

Characterization of Selected Lactic Acid Bacteria from Tapay: A Starter for Maguindanao Ethnic Delicacy in Cotabato City, Philippines

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Abstract

This study isolated and characterized lactic acid bacteria (LAB) from *Tapay*, a traditional ethnic starter culture sourced from Dalumangcob, Sultan Kudarat, Maguindanao (BARMM), Philippines. Phenotypic characterization revealed that the isolates were Gram-positive, non-spore-forming cocci capable of thriving under diverse environmental stresses, including broad pH (5.0–9.0) and salinity (0–8% NaCl) thresholds, as well as mesophilic temperatures 25–45°C. Biochemical profiling identified all isolates as homofermentative lactic acid producers. While extracellular α -amylase and nitrate reductase production were generally absent across most strains, isolate TLB2 uniquely exhibited both nitrate and nitrite reductase activities. Crucially, the cell-free supernatants of these isolates demonstrated robust antimicrobial efficacy, mediated by bioactive metabolites such as organic acids and bacteriocins, which effectively inhibited key foodborne pathogens: *Escherichia coli* Biotech 1634, *Bacillus cereus* Biotech 1509, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* Biotech 1335. Genotypic validation via 16S rRNA gene sequencing confirmed that isolates TLB2, TLB3, TLB4, and TLB6 shared an 85% sequence similarity with *Pediococcus pentosaceus*, consistent with the typical profile of an acid-tolerant, anaerobic, sugar-fermenting bacterium native to fermented matrices. Meanwhile, isolates TLB1 and TLB5 were classified at the *Pediococcus* genus level. These findings underscore the biopreservative potential of indigenous *Tapay* LAB as functional starter cultures for regional food optimization and safety.

Introduction

Traditional fermented foods have a long history of significant cultural and dietary importance in Asian societies. Knowledge of their preparation has been passed down through generations, often incorporating improvements while maintaining the original consistency, organoleptic, and nutritional value [27]. These foods have subsequently undergone scientific study and optimization in terms of their shelf life stability. One such popular fermented food in the Southern Philippines and other parts of Asia is *TAPAY* or *TAPE*. Variations in these products primarily arise from the specific indigenous microorganisms employed in their fermentation processes [44].

In the Philippines, “*Tapay*” signifies a traditional fermented rice snack originating from the Maranao and Maguindanaon communities. This delicacy embodies the community's culinary ingenuity and cultural heritage, serving as a flavorful representation of their preserved traditions. *Tapay* production involves the fermentation of ordinary rice, initially boiled in water or coconut milk, using a locally produced starter called “*Tapay*”. This fermentation process imparts a characteristic liquor-like aroma and taste to the final product. *Tapay* is often enjoyed chilled with coconut milk and a sweetener like condensed milk. Additionally, a condiment called “*tiyolo*”, prepared from pounded, roasted, and grated coconut meat and brown sugar, is commonly incorporated to further enhance the distinct aroma and flavor profile of *Tapay* [41].

Tapay is a traditional fermented food throughout Southeast Asia, especially in Austronesian cultures and other parts of East Asia. It refers to both the fermented paste and the alcoholic beverage that is derived from it. This product exhibits a sweet or sour taste (Fermented Cereals: A Global Perspective) and can be consumed directly. It is used as an ingredient in various dishes or further fermented to produce rice wine, which in some cultures is also called “*Tapay*”. Primarily made from white or glutinous rice, “*Tapay*” can also utilize diverse carbohydrate sources like cassava and potatoes [10]. The fermentation process relies on a consortium of molds, including *Aspergillus oryzae*, *Rhizopus oryzae*, *Amylomyces rouxii*, and *Mucor* species, alongside yeasts such as *Saccharomyces cerevisiae*, *Saccharomycopsis fibuliger*, *Endomycopsis burtonii*, and some bacteria [1]. Additionally, bacteria play a minor role due to their suppression by ingredients incorporated during the preparation of “*ragi Tapay*”. The addition of the ingredients, the garlic and chili possess antibacterial properties [38], potentially eliminating bacteria within the fermented product. Determining the identity of bacteria that are dominant in *Tapay* starter before the pre-fermentation of “*Tapay*” using cultural and molecular techniques provides information that can ultimately be used to manipulate pre-existing microbial communities or positively classify them.

Lactic Acid Bacteria (LAB) are versatile facultative anaerobes thriving in various natural environments like fruits, vegetables, and meat [38]. They hold significant value in food preservation due to their natural ability to inhibit the growth of spoilage and pathogenic microorganisms [8]. Classified as food-grade, LABs are safe for consumption as they lack toxin production and actively contribute to health by suppressing the growth of harmful microorganisms. Notably, LAB produces lactic acid, which effectively lowers the surrounding pH [8]. This acidic environment created by LAB hinders the proliferation of spoilage microorganisms and eliminates pathogenic bacteria, particularly within the human body.

Characterizing selected lactic acid bacteria (LAB) from *Tapay* starters is a key element in this traditional Maguindanao fermented rice product that contributes to preserving this cultural identity. Identification of selected LAB species can be achieved through phenotypic and genotypic analyses. While phenotypic methods are less accurate due to potential misidentification under controlled laboratory conditions, advancements in molecular biology offer a more precise and rapid approach through 16s rRNA profiling. This technique, specific to individual microorganisms, allows for accurate environmental identification [8].

Scientific investigation into the traditional fermentation practices of *Tapay* starters presents an opportunity to identify specific LAB strains crucial for shaping the final product. This pursuit bridges empirical knowledge with cultural heritage, fostering a deeper understanding of fermented foods while aiding in the sustainable preservation of Maguindanao culinary traditions. In this study, isolation and phenotypic characterization of lactic acid bacteria from *Tapay* starter samples collected from Dalumangcob, Sultan Kudarat, Maguindanao, Philippines were determined.

Materials and Methodology

Sampling and lactic acid bacteria isolation

A total of five samples from different stalls in the markets of Dalumangcob, Sultan Kudarat, Maguindanao, Philippines, were brought to the laboratory under aseptic conditions and kept at + 4°C until use. Enrichment, isolation, and purification methods were adapted and based on the study of [38] by Bergey's Manual of Determinative Bacteriology. The LAB isolation process by enriching the samples begins by taking 5 grams of *Tapay* starter and then mashing it using a sterile mortar. The mashed *Tapay* starter was put into 45 mL of MRS Broth. and then incubated at 37°C for 24 hours. After incubation, the samples were diluted using 9 mL Tryptone Soya Broth to obtain 10⁻¹. Serial dilution up to 10⁻⁷ was performed. Inocula were plated using the double-layer plate technique using MRSA with Bromocresol purple as a color indicator, incubated at 37°C for 48 hours, with 3 replications per dilution. The isolated colonies with changes of color indicating acidification were purified on their respective isolation media using the 4-quadrant streak method. Purification plates were incubated at 35°C for 24–48 hours. Pure isolates produced were stored on slanted MRSA media for further testing.

Phenotypic Characterization

To determine phenotypic characterization, test strains were grown in MRS broth medium at different temperatures, 25°C to 50°C (with 5°C intervals) for up to 72 h. The pH ranges were analyzed in MRS broth at pH 3.0–11.0 (2 pH unit intervals), and tolerance of NaCl was determined using MRS broth supplemented with 0–12% NaCl (at intervals of 1.0%) for 72 h. All experiments were tested in triplicate, and growth was measured at OD_{600 nm} [2]. Gram-staining of the test strains was performed in accordance with previously described methods [14]. Catalase activity was measured by the production of bubbles from a drop of 3% H₂O₂ (v/v). The nitrate reduction test was used to test the ability to reduce nitrate (NO₃⁻) to nitrite (NO₂⁻), incubating the isolated LAB in nitrate broth and incubating at 37°C for 24 hours, then it was supplemented with the addition of 0.1 mL of 0.8% sulfanilic acid, and then adding 0.1 mL of 0.5% naphthol. Furthermore, biochemical characterization of isolates was conducted using carbohydrate utilization profile [38]. A starch hydrolysis test was used to determine the potential degradation of isolated LAB. Inoculating the isolated LAB in starch agar and incubating at 37°C for 72 hours, and then the Gram-iodine solution was [44].

Evaluation of Antibacterial Activity

The antibacterial potential of the isolated lactic acid bacteria (LAB) was evaluated using the disc diffusion assay, following the protocols described by Mezaini et al [22]. The target pathogenic strains utilized in this study included *Escherichia coli* (Biotech 1634), *Bacillus cereus* (Biotech 1509), *Staphylococcus aureus* (ATCC 25923), and *Pseudomonas aeruginosa* (Biotech 1335).

To prepare the inoculum, pathogenic bacteria were suspended in sterile saline solution to achieve a concentration of 10⁶ CFU/mL. A 2 mL aliquot of this suspension was uniformly spread onto the surface of Mueller-Hinton agar (MHA) plates. The inoculated plates were maintained at room temperature (30°C) for approximately 3–5 minutes to ensure sufficient surface desiccation before treatment.

To obtain the cell-free supernatant (CFS), the LAB strains were cultured overnight in MRS broth and subsequently centrifuged at 15,000 x g for 10 minutes at 4°C. The resulting supernatant, which contains various bioactive metabolites responsible for antagonistic activity [20], was passed through a 0.2 µm cellulose acetate membrane filter to ensure the complete removal of residual viable cells.

Under aseptic conditions, sterile filter paper discs were immersed in the CFS and placed at the center of the pre-inoculated MHA plates. Positive controls consisted of standard antibiotic discs, specifically penicillin, streptomycin, and gentamicin. All assays were performed in triplicate. The plates were incubated at 37°C for 24 hours, after which the antagonistic effects were quantified by measuring the diameter of the zones of inhibition (mm) using a digital caliper.

Genotypic Characterization

Following the assessment of physiological and biochemical characteristics, as well as the determination of inhibitory properties against the selected pathogens, the candidate LAB isolates were subjected to genotypic identification. Molecular characterization was performed via 16s rRNA gene sequencing to confirm the identity of the isolates at the species level.

Chromosomal DNA extraction. A 10 mL liquid culture was centrifuged at 12,000 rpm for one (1) minute in a 1.5 mL microtube. The pellet was resuspended in 467 µL of 1× lysis buffer, 3 µL of proteinase K, and 5 µL of nuclease-free water, then incubated at 55°C for 1 hour. Enzyme inactivation was performed at 95°C for 10 minutes, followed by centrifugation at 12,000 rpm for 10 minutes [42].

Agarose gel making. A total of 0.5 grams of agarose powder was dissolved in 50 mL of 1x TAE buffer with the help of a hot plate. The agarose solution was left to warm, and then five (5) L of red gel was added and poured into the agarose gel printer. The agarose gel was left to solidify and then put into the electrophoresis pan. A 1x TAE buffer solution was poured into the pan until the gel was submerged [42].

16s rRNA fragment amplification. A 16s rRNA analysis was carried out by extracting chromosomal DNA from selected bacteria, which was amplified by the PCR technique. The sample solution from DNA extraction was put into a 0.2 mL PCR tube with 1.5 L, 7.5 L of PCR master mix reagent, BactF1 forward primer (5'-AGA GTT TGA TC (G/C) TGG CTC AG-3 ') 1 L, Uni B1 reverse primer (5'-GGT TAC (G/C) TTG TTA CGA CTT-3') 1 L and 4.8 L nuclease-free water (NFW). The mixture was placed into the PCR apparatus, beginning with a pre-separation step at 95°C for 5 minutes, followed by separation at 95°C for 30 seconds, attachment at 60°C for 30 seconds, polymerization at 72°C for 1 minute, and a final polymerization at 72°C for 4 minutes. The PCR process was carried out for 30 cycles [42].

Verification of DNA Extraction. Verification of DNA extraction results was carried out by agarose gel electrophoresis. A total of five (5) mL of the DNA-isolated solution was added to one (1) mL of loading dye and homogenized. The DNA marker was made from a mixture of four (4) mL NFW, one (1) mL DNA marker, one (1) kb size, and one (1) mL loading dye. The sample mixture and DNA markers were inserted into the wells on the agarose gel and then electrophoresed for 35 minutes with a voltage of 100 V/cm

[12]. To verify the results of PCR amplification, agarose gel electrophoresis was also carried out. A total of six (6) mL of the amplified solution was added. Figure 4 shows the outline of the 16S rRNA sequencing. DNA extraction was followed by 16S rRNA gene amplification, then the PCR product was cloned and sequenced. Finally, the sequences were blasted, and bioinformatics tools were used to illustrate the phylogenetic tree.

Statistical Analysis

All data of antimicrobial activity experiments were analyzed by one-way analysis of variance (one-way ANOVA) using the Statistical Package for the Social Sciences (SPSS), version 17.0 (IBM Co.). All results were presented as mean $\pm$ S.D. Differences among the treatments were performed using Tukey HSD. Probabilities ($p < 0.05$) were considered significant.

Results and Discussion

Isolation and Characterization of Morphology

The initial screening of the *Tapay* starter sample from Dalumangcob, Sultan Kudarat, was used. On MRS agar containing 0.004% bromocresol purple, the colony was selected based on its macroscopic features, such as shape, color, elevation, type of growth environment, and optimal colony density (30–300 colonies). According to colony morphology assessment, six (6) isolates producing acid bacteria were isolated. Morphologically, the colonies were milky white and grew (2) microaerophilic and (4) anaerobic. The yellow color around the colony indicates it is a suspected LAB (see Fig. 1).

The yellow edge or yellow halo around lactic acid bacteria (LAB) colonies isolates on MRS agar containing bromocresol purple (BCP) is due to the production of lactic acid by the bacteria, which lowers the pH of the medium around the colony. As LAB ferment sugars and produce lactic acid, the localized pH drops, causing the bromocresol purple in the medium near the colony to change from purple to yellow, resulting in the yellow edge or halo that is observed around the colony [13]. Microscopically, the bacteria were all cocci with purple reactions, namely, gram-positive and non-spore-forming groups (see Fig. 2).

Lactic acid bacteria (LAB) are classified as gram-positive due to their thick peptidoglycan cell wall, which retains the crystal violet stain during gram staining [11]. This structural feature also enhances their acid tolerance, allowing them to thrive in fermented environments by outcompeting other microbes [9]. Their non-spore-forming nature arises from the absence of sporulation genes. LAB rely on metabolic adaptations (e.g., homofermentative lactic acid production) rather than forming resistant spores to survive harsh conditions [11]. This trait is consistent across genera like *Lactobacillus* and *Streptococcus*, which prioritize rapid carbohydrate fermentation over sporulation [9]. Cocci LAB originating from the starter of fermented rice form small, circular, whitish or cream-colored colonies with smooth edges [26]

Biochemical Characterization of Isolated LAB

Biochemical characterization of lactic acid bacteria (LAB) involves a series of tests to identify and understand their metabolic and physiological properties, which are crucial for their application in food fermentation, probiotics, and biotechnology.

The catalase test is a key biochemical test used to differentiate lactic acid bacteria (LAB) from other bacteria based on the presence or absence of the enzyme catalase. Catalase test showed that all six isolates were not able to produce bubbles when mixed with 3% H_2O_2 (see Fig. 3). This showed that the catalase enzyme was absent. Lactic acid bacteria are typically catalase-negative, meaning they do not produce the enzyme catalase and thus do not produce bubbles when hydrogen peroxide is applied. This is because LAB are generally anaerobic or microaerophilic and do not require catalase to break down hydrogen peroxide [14]. The cocci LAB cell, when hydrogen peroxide is added, there are no bubbles (oxygen release) because these bacteria lack the catalase enzyme activity. This differentiates them from catalase-positive and gram-positive cocci like *Staphylococcus* species, which produce bubbles due to catalase activity [1].

Nitrate Reduction Test

The nitrate reduction test in lactic acid bacteria (LAB) assesses their ability to reduce nitrate (NO_3^-) to nitrite (NO_2^-) or further to other nitrogenous compounds, through nitrate reductase and nitrite reductase enzymes. This test is important because it reveals aspects of LAB metabolism related to anaerobic respiration and nitrogen cycling. Nitrate reduction test showed that only five isolates do not possess the classical nitrate (NO_3^-) to nitrite (NO_2^-) (see Fig. 4). This is because LAB are facultative anaerobes with fermentative metabolism, lacking the respiratory electron transport chain components, and use nitrate as a terminal electron acceptor [7]. The isolate 2 (TLB2) shows positive in nitrate reductase. Some LAB strains, such as cocci, have nitrate reductase and nitrite reductase enzymes that allow them to enzymatically reduce nitrate (NO_3^-) to nitrite (NO_2^-) and further convert nitrite to nitric oxide (NO), nitrous oxide (N_2O), or nitrogen gas (N_2). This enzymatic activity helps detoxify nitrite and contributes to nitrogen metabolism during fermentation [34]. Nitrate reduction can serve as an alternative electron acceptor mechanism under low-oxygen conditions, facilitating energy metabolism in some LAB strains [38]. This is especially relevant in fermentation environments where oxygen is limited.

Growth on different pH

The growth of the isolated LAB indicated their tolerance to acid pH varied according to the isolates screened. In general, all the isolates can grow at 5–9 pH, indicating that all isolated LAB can tolerate low and high pH. Lactic acid bacteria can grow in a slightly acidic to near-neutral range, approximately pH 5.5–6.5 [38], and tolerate and grow at pH 2.5–3.0, which is relevant for surviving the stomach acidity [16], and can tolerate and grow above pH 7.0 usually less favorable and less efficient in production in various bioactive compounds. Lactic acid bacteria are acidophilic, which means they tolerate low pH

through specialized mechanisms that maintain intracellular pH homeostasis and activate stress responses. LAB actively pumps excess protons (H^+) out of the cell using membrane bound H^+ -ATPase, preserving a near-neutral cytoplasmic pH (pHi) even in acidic environments [24]. This prevents acidification of critical enzymes and cellular components. In addition, exposure to mild acid triggers ATR (Acid Tolerance Response), a protective mechanism involving protein synthesis. ATR enhances survival under extreme acid stress and confers cross-protection against heat, salt, and oxidative damage [24]. LAB adjusts fermentation pathways to minimize acid accumulation. Some species reduce lactic acid production under prolonged acid stress, balancing cytoplasmic pH [21]. In alkaline conditions, LAB maintain an acidic cytoplasm relative to the external environment. This reversed ΔpH is stabilized by K^+/H^+ antiporters and Na^+/H^+ antiporters, which expel excess cations to counteract alkalinity [23]. In addition, ATPase enzymes work in reverse under high pH, importing H^+ ions to acidify the cytoplasm and sustain metabolic activity. Menconi et al [21] reported that Alkali-tolerant LAB, such as plant-associated *Lactobacillus casei*, retain glycolysis activity at pH 8.5–9.0, ensuring energy production despite external alkalinity. On the other hand, the non-tolerant species lack this adaptability [23].

Tolerance of NaCl

In different concentrations of NaCl of the isolated LAB, it was noted that all isolates can grow in the range of 0–8% NaCl, indicating their ability to tolerate significant osmotic stress. According to the results of Rao et al [38], lactic acid bacteria could grow and produce lactic acid efficiently at salt concentrations up to 8%, with reasonable biomass yields and acid production even at these elevated salt levels. This demonstrates that many LAB strains can maintain metabolic activity and growth in environments with high salinity. In addition, Menconi et al [21], reported that LAB cope with high NaCl concentrations by regulating osmotic pressure, maintaining cellular turgor, and adjusting metabolism to prevent damage from osmotic stress. This includes the synthesis of compatible solutes and activation of stress response pathways that protect enzymes and cellular structures.

Growth on Elevated Temperatures Test

On the growth of isolated LAB in different temperatures, all isolated LAB are able to grow in a temperature range of 25–45 °C, indicating that the isolated LAB are mesophilic, allowing efficient metabolism and replication. According to the study of Śliżewska & Chlebicz-Wójcik [44], LAB can grow slowly or stop below 20°C or above 45°C. For many LAB, some strains can survive and even grow at temperatures as low as 2–5°C or as high as 50–53°C, though with reduced efficiency. It supports that the 25–45°C range represents a practical and reliable window for the robust growth of isolated LAB.

Carbohydrates utilization

The isolated LAB was tested for its ability to ferment carbohydrates from various carbon sources. All isolated LAB can ferment the sugars and cannot produce gas (CO_2), indicating that the isolated LAB can ferment sugars and homofermentative LAB, as they possess metabolic pathways that convert carbohydrates, primarily glucose and other hexoses, into energy (ATP) and lactic acid under anaerobic conditions [8]. This fermentation process allows LAB to generate cellular energy without using oxygen. In

addition, Kandler [15] reported in their study of carbohydrate metabolism in lactic acid bacteria that the metabolic pathway called the Embden-Meyerhof-Parnas (glycolytic) pathway, which one mole of glucose is converted into two moles of pyruvate, which are then reduced to lactic acid to maintain redox balance, yielding two moles of ATP per mole of glucose. This process produces lactic acid as the almost exclusive end product, with no gas formation.

Starch Hydrolysis Test

Starch hydrolysis test of LAB isolates to determine their ability to produce amylase. All of the isolates did not have amylolytic activity, which breaks down the starch into glucose, maltose, or dextrans to leave a clear zone around enzyme-producing colonies (see Fig. 5). Unhydrolyzed starch in the medium binds iodine, creating a dark blue or black complex background, due to the interaction between iodine molecules and the helical structure of amylose (a linear starch component) [18]. Starch hydrolysis requires α -amylase and related enzymes to cleave α -(1→4) and α -(1→6) glycosidic bonds [43]. Some LAB do not produce an amylase enzyme capable of hydrolyzing starch. This is supported by enzyme activity studies of Kim et al [17], showing that some LAB lack α -amylase activity, which is necessary to break down starch into simpler sugars. Instead, they primarily ferment hexose sugars such as glucose via glycolysis (Embden-Meyerhof pathway) and can utilize maltose, but it does not secrete amylase to hydrolyze starch extracellularly [28].

Potential Antimicrobial Assay

E. Coli Biotech 1634. A One-way analysis of variance (ANOVA) was conducted to evaluate the effect of isolated LAB on the pathogen microorganisms. The ANOVA (Table 1) shows that statistically no significant difference with the isolated LAB in terms of antimicrobial activity (F-value of (5,12) = 1.34, p-value of .310). The TLB5 shows a wide range of inhibition zones with Ø 9.6 mm, and the TLB3 shows a smaller inhibition zone with Ø 7.3 mm (Fig. 6). LAB produce lactic acid and other organic acids (e.g., acetic, formic) as their metabolic byproducts. Lactic acid disrupts the outer membrane of gram-negative bacteria like *E. coli*, releasing lipopolysaccharides (LPS) and increasing permeability to harmful agents [38].

Table 1 Antimicrobial activity of isolated LAB against pathogenic bacteria

ISOLATED LAB	<i>E. Coli</i> Biotech 1634 ^{ns}	<i>Bacillus cereus</i> Biotech 1509 ^{ns}	<i>S. aureus</i> ATCC 25923 ^{ns}	<i>P. aeruginosa</i> Biotech 1335 ^{ns}
TLB1	8.33 ± 0.8819	7.00 ± 0.0000	8.33 ± 0.3333	12.00 ± 4.0415
TLB2	7.67 ± 0.8819	7.67 ± 0.6667	9.00 ± 0.0000	8.67 ± 0.6667
TLB3	7.33 ± 0.8819	7.00 ± 0.0000	8.00 ± 0.5664	11.00 ± 3.0551
TLB4	9.33 ± 0.6667	7.67 ± 0.3333	9.00 ± 0.0000	12.67 ± 4.7022
TLB5	9.67 ± 0.3333	8.00 ± 0.5774	7.67 ± 0.6667	10.67 ± 1.7638
TLB6	9.00 ± 1.0000	7.00 ± 0.0000	8.33 ± 0.3333	8.00 ± 0.5774

**-highly significant at 95% level of significance

*-significant at 95% level of significance

^{ns}-no significant

B. cereus Biotech 1509. The inhibition effect of isolated LAB in *B. cereus* Biotech 1509 shows that all of the isolates affect the pathogen; however statistically results show that all the isolated LAB have no significant difference at a $p < 0.05$. The TLB5 shows a wide range of inhibition zones with a Ø 8 mm, and the TLB1 shows a smaller inhibition zone with a Ø 7 mm. Lactic acid bacteria (LAB) inhibit *Bacillus cereus* primarily through the production of antimicrobial metabolites and environmental acidification. LAB synthesize lactic acid, acetic acid, and other organic acids that lower the pH of the environment. This acidic condition inhibits the growth of *B. cereus* vegetative cells and spores. Soria and [1] study shows that the antimicrobial effect is strong at low pH (around 3.5 to 5.0) but disappears when the pH is neutralized (around 6.5), indicating that acidification is a key factor in inhibition.

S. aureus ATCC 25923. The TLB4 shows a wide range of inhibition zones with Ø 9 mm, and the TLB5 shows a smaller inhibition zone with Ø 7.6 mm. The inhibition effect of isolated LAB in *S. aureus* ATCC 25923 shows that all the isolated LAB can inhibit the pathogen with a f-value (5,12) = 1.711, p-value of 0.207, indicating that all the isolated LAB have no significant difference in terms of the inhibition effect on the pathogen. Many LAB strains secrete bacteriocins-heat-stable, proteinaceous antimicrobial peptides-that directly kill or inhibit *S. aureus*. The bacteriocins from LAB strains in fermented foods have shown strong inhibitory activity against *S. aureus*, with some having molecular weights around 14.4 kDa. These bacteriocins can disrupt *S. aureus* cell membranes and interfere with its growth [8].

P. aeruginosa Biotech 1335. Statistical analysis revealed no significant difference in the inhibitory activity among the isolated Lactic Acid Bacteria (LAB) against *P. aeruginosa*, with an F-value of (5, 12) = 0.139 and a p-value of 0.846. Although the overall differences were not statistically significant at the $\alpha = 0.05$ level, the isolates exhibited a range of inhibitory activity. Notably, isolate TLB4 produced the largest zone of inhibition (Ø 12.6 mm), while TLB6 yielded a smaller zone (Ø 8 mm). LAB strains strongly inhibit the growth and biofilm formation of *P. aeruginosa*. Biofilms protect *P. aeruginosa* from antibiotics and immune responses, so disrupting biofilms reduces its virulence and persistence [36]. LAB reduces the production of pyocyanin, a key virulence factor of *P. aeruginosa* that contributes to tissue damage and infection severity [19].

Genotypic Characterization

A 16S rRNA sequence analysis is used as a powerful tool in determining the prokaryotic diversity in almost every environment [6]. To determine the identification of isolated LAB from *Tapay* starter, the molecular identification of lactic acid bacteria based on 16S rRNA was performed. The Isolated TLB1 and TLB5 are unresolved or uncharacterized species, but within the *Pediococcus* genus, as shown in Fig. 7. Isolates can be confidently placed within the *Pediococcus* genus based on conserved core features such as Gram-positive cocci morphology, lactic acid fermentation, and 16S rRNA gene sequences. However, species-level discrimination within *Pediococcus* requires finer resolution beyond these conserved traits, which is often achieved through genomic and proteomic analyses, including plasmid and phage content profiling [3]. A study of [4], analyzing 130 *Pediococcus* isolates, found that 64.6% were identified to species level by MALDI-TOF MS supplemented with a local database, 35.4% remained identified only to genus level, partly due to insufficient genomic characterization, including plasmid/phage profiling.

The isolated TLB2, TLB3, TLB4, and TLB6 were found to be 85% similar to standard type strains. The five isolates are similar to *Pediococcus pentosaceus*, as shown in the Fig. 16S rRNA gene-based phylogenetic tree. *Pediococcus pentosaceus* is a Gram-positive, non-motile, non-spore-forming bacterium that belongs to the lactic acid bacteria (LAB) group. It is characterized by its spherical shape, typically found in pairs or tetrads, and is a facultative anaerobe, meaning it can survive in both aerobic and anaerobic environments [28]. Additionally, it is used as a starter culture in the fermentation of various foods, contributing to flavor, texture, and safety.

P. pentosaceus can produce an antimicrobial agent known as bacteriocins [43] “against several species of *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Staphylococcus*, *Enterococcus*, *Bacillus*, and *Listeria*” [25]. Antimicrobial agents are produced by bacteria to limit the growth of competitors and/or other harmful bacteria. The bacteriocin isolated in *Pediococcus pentosaceus* was labeled pediocin P. [25]. The bacteriocin produced by *Pediococcus pentosaceus* can be used as a food preservative. The bacteriocins only inhibit Gram-positive microbes [25]. Gram-negative bacteria were not inhibited. Pediocin P. inhibited several species of food pathogens such as *Listeria monocytogenes*, which can cause Listeriosis (Osmanağaoğlu et al [25]. The end product of fermentation is lactic acid, which lowers the environmental pH, which can also contribute as a preservatives and inhibit pathogens.

Conclusion

The lactic acid bacteria (LAB) isolated from a *Tapay* starter sample collected in Dalumangcob, Sultan Kudarat, Maguindanao (BARMM), demonstrated characteristics consistent with homofermentative LAB, particularly *Pediococcus pentosaceus*. *Pediococcus pentosaceus* are Gram-positive, cocci, non-spore forming, and capable of tolerating a broad range of environmental conditions, including pH 5–9, 0–8% NaCl, and temperatures from 25–45°C, classifying them as mesophilic. While it is generally unable to reduce nitrate or produce α -amylase. Significantly, the *Pediococcus pentosaceus* produced bioactive compounds, including organic acids and bacteriocins, which effectively inhibited the growth of various pathogenic bacteria such as *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, and *Pseudomonas*

aeruginosa. These findings highlight the potential of these *Tapay*-derived LAB, specifically *Pediococcus pentosaceus*, as a source of beneficial microorganisms with antimicrobial properties for various applications.

Declarations

This manuscript represents original work and has not been published previously, nor is it currently under consideration for publication, in whole or in part, by any other peer-reviewed scientific journal or indexing platform.

The sourcing of traditional starter cultures was handled with absolute respect for local cultural communities, purposefully contributing to the preservation and sustainable optimization of the historical food systems and dietary heritage of the Maranao and Maguindanaon populations.

The authors declare no competing financial, personal, or institutional conflicts of interest that could influence or bias the objective presentation and interpretation of the research outputs.

Ethics Approval and Consent to Participate

“Not Applicable”

Consent for Publication

“Not Applicable”

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Authors' Contribution

Mark Winston C. Ladrillo: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing – original draft, Visualization, Project administration. **Lynette C. Cimafranca:** Supervision, Validation, Writing – review & editing. **Inish Chris P. Mesias:** Writing – review & editing. **Lijueraj J. Cuadra:** Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

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Figures

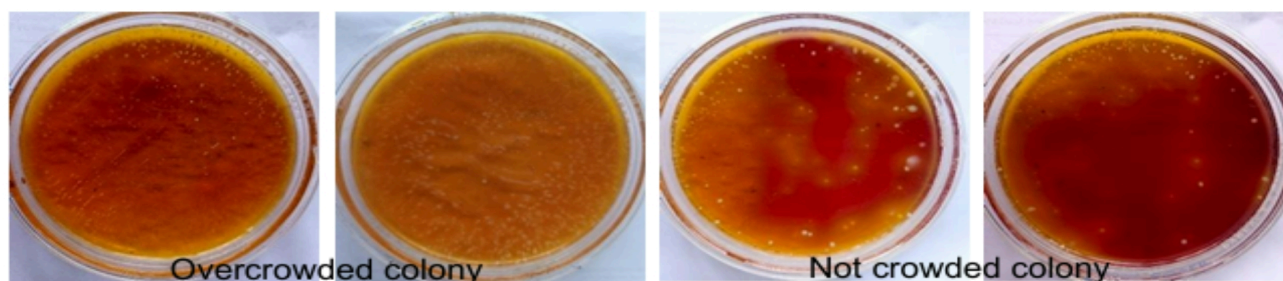


Figure 1

Mother culture of isolated LAB

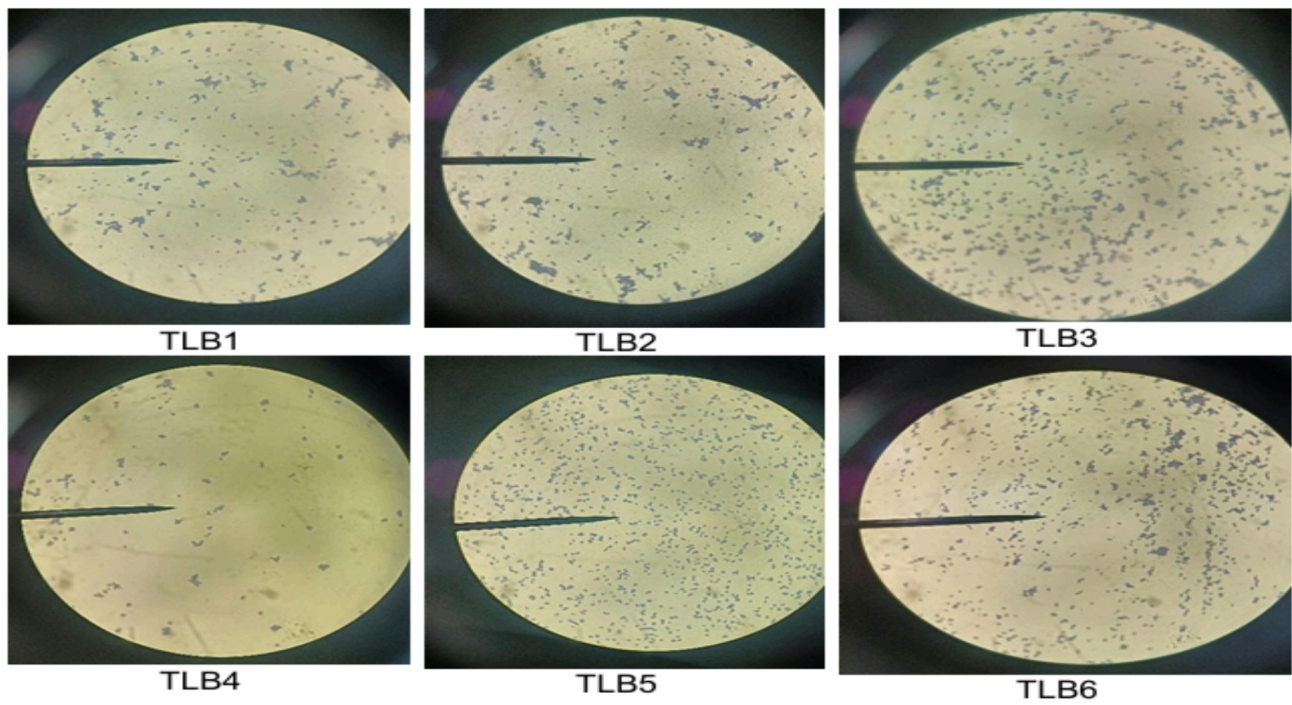


Figure 2

Gram-staining of isolated LAB

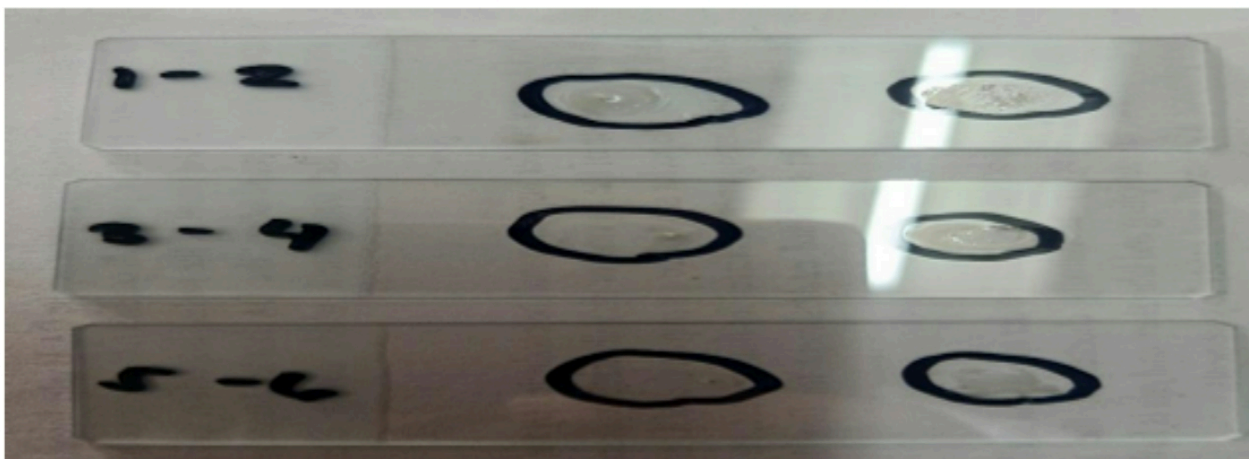


Figure 3

Catalase test result of isolated LAB

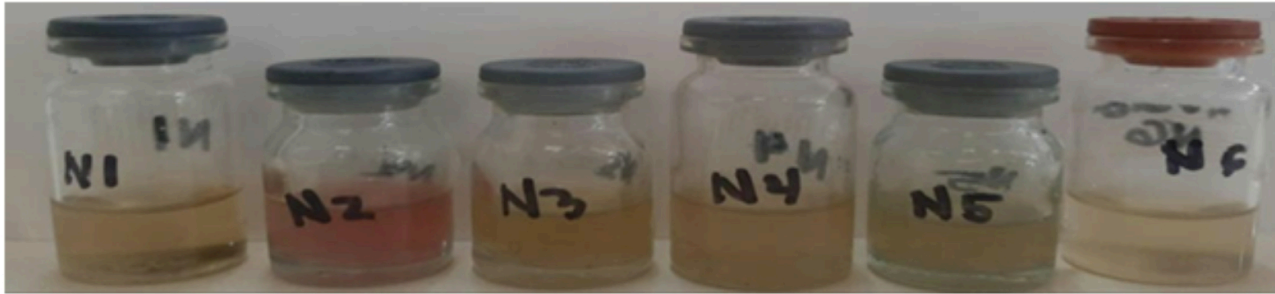


Figure 4

Nitrate reduction result of isolated LAB

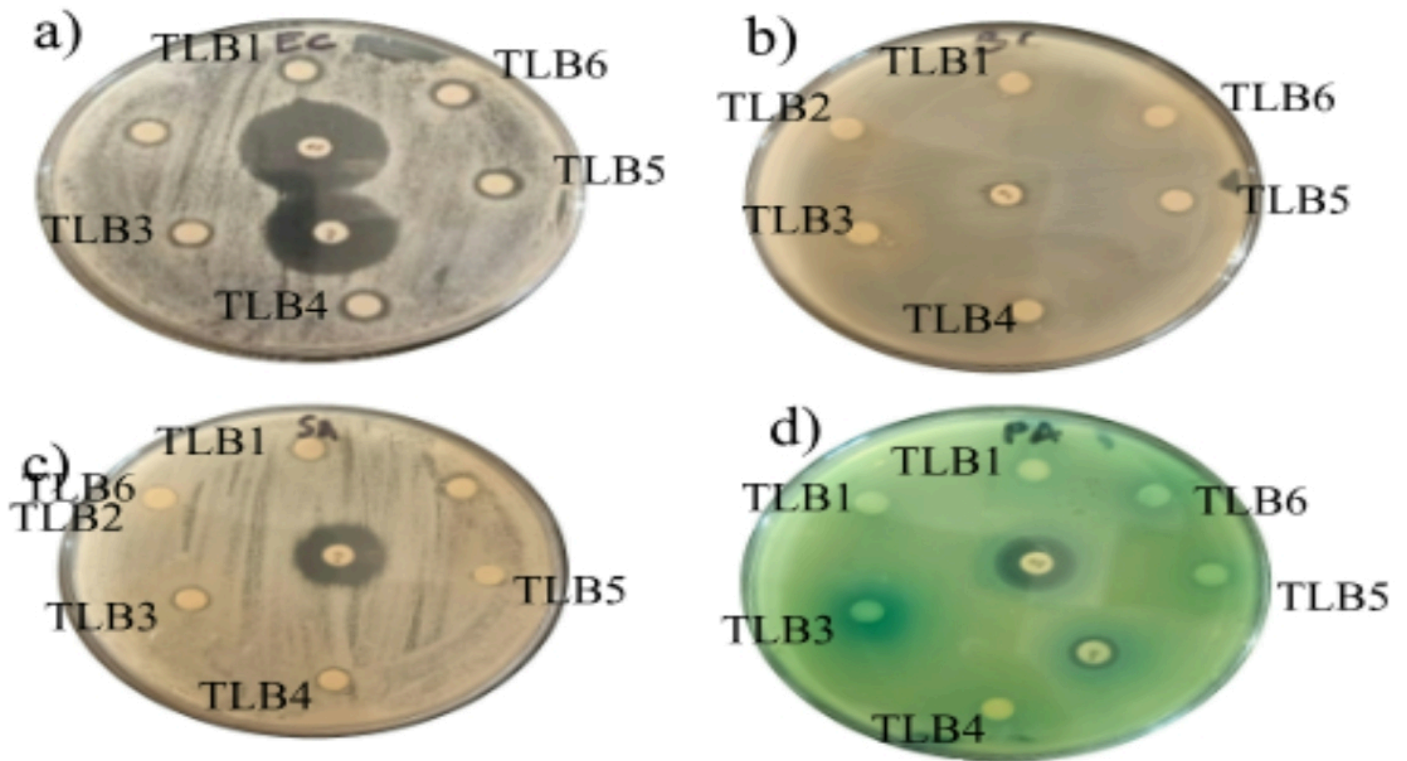


Figure 5

Potential antibacterial activity of isolated LAB a) Petri image of *E. coli* Biotech 1634 b) Petri image of *B. cereus* Biotech 1509 c) Petri image of *S. aureus* ATCC 25923 d) Petri image of *P. aeruginosa* Biotech 1335



Before adding KI+I₂ reagent



After adding KI+I₂ reagent

Figure 6

Starch hydrolysis results of isolated LAB

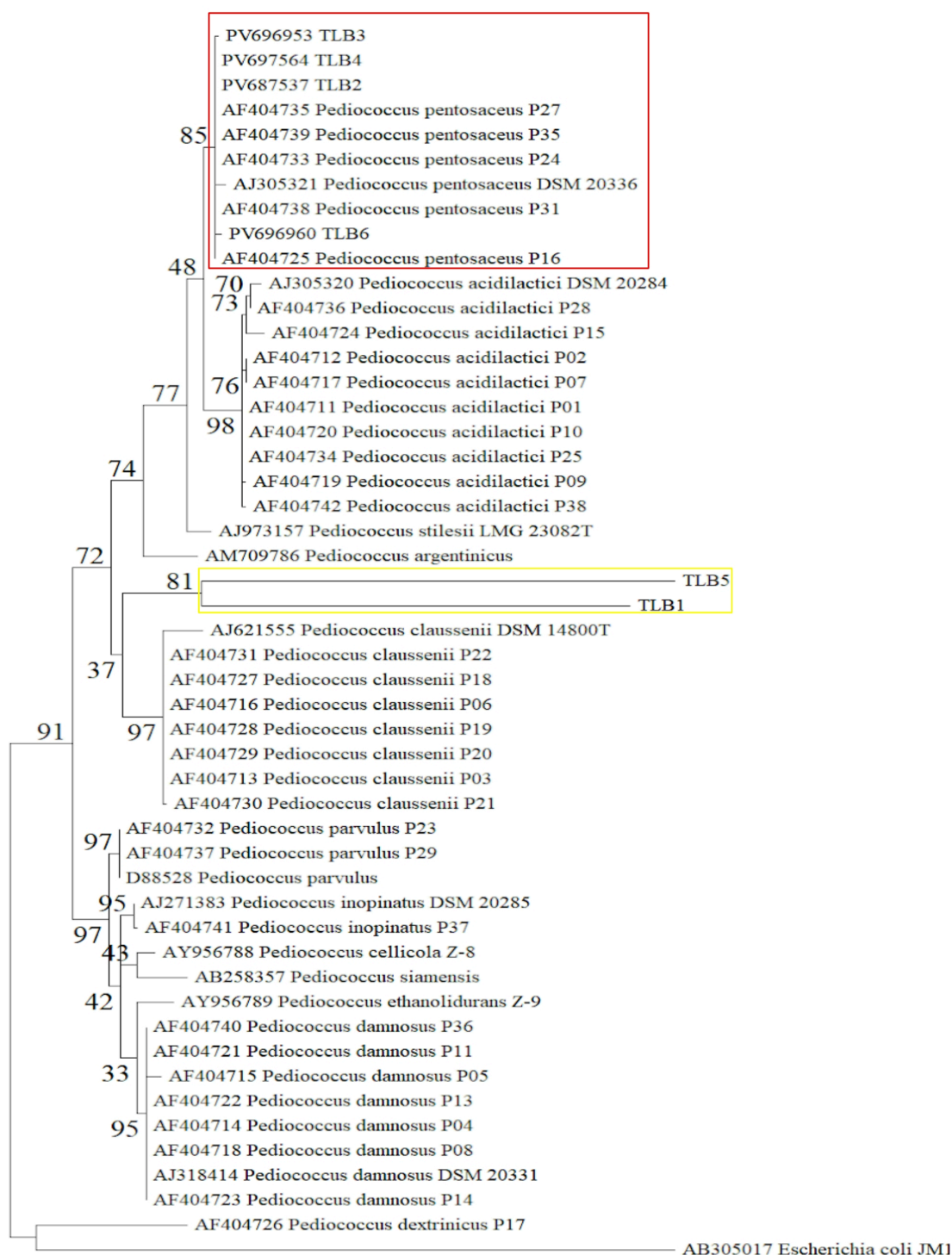


Figure 7

Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences of test strains and related type species. *Escherichia coli* JM109 was used as an out-group. Bootstrap values based on 1000 replications are listed as percentages at branching points. The accession numbers are given with the species. The scale bar represented 0.5% divergence

Supplementary Files

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