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Original Research Paper

ISOLATION, IDENTIFICATION AND SAFETY PROFILING OF POTENTIAL PROBIOTIC LACTIC ACID BACTERIA FROM INDIGENOUS FOOD PLANTS IN ILOCOS SUR

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ABSTRACT

This study investigated lactic acid bacteria (LAB) associated with Indigenous Food Plants (IFPs) of Ilocos Sur-Yacon (Smallanthus sonchifolius), Tebbeg (Ficus nota), and Allagat (Uvaria rufa)-through isolation, characterization, molecular identification, and safety evaluation. Samples were enriched in MRS broth, which yielded the highest LAB recovery, followed by serial dilution and plating. Out of 83 isolates, ten were selected based on Gram-positive, catalase-negative, and typical LAB morphology. All isolates exhibited non-hemolytic activity, indicating favorable safety profiles, while antibiotic susceptibility tests revealed significant variation ($p < 0.001$), with several isolates showing comparable or higher susceptibility than the Lactiplantibacillus plantarum ATCC reference strain. 16S rRNA gene sequencing revealed high sequence similarity (96.11–100%), identifying the isolates as Enterococcus spp., Pediococcus pentosaceus, Streptococcus Suis, and Lactiplantibacillus plantarum. The generated sequences were deposited in the NCBI GenBank database under accession numbers PZ235148, PZ231870, PZ231868, PZ231865, PZ231791, PZ231582, PZ231487, and PZ231317. Phylogenetic analysis using the Maximum Likelihood method confirmed clear clustering consistent with BLAST results. Notably, ISPSC-AL8B grouped with Pediococcus pentosaceus, while ISPSC-XY13 clustered within the Lactiplantibacillus plantarum lineage. Overall, the results confirm the successful identification of LAB from IFPs and highlight selected isolates as potential candidates for probiotic and food biotechnology applications.

Keywords: *Lactic acid bacteria; Indigenous food plants; Characterization; Hemolytic activity; Antibiotic susceptibility, 16s rRNA, Phylogenetic Tree*

1. INTRODUCTION

Lactic acid bacteria (LAB), particularly those of the genus Lactobacillus (now primarily classified under genera such as *Lactiplantibacillus*), are among the most recognized and researched microorganisms in food fermentation, preservation, and health enhancement. These microorganisms generally derive from plant materials, where they proliferate on fermentable sugars and bioactive compounds. They demonstrate considerable potential for the advancement of probiotic and functional food products due to their acid adaptability, production of antimicrobial metabolites, and capacity to metabolize complex carbohydrates (Lee et al., 2022; Baek et al., 2024).

Indigenous Food Plants (IFPs) represent an underutilized reservoir of advantageous microorganisms. Historically, these plants were utilized for consumption and in herbal medicine across numerous Filipino communities, providing both nutritional and therapeutic advantages. Highlighting that plant-associated lactobacilli (LAB) serve as reservoirs of functional strains adapted to the chemical diversity present in the endogenous environment, including phytochemicals and prebiotic substrates (Xiao et al., 2024; Balungaya et al., 2024).

In Ilocos Sur, Yacon (*Smallanthus sonchifolius*), Tebbeg (*Ficus nota*), and Allagat (*Uvaria rufa*) are prevalent indigenous food plants with distinct biochemical compositions. Yacon is acknowledged for its high concentrations of fructooligosaccharides (FOS) and inulin, which function as prebiotic agents that foster advantageous gut microbiota (Lachman et al., 2003; Ojansivu et al., 2011). It contains phenolic compounds and antioxidants that assist microbes in selection and adaptation (Paula et al., 2015). Tebbeg and Allagat contain phytochemicals such as flavonoids, alkaloids, tannins, and terpenoids that

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combat pathogens and safeguard cells from damage (Tip-pyang et al., 2011; Rosandy et al., 2013; Mapatac, 2015). These chemicals create chemically complex environments, influencing microbial diversity and facilitating the survival of robust LAB populations.

Previous studies have demonstrated that Indigenous Food Plants, particularly Yacon, possess naturally occurring lactic acid bacteria, albeit in relatively low quantities initially. Microbial counts in fresh plant tissues significantly increase following enrichment or fermentation, indicating that plant matrices can promote LAB proliferation under optimal conditions (Molina et al., 2025). The distribution of LAB may vary among plant parts, with higher populations often observed in root-associated environments. These findings highlight the importance of enrichment strategies and confirm that Indigenous Food Plants are viable sources for isolating potential probiotic bacteria.

Despite the growing interest in plant-derived lactic acid bacteria (LAB), research on Indigenous Food Plants in the Philippines remains limited, particularly concerning systematic isolation, safety evaluation, and molecular identification. Recent research has primarily focused on fermented foods, leading to a lack of investigation into plant-associated lactic acid bacteria from indigenous species (Bedada et al., 2025; Duche et al., 2023). Moreover, there is a lack of comprehensive studies that combine microbial enumeration, phenotypic characterization, safety assessment, and molecular identification.

This study aimed to isolate lactic acid bacteria (LAB) from particular Indigenous Food Plants and evaluate their safety profile for potential application in functional foods. The research quantified the colony-forming units (CFU) of lactic acid bacteria (LAB) in plant samples, characterized the cultural and morphological properties of the isolates, evaluated their hemolytic activity, assessed their antibiotic susceptibility profiles, and identified the isolates via 16S rRNA gene sequencing. The results are expected to promote the development of locally sourced probiotic strains and support the use of indigenous biodiversity for functional food and biotechnological purposes.

2. METHODOLOGY

2.1. Research Methodology

The study employed an experimental laboratory design supplemented by descriptive analyses. Experimental techniques were used to isolate, identify, and characterize lactic acid bacteria (LAB) from designated Indigenous Food Plants (IFPs), while descriptive methods documented their cultural, morphological, and safety characteristics. This integrated methodology enabled the quantitative assessment of microbial load and the qualitative analysis of bacterial traits relevant to potential functional food applications.

2.2. Materials and Equipment

The plant samples included Yacon (*Smallanthus sonchifolius*), Tebbeg (*Ficus nota*), and Allagat (*Uvaria rufa*). The research employed an autoclave, a biosafety cabinet, an incubator, an analytical balance, a compound microscope, a colony counter, a hot plate, micropipettes, and a vernier caliper as laboratory apparatus. The collection included sterile gloves, scalpels, scissors, ziplock bags, an icebox, inoculating loops, an alcohol lamp, microcentrifuge tubes (MCTs), sterile pipette tips, applicator sticks, and various glassware, including beakers, test tubes, reagent bottles, and graduated cylinders. For isolation and characterization, we employed de Man, Rogosa and Sharpe (MRS) broth and agar, saline solution, 10% peptone water, Mueller–Hinton agar (MHA), blood agar (5% sheep blood), and antibiotic discs (Penicillin, Clindamycin, Erythromycin, Trimethoprim–Sulfamethoxazole, Ampicillin–Sulbactam, and Amoxicillin). The reagents utilized for Gram staining and catalase tests comprised crystal violet, Gram's iodine, safranin, decolorizer, and 3% hydrogen peroxide.

2.3. Acquisition of Botanical Specimens

Tebbeg and Allagat samples were aseptically collected from Barangay Silag, Santa Maria, Ilocos Sur, while Yacon was sourced from Barangay Malaya, Cervantes, Ilocos Sur. All plants were harvested with sterile scissors and scalpels while donning gloves to reduce contamination. Samples were promptly placed in sterile ziplock bags and stored in an icebox to maintain microbial viability during transport. Representative samples of each specimen were prepared as herbarium vouchers and submitted to the Herbarium of Northwestern Luzon (HNUL) in San Nicolas, Ilocos Norte, for taxonomic verification. Subsequent to identification, all samples were conveyed to the Microbiology Laboratory of Mariano Marcos State University (MMSU) for processing.

2.4. Preparation of Samples

Upon arrival at the laboratory, plant samples were washed with sterile distilled water to eliminate surface contaminants. Each sample type underwent triplicate analysis. One gram of plant tissue was aseptically measured and placed into test tubes containing 9 mL of sterile MRS broth, 10% peptone water, or saline solution, according to the enrichment needs. All media, instruments, and glassware were sterilized via autoclaving at 121 °C for 15 minutes before utilization. The inoculated enrichment tubes were incubated at 37 °C for 24 to 48 hours to promote the proliferation of potential lactic acid bacteria populations naturally found in the plant material.

2.5. Microbial Assessment and Quantification

Following enrichment, serial dilution procedures were conducted aseptically within a biosafety cabinet. Serial dilutions were prepared using sterile saline, reaching a dilution of 10^{-8}. Aliquots of 100 µL from the 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} dilutions were spread onto MRS agar plates with sterile spreaders. Plates were incubated at 37.5 °C for 24 to 48 hours under microaerophilic conditions (0% oxygen). Microbial proliferation was measured by enumerating colony-forming units (CFU) with a digital colony counter, and results were reported as CFU per gram of plant tissue.

2.6. Screening and Purification of Presumptive Lactic Acid Bacteria

Presumptive LAB colonies were identified based on distinct macroscopic traits, including creamy to white pigmentation, smooth convex morphology, and circular colony formation. A total of 83 colonies were isolated via successive streak plating onto fresh MRS agar. Gram staining was conducted according to standard microbiological protocols to verify the Gram-positive, rod- or coccus-shaped morphology typical of LAB. Catalase testing involved the application of 3% hydrogen peroxide to freshly prepared bacterial smears; isolates that failed to generate effervescence were classified as catalase-negative and preserved for subsequent analyses.

2.7. Evaluation of Hemolytic Activity

Ten isolates that satisfied the phenotypic criteria (creamy appearance, Gram-positive, and catalase-negative) were chosen for hemolytic assessment. Recently cultivated isolates were inoculated onto 5% sheep blood agar plates. *Enterococcus faecalis* functioned as the positive control for hemolysis. Plates were incubated at 37 °C for a duration of 24 hours. Hemolytic patterns were evaluated visually: β-hemolysis was characterized by transparent zones of complete red blood cell lysis, α-hemolysis by greenish discoloration indicating partial lysis, and γ-hemolysis by the absence of any halo around colonies. Only isolates demonstrating γ-hemolysis were deemed non-hemolytic and potentially safe for probiotic evaluation.

2.8. Antibiotic Susceptibility Assessment

The ten chosen isolates were assessed for antibiotic susceptibility employing the Kirby–Bauer disk diffusion technique. Each isolate was inoculated onto Mueller–Hinton agar plates employing the lawn culture technique to guarantee uniform bacterial proliferation. Antibiotic discs comprising Penicillin (P), Clindamycin (DA), Erythromycin (ERT), Trimethoprim–Sulfamethoxazole (SXT), Ampicillin–Sulbactam (SAM), and Amoxicillin (AMOX) were aseptically positioned on the agar surface. *Lactobacillus plantarum* ATCC was utilized as a quality control strain. Plates were incubated at 37 °C for a duration of 24 hours. Zones of inhibition (ZOI) were quantified in millimeters with a vernier caliper, and susceptibility classifications were assessed according to established criteria.

2.9. Molecular Characterization and Phylogenetic Assessment

The Genomics and Genetic Engineering Laboratory at Mariano Marcos State University in Ilocos Norte, Philippines, conducted genomic DNA extraction, amplification of the 16S rRNA gene, and sequencing in accordance with standardized laboratory protocols (Client ID: 25-007). Genomic DNA was extracted from bacterial isolates using the GF-1 Bacterial DNA Extraction Kit (Vivantis; Cat. No. GF-BA-100) according to the manufacturer's guidelines, with slight modifications. The integrity of the DNA was assessed by agarose gel electrophoresis, which revealed that all samples displayed a single distinct band exceeding 10,000 bp, indicating high-molecular-weight DNA. The concentration of DNA was quantified using the Quant-iT dsDNA System (Promega) and assessed with a Quantus Fluorometer after dilution in preheated elution buffer. The bacterial 16S rRNA gene was amplified using universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). The polymerase chain reaction (PCR) was performed using Emerald Green Master Mix with 1 µL of DNA template, with 35 amplification cycles. PCR amplicons were validated by agarose gel electrophoresis, yielding a single distinct band of approximately 1,500 bp.

Sanger sequencing was performed utilizing the ABI BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions adhered to cycling conditions that included an initial denaturation at 96 °C for 1 minute, followed by 25 cycles at 96 °C for 10 seconds, 50 °C for 5 seconds, and 62 °C for 4 minutes. Sequencing reaction purification was performed using the BigDye XTerminator kit before capillary electrophoresis on an ABI 3730xl DNA Analyzer, equipped with a 50-cm capillary array and POP7 polymer. Base calling was conducted utilizing Sequencing Analysis Software version 5.4.

2.10. Data Analysis

The data acquired from microbial enumeration, morphological and cultural characterization, hemolytic testing, and antibiotic susceptibility analysis were systematically analyzed through both quantitative and qualitative methods to evaluate the presence, characteristics, and safety of LAB isolates from Indigenous Food Plants. Microbial counts were assessed from MRS agar plates utilizing a digital colony counter, with only plates containing 30–300 colonies deemed valid to ensure

precision; CFU per gram of sample was computed using the formula CFU/g = (Number of Colonies × Dilution Factor) / Volume Plated, and results were presented as mean ± standard deviation from triplicate samples. Cultural and morphological attributes—color, Gram reaction, and cell shape—were recorded descriptively, and isolates displaying Gram-positive, rod or coccoid, non-spore-forming characteristics were classified as consistent with LAB. Catalase activity was evaluated by introducing 3% H_2O_2 to fresh cultures, with reactions classified as positive or negative based on bubble production, serving as a safety screening criterion. Hemolytic activity on 5% sheep blood agar was categorized as β-hemolysis (clear zone), α-hemolysis (greenish zone), or γ-hemolysis (no zone), with only non-hemolytic (γ) isolates deemed safe for probiotic applications. Antibiotic susceptibility was assessed by measuring inhibition zones with a vernier caliper, and isolates were classified as Resistant, Intermediate, or Susceptible based on established interpretive criteria. Statistical analysis was conducted utilizing Microsoft Excel, IBM SPSS, and RStudio for data computation, organization, and inferential testing.

Consensus sequences underwent molecular identification via the Basic Local Alignment Search Tool (BLASTn) against the National Center for Biotechnology Information (NCBI) nucleotide database to ascertain species identity based on percentage sequence similarity. A multiple sequence alignment of the acquired sequences and closely related reference sequences obtained from GenBank was conducted using MEGA version 12. Phylogenetic relationships were deduced employing the Neighbor-Joining method based on the Kimura two-parameter (K2P) nucleotide substitution model. The robustness of the tree was evaluated using bootstrap analysis with 1,000 replicates, and bootstrap support values exceeding 50% were indicated at the nodes. *Escherichia coli* ATCC 11775 served as an outgroup for the phylogenetic tree's rooting.

3. RESULTS AND DISCUSSION

3.1. Colony Forming Units (CFU) of Lactic Acid Bacteria

The quantification of lactic acid bacteria (LAB) extracted from Yacon, Tebbeg, and Allagat across three enrichment media demonstrated significant disparities in microbial yield. The 10^{-5} and 10^{-6} dilution plates were classified as TNTC (too numerous to count), signifying high microbial densities in all plant samples after enrichment. The 10^{-7} dilution consistently produced measurable colony counts and thus constituted the principal foundation for comparative analysis. The 10^{-8} dilution demonstrated significantly reduced growth, with multiple replicates exhibiting no colonies (NC) or contamination (CONT), indicating anticipated constraints linked to extreme dilutions.

MRS broth exhibited the highest recovery of lactic acid bacteria across all three Indigenous Food Plants, confirming its established function as a selective medium optimized for these microorganisms. In Yacon, the mean CFU per replicate in MRS broth varied from 90.0 to 106.0, with colony counts at 10^{-7} attaining 168–195 CFU per plate. Tebbeg exhibited a comparable pattern, with MRS-derived averages ranging from 88.0 to 188.0, signifying robust enrichment potential. Allagat demonstrated significant growth in MRS broth, with replicate means ranging from 83.0 to 169.0. The results collectively demonstrate that MRS broth yielded the most vigorous and uniform

LAB populations across all tested plant species.

Conversely, Peptone Water and NaCl solution produced moderate but discernible levels of LAB growth. For Yacon, Peptone Water yielded replicate averages ranging from 51.0 to 102.0, while NaCl solution produced averages between 50.0 and 100.0. Tebbeg exhibited a comparable trend: Peptone Water generated values between 47.0 and 94.0, while NaCl solution resulted in values ranging from 50.5 to 96.0. Allagat exhibited a similar pattern, with Peptone Water averages ranging from 49.5 to 100.0 and NaCl solution averages spanning from 47.5 to 98.0. The findings indicate that although LAB are inherently present in the three plant types, their growth is significantly affected by the nutritional and selective characteristics of the enrichment medium.

The enhanced efficacy of MRS broth is due to its optimized formulation, comprising dextrose, polysorbate, magnesium sulfate, and beef extract, which collectively promote the proliferation of acid-tolerant, fastidious lactic acid bacteria (LAB). Peptone Water and NaCl solution do not contain the specific growth factors necessary for optimal LAB proliferation, leading to reduced colony yields. This disparity highlights the significance of medium selection in microbial isolation and corroborates the experimental methodology employed in this study.

Occasional contamination and the absence of colonies at a 10^{-8} dilution indicate the natural variability present in microbiological assays. These observations substantiate the dependability of the 10^{-7} dilution as the standard quantification reference. The distinct gradient in colony counts—from TNTC at 10^{-5} and 10^{-6} to quantifiable levels at 10^{-7}—illustrates proper serial dilution and effective enrichment.

The data indicate that MRS broth is the most effective medium for isolating LAB from Yacon, Tebbeg, and Allagat, with Yacon exhibiting the highest LAB density among the three plants. This indicates that Indigenous Food Plants, especially Yacon, may act as abundant sources of lactic acid bacteria, with potential uses in probiotic and functional food innovation. The findings establish a robust basis for the subsequent characterization and safety assessment of LAB isolates acquired in this study.

3.2. Analysis of LABS Culture

A total of 83 bacterial isolates were successfully acquired from Yacon, Tebbeg, and Allagat through enrichment and plating on MRS agar. All isolates displayed consistent colony morphology, defined by a circular shape and creamy white pigmentation. The significant morphological consistency implies that the isolates probably belong to a related physiological group, strongly indicating the prevalence of lactic acid bacteria (LAB) among all plant sources.

The circular colony morphology is a characteristic trait of LAB, which generally yield smooth, round colonies owing to their non-motile properties and consistent cell division. The creamy white colony color noted in all isolates aligns with the anticipated pigmentation of lactic acid bacteria (LAB), specifically members of the *Lactobacillus, Enterococcus, Leuconostoc, Pediococcus,* and *Weissella* genera, which typically exhibit minimal pigment production and appear as white to off-white colonies on selective media. The lack of colored, irregular, or mucoid colonies further corroborates the effective selective isolation of LAB and reduces the probability of contamination by non-LAB organisms.

The dataset further illustrates effective isolation from various plant sources, as denoted by the naming codes (A1–A22 for Yacon, T1–T32 for Tebbeg, and AF/AS/AL/XY codes for Allagat). Notwithstanding the varied origins of the plants, the isolates exhibited remarkably uniform macroscopic morphology, underscoring the efficacy of MRS medium in selectively enriching LAB populations irrespective of sample type. This consistency underscores the natural prevalence of LAB in Indigenous Food Plants and suggests their potential as dependable microbial reservoirs for probiotic investigation.

The absence of morphological variation among isolates may also signify the efficacy of purification procedures, confirming that each isolate corresponds to a singular, distinct colony devoid of contamination. This consistency is crucial for subsequent characterization procedures (Gram staining, catalase reaction, hemolytic assay, and antibiotic susceptibility testing), as it verifies that the working isolates are pure cultures derived from LAB-like colonies.

3.3. Morphological Examination and Catalase Assay

The Gram staining results indicated that the majority of isolates were Gram-positive, aligning with the anticipated traits of LAB; however, several isolates displayed Gram-negative reactions, suggesting the presence of non-LAB organisms that were initially chosen based solely on colony morphology. Morphological evaluation revealed a distribution of cocci, bacilli, paired cocci, and yeast-like entities among the isolates. Rod-shaped and coccoid isolates, characteristic of genera such as *Lactobacillus, Pediococcus, Lactococcus,* and *Weissella*, were prevalent, while numerous yeast-like isolates were also identified, especially in the A-series and T-series isolates, indicating natural microbial diversity in indigenous food plants.

Catalase testing further distinguished the isolates: numerous exhibited catalase-positive reactions signifying the presence of organisms with aerobic metabolic pathways while several isolates were catalase-negative, consistent with the defining physiology of LAB. Only isolates that were Gram-positive, non-yeast in morphology (either rod or cocci), and catalase-negative were classified as presumptive LAB when the three screening parameters were evaluated collectively. The isolates comprised A3A, A3B, A7, A9, T6, T8, and various rod-forming strains including T10–T16, AF1, AS5, AS8, XY13, and AL17-B. The findings demonstrate that while visually analogous colonies were acquired during the preliminary isolation, only a fraction satisfied the microbiological standards for LAB, underscoring the necessity of multi-step verification. The findings affirm that indigenous food plants contain diverse microbial populations; however, only certain isolates display traits characteristic of LAB, indicating their potential for further evaluation as probiotics and functional foods.

Goa et al. (2022) effectively isolated LAB from fermented milk utilizing selective media, illustrating that MRS agar is among the most efficient media for recovering fastidious LAB species. Lee et al. (2022) utilized selective culturing methods to isolate lactic acid bacteria from kimchi, highlighting the reliable recovery of Gram-positive, catalase-negative rods and cocci characteristic of *Lactobacillus, Leuconostoc*, and *Pediococcus.* These studies underscore the efficacy of selective media in isolating viable lactic acid bacteria from intricate fermented products.

Arcales et al. (2018) examined lactic acid bacteria (LAB) from fermented milkfish-rice mixtures in

the Philippines, documenting a varied collection of LAB characterized by typical creamy-white, circular colonies, subsequently verified via Gram staining and catalase testing. Their multi-step screening methodology parallels that of Roselli et al. (2025), who similarly underscored the significance of integrating morphological, physiological, and biochemical assessments to precisely identify LAB candidates. These findings emphasize that the isolation of LAB from food necessitates several verification stages to differentiate them from morphologically analogous non-LAB microorganisms.

Xiao et al. (2024) broadened LAB isolation to plant-derived sources utilizing a novel selective medium, revealing that edible plants harbor extensive and diverse LAB communities. Balungaya et al. (2024) documented the successful isolation of lactic acid bacteria from homemade fermented foods in the Philippines, emphasizing microbial diversity and the presence of strains with beneficial probiotic characteristics. The literature consistently demonstrates that food and plant environments are abundant in LAB, and that conventional isolation methods—culture enrichment, selective plating, and physiological screening—effectively identify strains appropriate for functional food applications.

3.4. Hemolytic Assessment

Hemolytic activity was assessed for ten selected isolates—T15, T16, AF1, AS5, XY13, AF2-B, XY3-B, AL8-B, AS11-B, and AL17-B—utilizing 5% sheep blood agar to evaluate their safety as prospective probiotic organisms. All isolates demonstrated γ-hemolysis, indicated by the lack of clear or greenish zones surrounding the colonies. This signifies that none of the isolates were able to lyse red blood cells. Gamma hemolysis is the preferred hemolytic profile for lactic acid bacteria used in food or probiotic applications, as it indicates the absence of hemolysins—virulence factors commonly associated with pathogenic microorganisms. Conversely, the positive control, *Enterococcus faecalis*, exhibited β-hemolysis, revealing the anticipated clear zone of total red blood cell lysis and confirming the assay's efficacy and sensitivity.

The hemolytic analysis of the ten chosen isolates demonstrated consistent gamma (γ) hemolysis, signifying a total lack of hemolytic activity on 5% sheep blood agar. This observation is crucial for safety assessment, as hemolysis is regarded as a principal virulence factor in numerous pathogenic bacteria. The lack of α- or β-hemolysis in all examined isolates indicates that none of the strains have extracellular enzymes that can degrade red blood cell membranes. γ-hemolysis is acknowledged as a characteristic of safe, non-pathogenic lactic acid bacteria, and its occurrence in all isolates indicates that the Indigenous Food Plants examined—Yacon, Tebbeg, and Allagat—contain LAB populations that fulfill one of the essential criteria for probiotic eligibility.

The assay's reliability was validated by the positive control, *Enterococcus faecalis*, which exhibited β-hemolysis, resulting in a distinct, transparent zone of complete erythrocyte lysis surrounding the colony. This response affirms the experimental conditions and verifies that the blood agar medium was entirely proficient in detecting any hemolytic activity present. The pronounced disparity between the pathogenic control and the non-hemolytic isolates enhances confidence in the safety profile of the isolates obtained from Indigenous Food Plants. These findings conform to the established safety guidelines for probiotics set forth by international organizations, including the European Food Safety Authority (EFSA) and the Food and Agriculture Organization/World Health Organization (FAO/WHO), both of which underscore the necessity for candidate probiotic strains to be non-hemolytic. The consistent γ-hemolysis observed in all isolates suggests that none contain hemolysin genes or protein factors linked to cytotoxicity. This characteristic is especially significant for *Lactobacillus, Pediococcus, Lactococcus*, and *Weissella* species, which are frequently extracted from plant sources and extensively utilized in functional foods, fermented drinks, and dietary supplements. The results consequently offer robust evidence for the potential food-grade application of these isolates.

The persistent non-hemolytic characteristics observed in isolates from three distinct plant species indicate that Indigenous Food Plants in Ilocos Sur serve as inherently safe ecological environments for LAB, likely attributable to their historical significance in traditional diets and fermentation methods. This pattern may indicate evolutionary adaptation, in which LAB associated with consumable plants have preserved symbiotic or benign interactions with human consumers over generations. The lack of hemolytic activity suggests that these isolates are improbable contributors to gastrointestinal or systemic pathogenicity, a crucial safety criterion for microorganisms utilized in the production of probiotic beverages and functional foods.

Baek et al. (2024) demonstrated that lactic acid bacteria isolated from various food matrices consistently exhibited γ-hemolysis, indicating a total absence of red blood cell lysis and affirming their generally non-pathogenic characteristics. Their findings correspond with those of Goa et al. (2022), who indicated that plant-derived LAB exhibited solely gamma hemolysis on blood agar,

thereby reinforcing the established notion that LAB infrequently produce hemolysins. These studies collectively indicate that lactic acid bacteria derived from food and plant environments are generally biologically safe concerning hemolytic properties.

In a similar vein, the research conducted by Valdiviezo-Marcelo et al. (2023) demonstrated that indigenous LAB strains derived from plant-based fermentations exhibited neither β- nor α-hemolytic activity, indicating that hemolysin-related virulence factors are rare among environmental LAB. Bedada et al. (2025) further corroborated this by noting that none of the LAB isolates from fermented foods exhibited hemolytic activity, underscoring the safety of LAB populations in diverse traditional food systems. The uniformity of gamma hemolysis in these studies reinforces the assertion that LAB inherently lack hemolytic toxins.

Yasmin et al. (2020) incorporated hemolysis testing in their safety assessment and similarly identified non-hemolytic LAB strains, thus reinforcing the prevailing scientific consensus. All published studies consistently confirm that LAB are safe microorganisms appropriate for probiotic, food-grade, and fermentation applications through hemolysis assays. The literature collectively demonstrates a robust and consistent pattern indicating that LAB derived from foods and plants predominantly display non-hemolytic behavior, positioning them as promising candidates for further biotechnological applications.

Within the broader framework of the study, the hemolysis results augment prior findings from the cultural, morphological, and catalase assessments. These isolates exhibited typical characteristics of lactic acid bacteria (LAB) colonies and cells, as well as biochemical behavior indicative of safe, non-pathogenic lactic acid bacteria. The aggregated dataset suggests that isolates T15, T16, AF1, AS5, XY13, AF2-B, XY3-B, AL8-B, AS11-B, and AL17-B are robust preliminary candidates for probiotic development. Their non-hemolytic characteristics, along with Gram positivity and catalase negativity, render them suitable for additional molecular identification and functional assessment.

3.5. Antibiotic Susceptibility Assessment

The descriptive statistics for antibiotic susceptibility indicated clear variations in the response profiles of the ten selected isolates relative to the Lactobacillus plantarum ATCC reference strain. The mean inhibition zone diameters for Penicillin (P) varied from 15.83 mm (AS5) to 26.83 mm (XY13), while the ATCC strain exhibited a mean of 20.5 mm. The majority of isolates exhibited similar or marginally increased susceptibility compared to the ATCC strain, with the exception of AF1 and AS5, which displayed narrower zones of inhibition, indicating diminished sensitivity. The extensive confidence intervals noted in isolates like AF1 and XY3-B indicate biological variability among replicates.

The ATCC strain demonstrated a consistently high mean inhibition zone of 33.83 mm for Clindamycin (DA), indicating robust susceptibility. Multiple isolates—AF1 (32.17), AL17-B (33.5), and AL8 (28.67)—exhibited comparably extensive zones, signifying elevated sensitivity. Conversely, AS11-B exhibited the lowest mean zone (23.17 mm), indicating diminished susceptibility compared to the group. All isolates, however, generated inhibition zones that fell within the anticipated susceptibility range for Gram-positive lactic acid bacteria (LAB).

The Erythromycin (ERT) results exhibited significant variability among isolates. The ATCC strain exhibited a mean inhibition zone of 25.83 mm, whereas several isolates displayed significantly larger zones, including T15 (33.00 mm), T16 (33.00 mm), and XY3-B (37.17 mm), indicating exceptional susceptibility. In contrast, XY13 exhibited the lowest average inhibition zone (23.17 mm), albeit remaining within a susceptible range. The substantial standard deviations among isolates like AF1 and XY3-B signify heterogeneity in erythromycin response, potentially indicative of inherent variations in resistance mechanisms.

A more varied susceptibility profile developed for Trimethoprim–Sulfamethoxazole (SXT). The ATCC strain exhibited a mean of 31.5 mm, signifying pronounced susceptibility. The majority of isolates exhibited moderate to high susceptibility, with XY3-B (32.33 mm) and AL17-B (32.17 mm) comparable to the ATCC standard. Isolate XY13 demonstrated a markedly low mean inhibition zone of 9.17 mm, categorizing it within the resistant range. This significant divergence highlights the existence of inherent or developed resistance mechanisms in specific plant-derived LAB strains. The extensive range of means (9–33.5 mm) and substantial standard deviations underscore SXT as the antibiotic exhibiting the greatest variability in response among isolates.

All isolates of Ampicillin–Sulbactam (SAM), including the ATCC strain, exhibited a consistent mean inhibition zone of 4 mm with no variance, signifying total resistance or non-responsiveness. This uniformity indicates that the β-lactamase inhibitor combination exhibits minimal or no efficacy against these LAB strains, aligning with established intrinsic β-lactam resistance patterns in certain Lactobacillus species.

Ultimately, Amoxicillin (AMOX) exhibited a wide range of responses. The ATCC strain exhibited the greatest susceptibility (35.17 mm), closely succeeded by AL17-B (36.00 mm) and AY3-B (33.50 mm). Multiple isolates, including AF1, AS5, and AF2-B, demonstrated moderate susceptibility, whereas one isolate—AL8—exhibited a markedly low inhibition zone of 4.00 mm, signifying pronounced resistance. The overall mean for AMOX (27.33 mm) indicates a composite population of susceptible and resistant strains, corroborated by the substantial standard deviation (8.35 mm), the largest among all antibiotic categories.

The antibiotic susceptibility profile indicated that most isolates exhibited susceptibility patterns similar to or superior to the ATCC *L. plantarum* strain for nearly all antibiotics, with the exception of SAM, for which universal resistance was observed. The variability in responses to SXT and AMOX indicates the existence of strain-dependent intrinsic resistance characteristics. These findings are crucial for assessing the safety of prospective probiotic candidates, ensuring they do not possess transferable antibiotic resistance genes that could present risks in clinical or food contexts.

One-way ANOVA demonstrated highly significant disparities in antibiotic susceptibility among the eleven tested isolates, including the Lactobacillus plantarum ATCC reference strain, for Penicillin, Clindamycin, Erythromycin, Trimethoprim–Sulfamethoxazole, and Amoxicillin ($p < 0.001$), indicating substantial variation in inhibition zone diameters among the isolates. Only Ampicillin–Sulbactam demonstrated no variation, affirming consistent resistance among all strains. Post-hoc Tukey HSD comparisons revealed distinct clustering patterns for each antibiotic. For Penicillin, AS5 and AF1 consistently ranked among the least susceptible isolates, while XY13 exhibited significantly greater susceptibility, constituting the highest subset. Clindamycin exhibited a comparable pattern, with AS11-B demonstrating the lowest activity, while AF1, AL17-B, and the ATCC strain grouped within the highest susceptibility category. Erythromycin exhibited considerable variability, with XY13, AF1, and AF2-B constituting the least susceptible subset, whereas XY3-B, T15, and T16 revealed markedly larger inhibition zones. Trimethoprim–Sulfamethoxazole exhibited the broadest spectrum of responses, with XY13 demonstrating significant resistance (9.17 mm) and clustering independently, whereas T15, T16, XY3-B, AL17-B, and the ATCC strain constituted the most susceptible group. Amoxicillin demonstrated comparable variability, with AL8 displaying total resistance (4.00 mm), whereas XY3-B, AL17-B, and the ATCC strain consistently fell within the highest susceptibility category. The findings collectively suggest that while the isolates originate similarly and exhibit comparable phenotypic screening traits, they significantly differ in their intrinsic antibiotic resistance profiles. This isolate-dependent variation illustrates the inherent diversity in LAB antimicrobial resistance and underscores the necessity of strain-level characterization when assessing probiotic safety and selecting candidates for functional food applications.

Stefańska et al. (2021) documented significant variability in antibiotic susceptibility among LAB strains derived from animals, observing that LAB frequently display strain-specific resistance or sensitivity instead of consistent species-level patterns. This strain-dependent variability corresponds with findings by Sirichoat et al. (2020), who identified varied susceptibility profiles among vaginal LAB, especially regarding macrolides, β-lactams, and sulfonamides. Both studies emphasize the heterogeneity of antibiotic responses as a prevalent characteristic of LAB populations.

Liu et al. (2009) reported intrinsic resistance to specific β-lactam antibiotics in probiotic LAB, indicating that this resistance is generally chromosomally encoded and non-transferable. Nunziata et al. (2022) corroborated this observation, noting that LAB from both commercial and artisanal products typically exhibit consistent resistance to β-lactams while retaining susceptibility to macrolides and lincosamides. The observed patterns indicate that intrinsic resistance traits are firmly established in numerous LAB genera and typically do not represent a public health threat.

Duche et al. (2023) and Refaat et al. (2025) highlighted the significance of antibiotic susceptibility profiling in food-associated LAB for safety assessment, noting that LAB typically exhibit sensitivity to erythromycin, clindamycin, and amoxicillin, while selective resistance, particularly to trimethoprim-sulfamethoxazole, is commonly observed. These findings support previous literature indicating that LAB exhibit stable, distinctive resistance patterns, many of which are intrinsic and deemed acceptable for probiotic application. The studies collectively indicate that LAB populations from food and plants display varied yet predictable susceptibility patterns that require individual assessment for safe application.

3.6. Certain Isolates of Potential Probiotic Lactic Acid Bacteria (LAB)

The preliminary characterization of the chosen lactic acid bacteria (LAB) isolates (T15, T16, AF1, AS5, XY13, AF2-B, XY3-B, AL8-B, AS11-B, and

AL17-B) was based on their growth in agar and broth media and microscopic analysis. All isolates exhibited successful pure growth (Table 5) under laboratory conditions, signifying their viability and adaptability to standard culture media commonly employed for LAB cultivation.

The isolates typically developed visible colonies on agar plates after incubation, displaying characteristic LAB morphology, including small to medium-sized, circular, smooth, and convex colonies with creamy to whitish coloration. These characteristics align with previously documented colony morphologies of lactic acid bacteria, especially those within the genera *Enterococcus, Lactiplantibacillus,* and *Pediococcus*. Differences in colony size and opacity among isolates may indicate species-level distinctions or strain-specific growth traits.

In broth culture, all isolates exhibited pronounced turbidity, signifying active growth and metabolic activity. The consistent turbidity observed among isolates indicates effective nutrient utilization in liquid media, a trait typically linked to fermentative bacteria like LAB. Certain isolates may display minor discrepancies in turbidity intensity, attributable to fluctuations in growth rate and cell density.

Microscopic analysis demonstrated that the isolates displayed cellular morphologies characteristic of lactic acid bacteria. The majority of isolates exhibited cocci-shaped cells organized in pairs or short chains, characteristic of *Enterococcus* and *Pediococcus* species. Conversely, isolate XY13 displayed rod-shaped cells, typical of *Lactiplantibacillus plantarum*. These observations align with the molecular identification results derived from 16S rRNA gene sequencing and phylogenetic analysis.

3.7. Molecular Characterization

The genomic DNA extracted from the bacterial isolates exhibited satisfactory quality and concentration appropriate for subsequent molecular applications, as shown in Table 6. Fluorometric analysis of DNA quantification indicated concentrations between 18 and 81 ng/µL across the ten samples. Samples 4 and 8 demonstrated the highest DNA yields, measuring 81 ng/µL and 71 ng/µL, respectively, while Sample 9 exhibited the lowest concentration at 18 ng/µL. All samples surpassed the minimum DNA input thresholds for PCR amplification and sequencing, demonstrating effective genomic DNA extraction and maintenance of nucleic acid integrity. DNA concentrations within this range are deemed sufficient for dependable amplification of bacterial 16S rRNA genes and Sanger sequencing procedures.

PCR amplification with universal 16S rRNA primers yielded a single, distinct amplicon of approximately 1,500 bp, indicating specific amplification devoid of detectable contamination or nonspecific products. High-quality chromatograms produced via capillary sequencing facilitated precise consensus sequence assembly and subsequent molecular identification.

BLASTn analysis of the NCBI nucleotide database demonstrated high sequence similarity values between 96.11% and 100%, thereby affirming the successful taxonomic identification of the isolates. Multiple isolates exhibited robust species-level congruence surpassing the widely recognized 98.7–99% similarity threshold for bacterial species delineation via 16S rRNA gene sequences (Janda and Abbott, 2007). Isolates identified as ISPSC-XY13 (*Lactiplantibacillus plantarum*) and ISPSC-AL8B (*Pediococcus pentosaceus*) demonstrated nearly complete sequence identities (≥99.5–100%), signifying dependable molecular identification at the species level.

The 16S rRNA gene sequences have been submitted to the NCBI GenBank database with the following accession numbers: ISPSC-T16 (PZ232055), ISPSC-AF1 (PZ232042), ISPSC-AS11 (PZ231870), ISPSC-XY13 (PZ231317), ISPSC-AL8B (PZ231868), ISPSC-XY3B (PZ231865), ISPSC-AF2B (PZ231791), ISPSC-AS5 (PZ231582), and ISPSC-T15 (PZ231487), as detailed in Table 7. These publicly accessible sequence records guarantee reproducibility, traceability, and transparency in the molecular identification process.

The predominant isolates were associated with the genus *Enterococcus,* comprising *E. saigonensis, E. gallinarum*, *E. lactis, E. faecium,* and *E. italicus*, signifying that Enterococcus spp. prevail in the lactic acid bacterial community linked to the sampled indigenous plant substrates. This observation aligns with prior research indicating the ecological adaptability and versatility of Enterococcus species in plant-associated habitats and fermented environments, where they play a role in microbial succession and metabolite generation (Franz et al., 2011).

Phylogenetic reconstruction employing the Neighbor-Joining method under the Kimura two-parameter model corroborated the BLAST-based identification outcomes. The tree topology demonstrated distinct clustering of isolates into separate genera, including *Enterococcus, Pediococcus*, and *Lactiplantibacillus*, with clear delineation among principal clades. A substantial and cohesive cluster was established by *Enterococcus* isolates, within which multiple subclusters were identified corresponding to

species-level classifications. The isolates ISPSC-AS5, ISPSC-T15, ISPSC-XY3B, and ISPSC-T16 are closely clustered with *Enterococcus gallinarum* reference strains, indicating common evolutionary connections. Likewise, ISPSC-AF1 exhibited clustering with *Enterococcus lactis* and *Enterococcus faecium*, whereas ISPSC-AS11 demonstrated a relationship with *Enterococcus italicus*. The clustering patterns indicate the genetic diversity within the Enterococcus genus and align with the elevated sequence similarity values derived from BLAST analysis.

Conversely, isolate ISPSC-AL8B (Accession No. PZ231868) established a unique and robust lineage within the *Pediococcus pentosaceus* clade, closely clustering with several reference strains. The distinct phylogenetic separation of this clade from *Enterococcus* demonstrates robust taxonomic resolution and validates its identification. Members of P. pentosaceus are renowned for their involvement in food fermentation and their ability to synthesize bacteriocins, which enhance antimicrobial efficacy and food preservation (Holzapfel and Wood, 2014). Thus, ISPSC-AL8B constitutes a promising candidate for additional functional and genomic characterization.

Likewise, isolate ISPSC-XY13 (Accession No. PZ231317) is grouped within the *Lactiplantibacillus plantarum* lineage alongside closely related reference strains. The brief branch lengths and close clustering noted within this group signify significant sequence conservation among *L. plantarum* strains. This discovery aligns with earlier studies emphasizing the restricted discriminatory capacity of the 16S rRNA gene in differentiating closely related taxa within the *L. plantarum* complex (Kleerebezem et al., 2003; Martino et al., 2016). Consequently, although BLAST and phylogenetic analyses robustly endorse its taxonomic classification, more refined methodologies are required for strain-level distinction.

Notably, isolate ISPSC-AF2B established a distinct lineage linked to Streptococcus suis, signifying divergence from the predominant lactic acid bacterial groups identified in this research. This unique phylogenetic position indicates possible ecological or functional disparities relative to other isolates and necessitates further examination.

The incorporation of Vibrio parahaemolyticus and Saccharomyces cerevisiae as outgroup taxa facilitated the definitive rooting of the phylogenetic tree and underscored the evolutionary divergence between lactic acid bacteria and non-LAB organisms. The alignment between BLASTn results and phylogenetic clustering offers compelling evidence for the precise taxonomic identification of the isolates.

Due to its phylogenetic placement within the *Lactiplantibacillus plantarum* lineage, significant sequence similarity, and previously documented functional characteristics, ISPSC-XY13 was selected for whole-genome sequencing (WGS) to facilitate genome-based taxonomic validation through average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH). Whole-genome analysis will enable thorough safety assessments, encompassing the identification of antimicrobial resistance genes, virulence factors, plasmid components, and mobile genetic elements. Furthermore, it is advisable to conduct functional genomic analysis focused on probiotic-related characteristics, including stress tolerance, adhesion ability, bacteriocin production, and carbohydrate metabolism.

ISPSC-AL8B is a compelling candidate for additional genomic and functional analysis owing to its clearly defined phylogenetic position and recognized significance in fermented food systems. Complementary phenotypic assays, such as acid and bile tolerance, antimicrobial activity, adhesion capacity, and metabolic profiling, are essential to corroborate genomic predictions. The amalgamation of molecular, phylogenetic, and phenotypic data will establish a comprehensive framework for evaluating the probiotic potential and biotechnological applications of these isolates.

4. CONCLUSIONS AND RECOMMENDATIONS

The Indigenous Food Plants—Yacon (*Smallanthus sonchifolius*), Tebbeg (*Ficus nota*), and Allagat (*Uvaria rufa*)—exhibited diverse populations of lactic acid bacteria (LAB), with MRS broth consistently producing the highest colony-forming unit (CFU) counts in all replicates. A total of 83 isolates displaying creamy-white, circular colonies were obtained; however, only a subset exhibited classical LAB characteristics, including Gram-positive rods or cocci and catalase-negative reactions. All ten selected isolates demonstrated γ-hemolysis, signifying non-hemolytic activity, whereas the positive control (*Enterococcus faecalis*) exhibited β-hemolysis. The lack of hemolytic activity verifies the safety of the chosen isolates and endorses their appropriateness for prospective probiotic and food-related uses.

Antibiotic susceptibility analysis demonstrated considerable isolate-dependent variability, with certain strains displaying inhibition patterns akin to or surpassing those of the *Lactiplantibacillus plantarum* ATCC control. All isolates exhibited resistance to Ampicillin–Sulbactam, while differing degrees of susceptibility were noted for Penicillin,

Clindamycin, Erythromycin, Trimethoprim–Sulfamethoxazole, and Amoxicillin. Molecular identification via 16S rRNA gene sequencing revealed a high sequence similarity (96.11–100%) with reference strains primarily associated with the genera *Enterococcus, Lactiplantibacillus, Pediococcus*, and *Streptococcus*. Phylogenetic analysis employing the Maximum Likelihood method based on the Kimura two-parameter model further validated their taxonomic classification and evolutionary relationships, exhibiting robust bootstrap support across principal clades. ISPSC-XY13 and ISPSC-AL8B were accurately identified as *Lactiplantibacillus plantarum* and *Pediococcus pentosaceus*, respectively, underscoring their potential for additional genomic and functional analysis.

Additional research is advised to improve the characterization and application potential of these isolates. This encompasses sophisticated molecular identification via whole-genome sequencing to evaluate virulence factors, mobile antibiotic resistance genes, and probiotic-associated characteristics. Further assessment of probiotic characteristics, including acid and bile resistance, autoaggregation, coaggregation, antimicrobial efficacy, and exopolysaccharide synthesis, is essential. The isolates may be evaluated in food or beverage systems, including yacon-based drinks or fermented vegetables, to assess fermentation efficacy, sensory attributes, and consumer acceptance. Furthermore, in vivo safety evaluations and the screening for transferable antibiotic resistance genes are crucial to comply with international standards for probiotic application. Whole-genome sequencing of ISPSC-XY13 and ISPSC-AL8B is highly advisable for genome-based taxonomic verification, thorough safety assessment, and in-depth functional characterization, encompassing the examination of probiotic-related genes and metabolic functions.

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Table 1. Categorization of Hemolysin Testing

Hemolysis Type	Observation on Blood Agar	Interpretation / Meaning	Safety Implication
β-hemolysis (Beta)	Clear, transparent zone surrounding the colony	Complete lysis of red blood cells and hemoglobin	Potentially pathogenic; not safe for probiotic use
α-hemolysis (Alpha)	Greenish or brownish discoloration around the colony	Partial lysis of red blood cells (oxidation of hemoglobin to methemoglobin)	May indicate opportunistic traits; not ideal for probiotics
γ-hemolysis (Gamma) (also called Non-hemolytic)	No zone of clearing or discoloration; agar remains unchanged around the colony	No lysis of red blood cells	Considered safe; preferred phenotype for probiotic LAB

Table 2. Zone of Inhibition table.

Antibiotic	Resistant (mm)	Intermediate (mm)	Susceptible (mm)
Penicillin (P)	≤ 28	–	≥ 29
Amoxicillin (AMX)	≤ 13	14–17	≥ 18
Clindamycin (DA)	≤ 14	15–20	≥ 21
Erythromycin (ERT)	≤ 13	14–22	≥ 23
Trimethoprim–Sulfamethoxazole (SXT)	≤ 10	11–15	≥ 16
Ampicillin–Sulbactam (SAM)	≤ 11	12–14	≥ 15

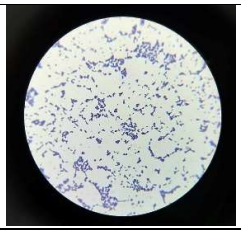
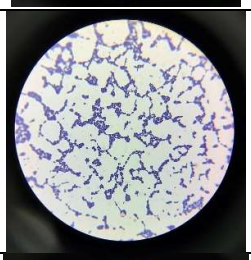
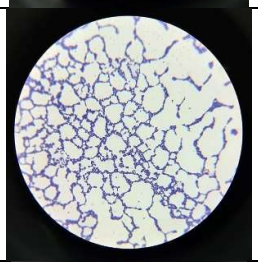
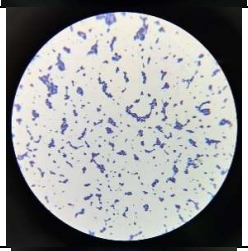
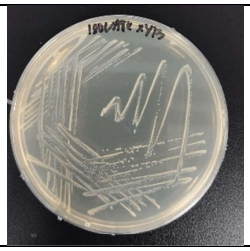
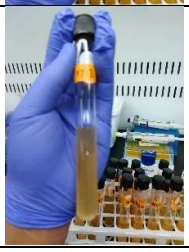
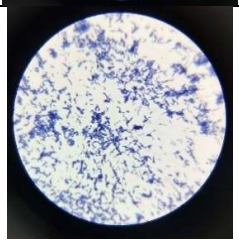
Table 3. Mean Colony Forming Counts (CFU) of Lactic Acid Bacteria Isolated from Indigenous Food Plants at 10^{-7} Dilution Across Different Enrichment Media

Plant	*MRS Mean (10^{-7})*	*Peptone Mean (10^{-7})*	*NaCl Mean (10^{-7})*
Yacon	181.7	98.3	96.0
Tebbeg	175.3	94.3	95.0
Allagat	167.3	95.7	96.0

Table 4. Hemolytic Activity of Selected LAB Isolates on 5% Sheep Blood Agar

No.	Isolate Code	Hemolysis Type	Interpretation
1	T15	γ (Gamma)	Non-hemolytic
2	T16	γ (Gamma)	Non-hemolytic
3	AF1	γ (Gamma)	Non-hemolytic
4	AS5	γ (Gamma)	Non-hemolytic
5	XY13	γ (Gamma)	Non-hemolytic
6	AF2-B	γ (Gamma)	Non-hemolytic
7	XY3-B	γ (Gamma)	Non-hemolytic
8	AL8-B	γ (Gamma)	Non-hemolytic
9	AS11-B	γ (Gamma)	Non-hemolytic
10	AL17-B	γ (Gamma)	Non-hemolytic
Positive Control	*Enterococcus faecalis*	β (Beta)	Complete hemolysis

Table 5. Growth of Putative LAB Isolates in MRS Agar and MRS Broth

Isolate Code	Growth in Agar	Growth in Broth	Under microscope
T15			
T16			
AF1			
AS5			
XY13			

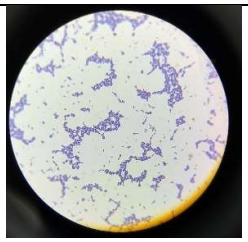
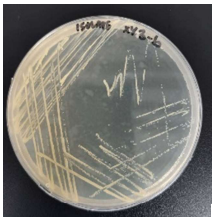
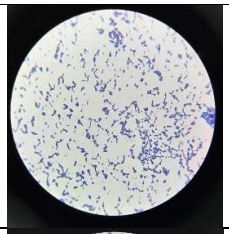
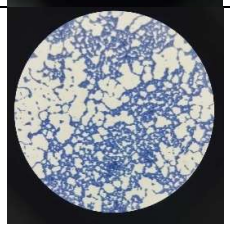
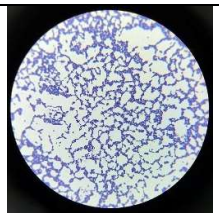
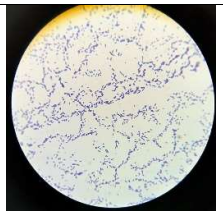
AF2-B			
xY3-B			
AL8-B			
AS11-B			
AF17-B			

Table 6. DNA Concentration of the Putative LABS

Sample	Concentration (ng/µL)
Sample 1	43 ng/µL
Sample 2	28 ng/µL
Sample 3	57 ng/µL
Sample 4	81 ng/µL
Sample 5	32 ng/µL
Sample 6	30 ng/µL
Sample 7	24 ng/µL
Sample 8	71 ng/µL
Sample 9	18 ng/µL
Sample 10	55 ng/µL

Table 7. Identification of the Putative LABS

No.	Isolate Code	Sample Source	Closest Identified Species	% Similarity	Accession Number
1	T15	Tebbeg fruit	*Enterococcus gallinarum*	~99%	PZ231487
2	T16	Tebbeg fruit	*Enterococcus gallinarum*	~99%	PZ232055
3	AF1	Allagat fruit	*Enterococcus lactis* /	~99%	PZ232042

			faecium		
4	AS5	Allagat stem	*Enterococcus saigonensis*	~99%	PZ231582
5	XY13	Yacon tubers	*Lactiplantibacillus plantarum*	100%	PZ231317
6	AF2-B	Allagat fruit	*Streptococcus suis*	~97–98%	PZ231791
7	XY3-B	Yacon tubers	*Enterococcus gallinarum*	~99%	PZ231865
8	AL8-B	Allagat leaf	*Pediococcus pentosaceus*	100%	PZ231868
9	AS11-B	Allagat stem	*Enterococcus italicus*	~99%	PZ231870
10	AL17-B	Allagat leaf	*Enterococcus lactis*	~99%	PZ235148

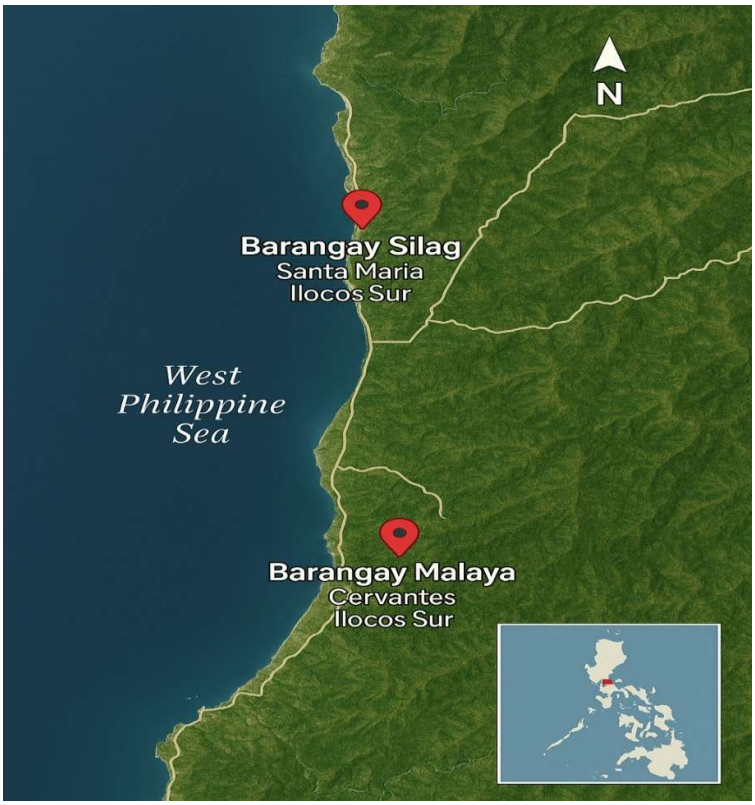


Fig 1. Collection Sites

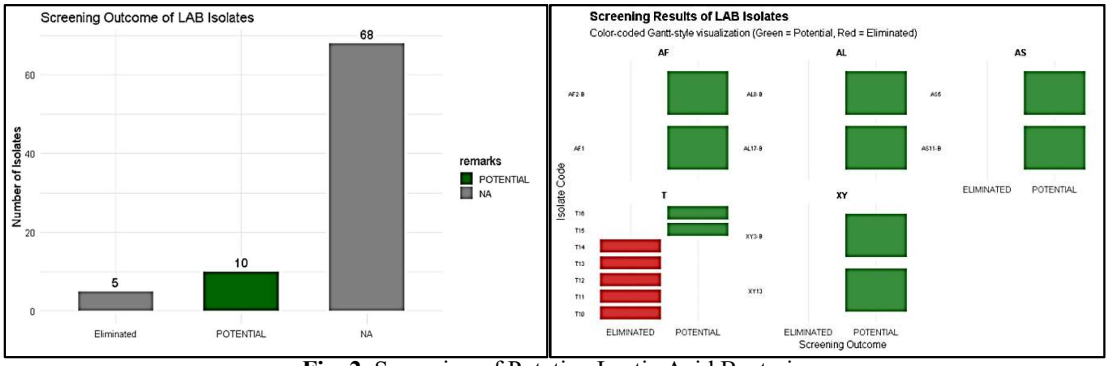


Fig. 2. Screening of Putative Lactic Acid Bacteria

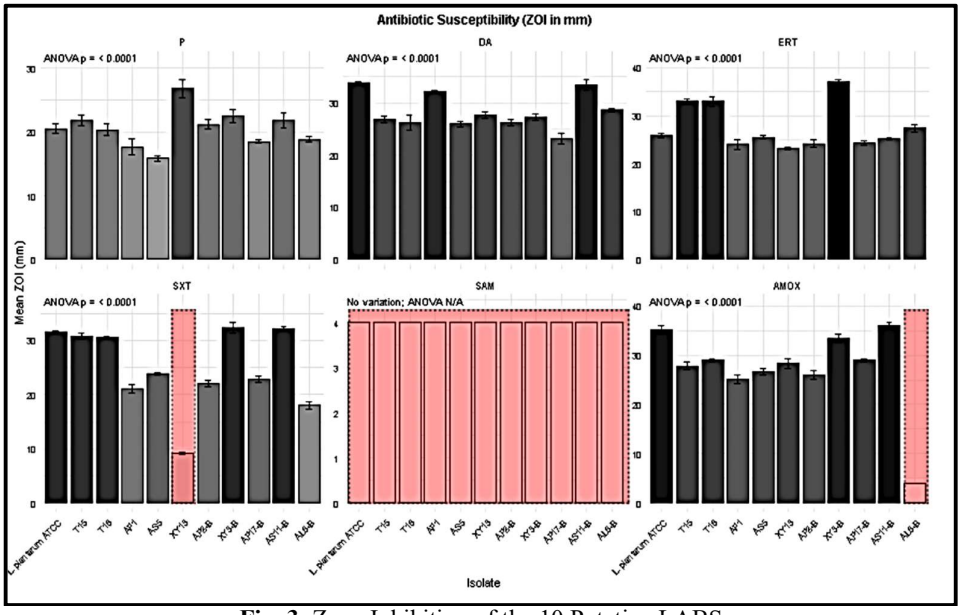


Fig. 3. Zone Inhibition of the 10 Putative LABS

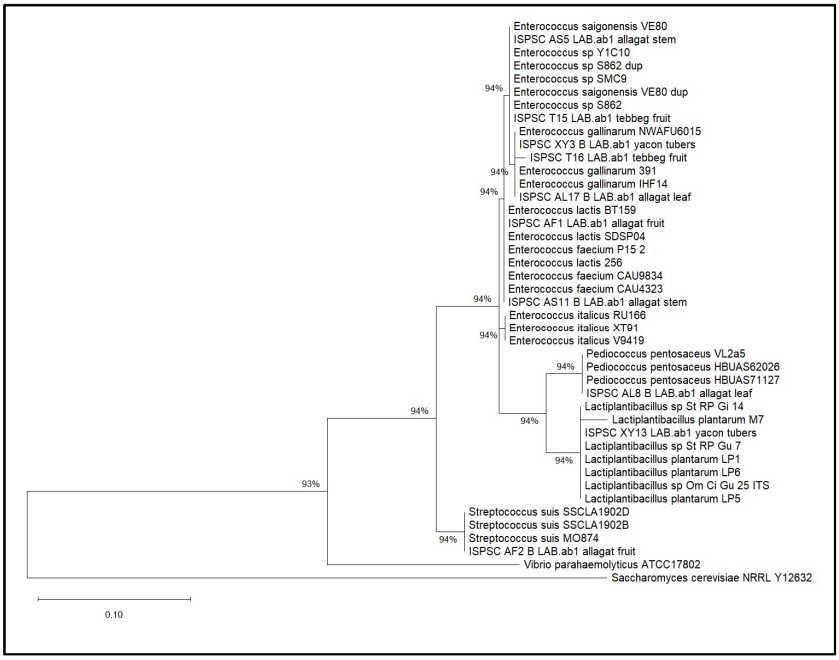


Fig. 4. Neighbor-joining Tree Analysis under the Kimura two-parameter model