

Antibacterial Potential of Lactic Acid Bacteria Isolated from Cassava *Tape* Against *Salmonella typhi*

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Abstract

Lactic acid bacteria (LAB) from traditional fermented foods, such as cassava *tape*, are known to produce metabolic compounds with potential inhibitory effects against pathogenic bacteria. This study aimed to identify LAB isolated from cassava *tape* and to evaluate their antibacterial activity against *Salmonella typhi* (*S. typhi*). This research was a descriptive experimental study using cassava *tape* samples obtained from three traditional markets in Palangka Raya City. LAB isolation was performed on de Man Rogosa Sharpe Agar supplemented with 1% calcium carbonate (CaCO₃), followed by macroscopic and microscopic observations and biochemical tests. Molecular identification was conducted using 16S rRNA gene analysis. Antibacterial activity against *S. typhi* ATCC 14028 was evaluated using the disk diffusion method on Mueller-Hinton agar. Three isolates were successfully obtained and coded as S1, S2, and S3. Molecular identification revealed that isolates S1 and S3 were identified as *Limosilactobacillus fermentum*, whereas isolate S2 was identified as *Myroides phaeus* and classified as a non-LAB. Antibacterial activity assays showed that none of the isolates produced inhibition zones against *S. typhi* at all tested concentrations. These results indicate that cassava *tape* contains LAB dominated by *L. fermentum*; however, under the experimental conditions of this study, the isolates did not exhibit antibacterial activity against *S. typhi*.

Keywords: Antibacterial activity; Cassava *tape*; Lactic acid bacteria; *Limosilactobacillus fermentum*; *Salmonella typhi*.

INTRODUCTION

Salmonella typhi (*S. typhi*) is a Gram-negative pathogenic bacterium that remains a public health concern, particularly in developing countries. Transmission of *S. typhi* mainly occurs through the fecal–oral route via the consumption of contaminated food or water, enabling the bacterium to penetrate the intestinal mucosa and survive within macrophages before disseminating systemically to organs such as the liver and spleen (Hartanto, 2021). The ability of *S. typhi* to persist in the environment and adapt to host defense mechanisms makes it a continuously relevant foodborne pathogen.

Control of *S. typhi* infection currently relies heavily on antibiotic therapy. However, the increasing reports of antimicrobial resistance have reduced treatment effectiveness. Multidrug-resistant *Salmonella typhi* (MDRST), which is resistant to first-line antibiotics such as ampicillin, trimethoprim–sulfamethoxazole, and chloramphenicol, as well as extensively drug-resistant (XDR) strains, have been widely reported (Sanjaya et al., 2022). In Indonesia, phenotypic evidence of multidrug-resistant *S. typhi* isolates has also been documented,

indicating the need for additional approaches that focus on controlling or inhibiting bacterial growth rather than replacing primary therapy (Jamilah et al., 2020)

One approach that has attracted considerable attention is the exploration of lactic acid bacteria (LAB) derived from fermented foods as potential sources of antibacterial compounds. LAB are Gram-positive, non-spore-forming bacteria with cocci or rod shapes, capable of tolerating acidic conditions and producing antimicrobial substances such as organic acids, bacteriocins, hydrogen peroxide, and other inhibitory metabolites (Ayivi & Ibrahim, 2022). It is important to emphasize that the antibacterial potential of LAB is evaluated in vitro against specific bacterial targets, including *S. typhi*, and does not directly indicate therapeutic effectiveness in humans.

Cassava *tape* is a traditional Indonesian fermented food produced through carbohydrate fermentation involving yeasts and LAB. Several previous studies have shown that cassava *tape* contains diverse LAB populations and has potential as a source of probiotic bacteria (Rahmah et al., 2021). Molecular identification using 16S rRNA gene sequencing has demonstrated that *Lactobacillus fermentum* is one of the most dominant LAB species found in cassava *tape* (Barus et al., 2017) In

addition, studies on fermented foods have reported the ability of LAB to inhibit the growth of other pathogenic bacteria, such as *Staphylococcus aureus*, suggesting the production of antibacterial metabolites (Rahmah et al., 2021)

Nevertheless, scientific evidence regarding the antibacterial activity of LAB isolated specifically from cassava *tape* against *S. typhi* remains limited. Previous studies generally focused on different food matrices, bacterial targets, or experimental conditions, which may influence metabolite production and the observed inhibitory effects. Therefore, direct evaluation of the antibacterial activity of LAB isolated from cassava *tape* against *S. typhi* under controlled laboratory conditions is required to clarify their actual potential.

Based on this background, this study aimed to identify LAB species isolated from cassava *tape* and to evaluate their antibacterial activity against *Salmonella typhi* through in vitro assays. This study specifically emphasizes bacterial growth inhibition and is not intended to recommend cassava *tape* consumption as a therapy or preventive measure for typhoid fever. The findings are expected to contribute scientific insight into the diversity of LAB in cassava *tape* and their potential role as sources of antibacterial compounds against *S. typhi*.

MATERIALS AND METHODS

Study Area

This study was conducted as a descriptive experimental laboratory study. Cassava *tape* samples were collected from three traditional markets located in Palangka Raya City, Central Kalimantan, Indonesia. All laboratory analyses were carried out under controlled laboratory conditions.

Procedures

Sample Collection and Preparation

Cassava *tape* samples were collected aseptically and placed into sterile containers. The samples were then transported immediately to the laboratory for further microbiological analysis.

Isolation of Lactic Acid Bacteria

Isolation of lactic acid bacteria (LAB) was performed using de Man Rogosa Sharpe Agar (MRSA) supplemented with 1% calcium carbonate (CaCO₃). One gram of cassava *tape* sample was homogenized in de Man Rogosa Sharpe Broth (MRSB) and subjected to serial dilution. Aliquots from appropriate dilutions were inoculated onto MRSA supplemented with CaCO₃ using the spread plate method. The plates were incubated at 37 °C for 24 hour under anaerobic conditions. Presumptive LAB colonies were purified, and pure isolates were coded as S1, S2, and S3 (Martani et al., 2025).

Macroscopic and Microscopic Characterization

Macroscopic characterization of LAB isolates was conducted by observing colony shape, margin, elevation, surface texture, and color. Microscopic characterization was performed using Gram staining to determine Gram reaction and cellular morphology (Kusnadi et al., 2025).

Biochemical Characterization

Biochemical tests were carried out for further characterization of the isolates. Catalase activity was determined by adding 3% hydrogen peroxide (H₂O₂) to bacterial colonies and observing bubble formation. Fermentation type was assessed using glucose fermentation tests to differentiate homofermentative and heterofermentative characteristics. Citrate utilization was evaluated using Simmons citrate agar by observing color changes after incubation. Triple Sugar Iron Agar (TSIA) tests were conducted to assess carbohydrate fermentation patterns and hydrogen sulfide production. Sulfide, indole, and motility (SIM) tests were performed to determine sulfide production, indole formation after the addition of Kovac's reagent, and bacterial motility. Methyl Red (MR) and Voges-Proskauer (VP) tests were conducted to evaluate acid production and acetoin formation during glucose fermentation. All biochemical tests were performed following standard microbiological procedures, and results were recorded based on observable reactions after incubation (Hafezi & Khamar, 2024).

Molecular Identification Using 16S Rrna Gene

Pure bacterial isolates were grown in de Man, Rogosa, and Sharpe Broth (MRSB) at 37 °C for 24 h. Genomic DNA extraction was performed using a Geneaid DNA Extraction Kit (Geneaid Biotech Ltd.). Briefly, 200 µL of the bacterial culture was placed into a microcentrifuge tube containing 200 µL of phosphate-buffered saline (PBS), followed by the addition of 20 µL of Proteinase K. The suspension was thoroughly mixed and incubated at 60 °C for 5 min. Subsequently, 200 µL of GSB buffer was added, vortexed, and incubated again at 60 °C for an additional 2 min. Absolute ethanol (96%) was then added, and the mixture was vortexed for 10 s. The resulting solution was transferred to a spin column and centrifuged at 14,000 × g for 1 min.

The collection tube beneath the spin column was discarded and replaced with a new one. Subsequently, 400 µL of W1 buffer was added, followed by centrifugation at the same speed for 30 s. The flow-through was discarded, and 600 µL of wash buffer was then added to the column and centrifuged for 30 s. After discarding the liquid in the collection tube, the column was centrifuged again for 3 min to remove residual buffer. The collection tube was then replaced with a sterile microcentrifuge tube. DNA was eluted by adding 100 µL of elution buffer and allowing the column to stand for 3 min, followed by centrifugation at the same

speed for 30 s. The eluted DNA was collected in the microcentrifuge tube and stored at $-40\text{ }^{\circ}\text{C}$ for subsequent use as a PCR template.

The 16S Rna gene was amplified using the universal primers 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and 1387R (5'-GGG CGG WGT GTA CAA GGC-3'), yielding an expected amplicon size of approximately 1,500 bp. PCR amplification was carried out under the following conditions: initial denaturation at $94\text{ }^{\circ}\text{C}$ for 2 min, followed by 30 cycles of denaturation at $94\text{ }^{\circ}\text{C}$ for 30 s, primer annealing at $55\text{ }^{\circ}\text{C}$ for 30 s, and extension at $72\text{ }^{\circ}\text{C}$ for 1.5 min, with a final extension step at $72\text{ }^{\circ}\text{C}$ for 10 min. The reaction was subsequently held at $12\text{ }^{\circ}\text{C}$ for 30 min. The amplified products were separated by electrophoresis on a 2% (w/v) agarose gel prepared in $0.5\times$ TBE buffer (100 g Tris base, 27.5 g boric acid, Ph 8.0, and 20 ml of 0.5 M EDTA). The gel was stained with ethidium bromide (1 μL ; 0.2 $\mu\text{g}/\text{ml}$) and visualized under ultraviolet illumination. The resulting 16S Rna gene amplicons were then subjected to sequencing analysis.

The PCR products were sequenced using a Genetic Analyzer (Applied Biosystems). The resulting nucleotide sequences were edited and aligned using BioEdit Sequence Alignment Editor software version 5.0.9. Sequence similarity analysis was performed by comparing the obtained 16S Rna gene sequences with those available in the GenBank database (<http://www.ncbi.nlm.nih.gov>) using BLASTn (Basic Local Alignment Search Tool for Nucleotide) to identify the closest related strains. Phylogenetic relationships were inferred using MEGA version 11.0.13 by

constructing phylogenetic trees with 1,000 bootstrap replications. This analysis was used to determine the evolutionary relatedness between the sequenced isolates and reference strains deposited in the GenBank database. (Martani et al., 2025).

Antibacterial Activity Assay

The antibacterial activity of LAB isolates against *Salmonella typhi* ATCC 14028 was evaluated using the disk diffusion method. Mueller Hinton Agar (MHA) was used as the test medium. A standardized suspension of *S. Typhi* was evenly spread onto the surface of MHA plates. Sterile paper disks impregnated with LAB cultures were placed on the agar surface and incubated at $37\text{ }^{\circ}\text{C}$ for 24 hours. Antibacterial activity was determined by measuring the diameter of inhibition zones formed around the disks (Saryono et al., 2023)

RESULTS AND DISCUSSION

Characterization of LAB

Macroscopic observation revealed that three purified isolates obtained from cassava tape exhibited colony characteristics commonly associated with lactic acid bacteria. All isolates formed circular colonies with smooth surfaces and entire margins, displaying convex elevation and a white coloration. The colonies were relatively small in size and showed a uniform appearance across isolates, indicating stable growth characteristics. A detailed summary of the macroscopic colony characteristics for each isolate is presented in Table 1.

Table 1 Macroscopic identification of lactic acid bacteria isolates.

Isolate	Color	Size	Shape	Elevation	Margin	Surface
S1	White	Small	Round	Covex	Entire	Smooth
S2	White	Small	Round	Covex	Entire	Smooth
S3	White	Small	Round	Covex	Entire	Smooth

Microscopic observation following Gram staining demonstrated that all three isolates exhibited Gram-positive characteristics, as indicated by the retention of crystal violet and the appearance of purple-stained cells. Despite this similarity, notable differences in cellular morphology were observed among the isolates. Isolate S1 displayed a rod-shaped morphology, whereas isolates S2 and S3 exhibited coccoid forms. The presence of Gram-positive rods in isolate S1 is consistent with the typical morphology of lactic acid bacteria. In contrast, the cocci morphology observed in isolates S2 and S3 suggests phenotypic diversity among the isolates. These morphological variations provide an important preliminary distinction among isolates and form the basis for subsequent biochemical and molecular analyses to confirm their taxonomic affiliation.

Subsequent biochemical profiling revealed that isolates S1, S2, and S3 shared several common characteristics. All isolates yielded negative results for catalase and citrate utilization tests, as well as for sulfide production, indole formation, and motility, indicating comparable basic metabolic traits among the isolates. In addition, the isolates showed similar responses in carbohydrate fermentation type tests. Differential reactions were observed in the Triple Sugar Iron Agar (TSIA) assay. While all isolates exhibited an acid reaction on the slant, isolate S1 produced an acid reaction in the butt, whereas isolates S2 and S3 showed an alkaline reaction. This variation suggests differences in carbohydrate fermentation pathways among the isolates. Further distinction was observed in the Methyl Red–Voges Proskauer (MR–VP) test. Although all isolates tested negative for the VP reaction, variability was

detected in the MR assay. Isolates S1 and S3 demonstrated positive MR results, indicating stable acid production, whereas isolate S2 was MR-negative, reflecting a lower capacity for mixed-acid fermentation. Despite sharing a largely similar biochemical profile,

specific differences in fermentation patterns and acid production levels were evident among the three isolates. The detailed biochemical characteristics of each isolate are summarized in Table 2.

Table 2 Result of LAB biochemical test.

Biochemical Test	Isolate		
	S1	S2	S3
Gram Staining	Gram-positive, rod-shaped	Gram-positive, circular-shaped	Gram-positive, circular-shaped
Catalase	-	-	-
Citrate	-	-	-
TSIA test	Acid/Acid Gas : (-)	Acid/Alkali Gas (-)	Acid/Alkali; Gas (-)
SIM test	H ₂ S: (-) Indole: (-) Motility: (-)	H ₂ S: (-) Indole: (-) Motility: (-)	H ₂ S: (-) Indole: (-) Motility: (-)
MR test	+	-	+
VP test	-	-	-
Fermentation type	Homofermentative	Homofermentative	Homofermentative

Identification Using 16S rRNA Gene

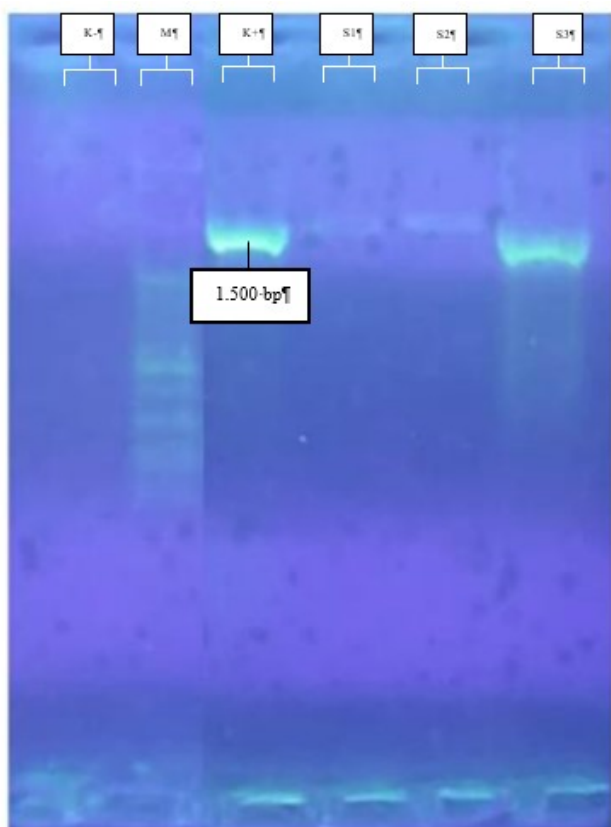


Figure 1 Amplification products of the 16S rRNA gene.

Table 3 Alignment of 16S rRNA gene sequences of the five LAB consortium isolates with available data in NCBI (BLAST-N).

Isolate Name	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession number
S1	<i>Limosilactobacillus fermentum</i> strain LDTM 7301 chromosome, complete genome	2237	11120	99%	0.0	98.43%	CP031195.1
	<i>Limosilactobacillus fermentum</i> strain a2-5 16S ribosomal RNA gene, partial sequence	2237	2237	99%	0.0	98.43%	OQ848047.1
	<i>Limosilactobacillus fermentum</i> strain KUMS-C18 16S ribosomal RNA gene, partial sequence	2237	2237	99%	0.0	98.43%	OP077202.1
S2	<i>Myroides phaeus</i> strain TS-2 16S ribosomal RNA gene, partial sequence	1175	1175	72%	0.0	87.31%	KX417283.1
	<i>Myroides phaeus</i> strain MY15 16S ribosomal RNA, partial sequence	1175	1175	72%	0.0	87.32%	NR_117475.1
	<i>Myroides phaeus</i> strain A6 16S ribosomal RNA gene, partial sequence	1175	1175	72%	0.0	87.31%	KU682196.1
S3	<i>Limosilactobacillus fermentum</i> strain SD-L-31 16S ribosomal RNA gene, partial sequence	2231	2231	97%	0.0	98.43%	MW583465.1
	<i>Limosilactobacillus fermentum</i> strain HBUAS51790 16S ribosomal RNA gene, partial sequence	2183	2183	97%	0.0	97.58%	MZ853337.1
	<i>Limosilactobacillus fermentum</i> strain SD-L-30 16S ribosomal RNA gene, partial sequence	2183	2183	96%	0.0	98.32%	MW582103.1

Sequence alignment of the 16S rRNA gene from the isolates against reference sequences available in the NCBI database (BLASTn) revealed varying levels of sequence similarity among the isolates. Isolate S1 exhibited a percent identity of 98.43%, indicating a close genetic relationship with the genus *Limosilactobacillus*, particularly *Limosilactobacillus fermentum*. In contrast, isolate S2 showed a lower percent identity of 87.31%, suggesting its affiliation with the genus *Myroides*, with the closest match to *Myroides phaeus*. Meanwhile, isolate S3 demonstrated high sequence similarity ranging from 97.50% to 98.43%, supporting its identification as *Limosilactobacillus fermentum*. These results confirm that while isolates S1 and S3 belong to lactic acid bacteria, isolate S2 represents a non-LAB bacterium. A detailed summary of the BLASTn alignment results is presented in Table 3.

Antibacterial Activity

The antibacterial activity assay showed that none of the tested lactic acid bacteria isolates exhibited inhibitory effects against *Salmonella typhi* at any of the evaluated concentrations (25%, 50%, 75%, and 100%). No inhibition zones were observed surrounding the discs impregnated with the isolates, indicating the absence of detectable antibacterial activity under the conditions applied in this study. A similar result was observed in the negative control, which also showed no zone of inhibition, confirming that the observed outcomes were not influenced by external inhibitory factors. These findings demonstrate that the tested isolates did not suppress the growth of *S. typhi* across all concentration levels. A summary of the antibacterial activity results is presented in Table 4.

Table 4 The Result of antibacterial activity.

Isolates	Concentration				
	Negative	25%	50%	75%	100%
S1	-	-	-	-	-
S2	-	-	-	-	-
S3	-	-	-	-	-

Discussion

The characterization of lactic acid bacteria in this study was conducted through macroscopic, microscopic, and biochemical observations, providing a comprehensive overview of the isolate profiles. Macroscopically, all isolates exhibited circular colonies with convex surfaces and smooth margins, which are characteristic features of lactic acid bacteria grown on MRSA supplemented with 1% CaCO. Similar morphological traits have been reported by (Finanda et al., 2021) who described LAB isolates from fermented substrates showing comparable colony appearances on the same selective medium. Microscopically, Gram staining revealed that all isolates were Gram-positive, although variations in cell morphology were observed. This finding is consistent with (Rahmah et al., 2021), who reported that LAB generally exhibit Gram-positive staining despite differences in cellular shape. Supporting this observation, (Amanda et al., 2025) demonstrated that LAB isolated from Mayas rice fermentation displayed both rod-shaped and coccoid morphologies, a pattern that parallels the morphological diversity observed among the isolates in the present study. Biochemically, all isolates tested negative for catalase activity, indicating the absence of the catalase enzyme responsible for decomposing hydrogen peroxide into water and oxygen. This result aligns with the fundamental biochemical characteristics of LAB and corroborates previous findings reported by

(Hairunnisa & Sari, 2019), further supporting the classification of the isolates as lactic acid bacteria.

The molecular identification based on 16S rRNA gene sequencing revealed that two isolates obtained from cassava tape, namely isolates S1 and S3, were identified as *Limosilactobacillus fermentum*, whereas isolate S2 was identified as *Myroides phaeus*. The identification of *L. fermentum* is consistent with its known phenotypic and biochemical characteristics, as this species is described as a Gram-positive, rod-shaped, non-spore-forming, and catalase-negative bacterium (Octaviana et al., 2023), which aligns with the results observed in this study. *Limosilactobacillus fermentum* is recognized as a lactic acid bacterium with notable probiotic potential and has been reported to exhibit various functional properties, including antibacterial, antioxidant, anti-inflammatory, and immunomodulatory activities, as well as roles in the prevention and management of hyperuricemia and uric acid-related disorders (Octaviana et al., 2023).

L. fermentum is widely distributed across diverse ecological niches and can be found on plant surfaces, as well as in the mucosal environments of humans and animals. This species is commonly isolated from a wide range of fermented foods, including fermented vegetables, dairy products, and traditional fermented foods, reflecting its strong adaptability to carbohydrate-rich and nutrient-dense substrates. Moreover, *L. fermentum* is generally regarded as a safe microorganism for food-related applications. This is supported by genomic analyses reported by (Yaikhan et al., 2025), which demonstrated the absence of harmful virulence genes in *L. fermentum*, thereby classifying this species as non-pathogenic and reinforcing its suitability for use in fermented food products.

The antibacterial activity assay demonstrated that lactic acid bacteria isolates obtained from cassava tape did not produce inhibition zones against the pathogenic bacterium *Salmonella typhi*. This finding indicates that antimicrobial compounds produced by the isolates, either in the form of viable cells or their metabolites, did not exhibit effective bactericidal or bacteriostatic activity under the experimental conditions applied in this study. In general, LAB are recognized for their probiotic potential due to their ability to synthesize various antimicrobial substances, such as bacteriocins, which play a role in suppressing pathogenic microorganisms. However, the absence of inhibitory activity observed in this study may be attributed to several factors, including insufficient concentrations of antimicrobial compounds or suboptimal growth and testing conditions. This result contrasts with findings reported by (Haro et al., 2020), who demonstrated that LAB isolated from traditional fermented foods such as naniura exhibited notable antibacterial activity against *S. typhi*.

Theoretically, LAB possess strong inhibitory effects against a wide range of pathogenic bacteria, particularly Gram-negative organisms such as *S. typhi*, through the

production of metabolites including lactic acid, acetic acid, hydrogen peroxide, diacetyl, and bacteriocins. Previous studies have confirmed that bacteriocins produced by LAB species, such as *Lactobacillus acidophilus*, exhibited antimicrobial activity against *S. typhi* using agar diffusion assays (Pratiwi et al., 2021). These findings suggest that LAB isolates derived from cassava tape may inherently possess antimicrobial components; however, their activity may not have been optimally expressed or detected in the present study. The lack of inhibition zones may also be associated with methodological limitations of the disc diffusion assay, as similarly reported by (Martani et al., 2025), where certain antimicrobial metabolites exhibited poor diffusion properties in agar media.

Differences in antibacterial effectiveness may be influenced by environmental conditions during LAB cultivation. In this study, anaerobic growth conditions were not fully optimized, which may have affected the metabolic activity of the isolates. Lactic acid bacteria are predominantly anaerobic microorganisms, and oxygen availability plays a crucial role in determining their metabolic pathways and the production of antimicrobial compounds (Cahyani & Agustini, 2025). Aerobic conditions may induce oxidative stress or alter fermentation pathways, leading to reduced synthesis of organic acids or bacteriocins compared to anaerobic environments, which are considered optimal for most LAB strains. Therefore, the absence of detectable antibacterial activity in this study highlights the need for further investigations focusing on bacteriocin detection and optimization of culture and assay conditions to better evaluate the antibacterial potential of LAB isolates from cassava tape.

CONCLUSION

This study demonstrated that lactic acid bacteria isolated from cassava tape were identified as *Limosilactobacillus fermentum* based on phenotypic characterization and molecular analysis. However, under the conditions applied in this study, the isolated bacteria did not exhibit antibacterial activity against *Salmonella typhi* as determined by the disc diffusion method. These findings indicate that although *L. fermentum* was successfully isolated from fermented cassava, its antibacterial potential against *S. typhi* was not detectable using the current assay conditions.

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Competing Interests: The authors declare that there are no competing interests.

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