantarum* AFI5 and $12.2 \pm 0.4\%$ with *Leuconostoc mesenteroides* GSL7, significantly higher than the $11.7 \pm 0.0\%$ reported for the chemically acidified control ($p < .05$). Total free amino acids reached approximately 402 mg/100 g with *Lactocaseibacillus paracasei* SK8, compared with 352.6

mg/100 g in the incubated, uninoculated control. The AFIS formulation also yielded 3,417 detected peptides, compared with 2,425 in that control. These measurements demonstrate differences in protein concentration, free amino acids, and peptide profiles across starter treatments. Kårlund et al. (2020) reviewed fermentation-associated changes in protein digestibility and nutrient availability across plant-derived foods, including cereals, legumes, and pseudocereals. One study covered in that review, Lorusso et al. (2018), reported that fermentation of quinoa beverages for 20 h at 30 °C increased *in vitro* protein digestibility from $71 \pm 1\%$ to $84 \pm 2\%$ with *L. plantarum* T6B10 and from $71 \pm 1\%$ to $86 \pm 2\%$ with *Lacticaseibacillus rhamnosus* SP1. These findings provide a quantitative example of protein-related changes evaluated through digestion assays and support further investigation of the nutritional profiles obtained with BSISO1 and BSISO14. Because these nutritional measurements were based on one representative sample per treatment, replicated analyses and assessments of amino acid composition, digestibility, and bioavailability would clarify reproducibility and potential implications for protein quality.

In summary, the analyzed samples showed numerical differences in nutritional composition, with the BSISO14 sample having the highest measured protein and crude fiber contents among the fermented samples. Together with the pH and TTA results, these descriptive observations provide preliminary information for evaluating BSISO1 and BSISO14 as indigenous starter-culture candidates for *B. spinosa* fermentation.

Further product development would involve evaluating acidification alongside processing conditions, hygiene, and applicable microbiological criteria. For products within its scope, the Codex standard for pickled fruits and vegetables specifies an equilibrium pH below 4.6 and compliance with applicable microbiological criteria (Codex Alimentarius Commission, 2023). These criteria provide a reference for future finished-product assessment, complementing fermentation monitoring through pH measurements in homogenized sample supernatants and TTA determination.

In the Philippine context, controlled fermentation could diversify the utilization of locally available labong beyond fresh vegetable consumption. With process standardization and confirmation of microbial safety and storage stability, this approach could offer a preservation option for freshly harvested shoots, allowing surplus harvests to be processed before deterioration and potentially reducing postharvest losses. Fermented bamboo products could also be explored as value-added foods for small-scale enterprises, with possible livelihood opportunities for local growers and processors. The study provides exploratory findings from pooled bamboo-shoot material collected at three locations, with broader geographic applicability requiring evaluation across additional independently collected samples. Replicated nutritional analyses are needed because one representative sample was tested per treatment, and microbial counts, strain persistence, finished-product equilibrium pH and safety, storage stability, postharvest outcomes, consumer acceptance, and economic feasibility remain to be evaluated.

CONCLUSION

Bambusa spinosa shoots yielded Gram-positive isolates consistent with presumptive LAB. Molecular analysis placed BSISO1 and BSISO14 within the *L. plantarum* group, with their respective assignments as *L. plantarum* and *L. pentosus* remaining provisional. During the 14-day fermentation trial, pH and total titratable acidity profiles characterized acidification in the

inoculated and spontaneous fermentation treatments, with the BSISO1-inoculated treatment generally exhibiting the strongest acidification response under the experimental conditions tested. Comparisons of fresh and fermented samples also revealed numerical differences in protein, crude fiber, reducing sugar, and fat contents. These nutritional findings provide descriptive information on the samples analyzed, as one representative sample per treatment was tested.

Overall, the acidification results support further evaluation of BSISO1 and BSISO14 as indigenous starter-culture candidates for controlled fermentation of *B. spinosa* shoots. Microbial counts, strain persistence, and safety-related measurements of the fermented product were not evaluated; therefore, strain survival, microbial dominance, preservation benefits, and product safety remain to be established. Further studies should confirm the isolates' taxonomic identities, quantify and standardize inoculum concentrations in CFU/mL, monitor strain persistence, and evaluate organic-acid production, replicated nutritional outcomes, sensory acceptability, microbial safety, shelf life, and pilot-scale performance before commercial application.

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AUTHOR CONTRIBUTIONS

J.E.H: Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original Draft, and Writing – Review and Editing. A.G.L.C: Supervision, Methodology, and Writing – Review and Editing.

DECLARATIONS

Informed consent statement

This study involved bamboo shoots and bacterial isolates and did not involve human participants.

Conflict of Interest

The authors declare no conflict of interest.

AI Declaration

During manuscript preparation, the authors used Grammarly to assist with grammar, spelling, punctuation, and language clarity, and ChatGPT (OpenAI) to assist with text organization and coherence. The authors reviewed all assisted content and take full responsibility for the final manuscript.

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