

Viability of *Lactobacillus spp.* from *Tuba* (Filipino Coconut Wine) in Rice Am Medium in Simulated Gastrointestinal Conditions

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Abstract

Background: Probiotic efficacy, although promising for overall health, has been reported to be reduced due to reduced viability after exposure to gastrointestinal conditions. Orally administered probiotics are significantly challenged by harsh conditions, including stomach acid, bile salts, and degrading enzymes, which pose a serious threat to their viability.

Objective: The present study aims to isolate *Lactobacillus* species from *tuba* (Filipino coconut wine) and inoculate the isolated bacteria into traditional rice am (*lanot*) Medium, commonly used in Filipino households, to assess their viability under gastrointestinal simulation. To test for its potential as a probiotic supplement, a simulated gastrointestinal condition was carried out to test for its survival during the gastrointestinal transit while maintaining a viable count sufficient to exert its probiotic effects

Methods: The researchers sourced *tuba* from Barangay Talakitok, Dawis, Digos City directly from a *tuba* seller, using the traditional fermentation process from freshly cut coconut sap to ensure authenticity and preserve the sample's natural microbial profile.

Results: After the identification process, the isolated strains were gram-positive and catalase-negative, characteristics typical of *Lactobacillus* species. In combination with the carbohydrate fermentation test results, the Advanced Bacterial Identification Software (ABIS) identified *Schleiferilactobacillus harbinensis* and *Levilactobacillus enshiensis* as the specific *Lactobacillus* species present in *tuba*. On the other hand, after the proliferation period, pre-simulation results showed that only two (2) of the samples reached the standard minimum 10⁶ CFU/mL, accounting for 6.46 log CFU/mL and 6.70 log CFU/mL. After the simulated gastrointestinal conditions, a marked reduction from the initial CFU/mL was

observed only in trials 2 and 3, with replicates 2 and 1 showing 3.79 log CFU/mL and 3.73 log CFU/mL post-simulation, respectively. Given that both the initial and post-simulation CFU/mL values failed to meet the established standard of at least 10^6 CFU/mL, these results highlight the incompatibility of *Lactobacillus* with the traditional *lanot* preparation as the proliferation medium.

Conclusion: Nonetheless, the successful isolation and identification of *Schleiferilactobacillus harbinensis* and *Levilactobacillus enshiensis* from *tuba* underscore their potential as novel indigenous probiotic candidates for future formulation and optimization studies.

Keywords: *Lactobacillus spp*, *tuba*, *rice am (lanot)*, *simulated gastrointestinal condition*, *probiotics*, *colony forming units (CFU/mL)*

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INTRODUCTION

Probiotic efficacy, although promising for overall health, has been reported to be lower due to reduced viability under gastrointestinal conditions. Orally administered probiotics are significantly challenged by harsh conditions, including stomach acid, bile salts, and degrading enzymes, which pose a serious threat to their viability (Yao et al., 2020). These factors directly affect the efficacy of probiotics, as fewer of them enter the small intestine during transit, indicating a lack of colonization capacity that might compromise their effectiveness.

Internationally, research on probiotic effects have repeatedly shown that probiotics face serious viability issues when exposed to gastrointestinal fluids, with numerous strains, including *Lactobacillus* and *Bifidobacterium*, showing drastic reductions in viability during gastrointestinal transit. da Silva et al. (2021) showed that out of 11 commercially available probiotic products, only 2 probiotics retained their indicated probiotic concentration, while only 6 exhibited a concentration in the ileum over 10^6 CFU/mL, which is thought to be the optimal number to support the host's probiotic advantages. The same study reported that only six of the eleven probiotics are capable of exerting an effect, while five are less likely due to insufficient viable bacteria to colonize the gut and exert their effects.

In Davao City, probiotics are perceived as valuable commodities for various commercial industries, where they are used as ingredients in food recipes and formulations. Currently, there is a lack of local studies and investigations on probiotic discovery and probiotic viability. However, while there are admittedly many studies discussing the potential of probiotics in areas such as their contributions to human gut health, cognitive function, and cultivation (Sanchez et al., 2016; Sharma & Bajwa, 2022), the field of probiotic research still has unexplored themes.

In the case of probiotic sources, although numerous studies on plant-based sources are present in recent literature, there have been few to no studies aimed at isolating *Lactobacillus* from fermented traditional Filipino recipes as a source of probiotics, such as *tuba*. According to Tantiado et al. (2016), *tuba* contains *Lactobacillus* strains, which are among the most commonly used probiotics. Moreover, there is a lack of investigation into the retention of standard CFU counts after consumption. Consequently, there has been little research on the viability of probiotics after exposure to gastrointestinal fluids. This parameter is important because it directly determines the effectiveness of the probiotic, as a reduced CFU count after passing through the body's fluids would indicate reduced colonization and lower efficacy, potentially suggesting an ineffective probiotic supplement.

The use of rice am, locally known as *lanot*, as a medium for the proliferation of *Lactobacillus* was considered based on the growing interest in non-dairy and plant-based matrices as alternatives for probiotic delivery. Dairy products such as milk, cheese, and yogurt remain common probiotic carriers; however, plant-based matrices have increasingly been explored as alternatives due to their accessibility and potential nutritional advantages (Ranadheera et al., 2017). In particular, cereal-and rice-based products have demonstrated potential in supporting the growth of probiotic microorganisms. Rasika et al. (2021) reported that cereal-based products can serve as sources and matrices for viable probiotic strains, while recent studies have demonstrated that fermented rice extract can provide a cost-efficient carbon source that supports the growth, viability, and metabolic activity of probiotic microorganisms (Sasikumar et al., 2025). Thus, the use of *lanot* provides an opportunity to investigate a locally available, economical, and culturally relevant rice-based medium for the proliferation and delivery of *Lactobacillus* isolated from *tuba*. This approach also addresses the limited investigation of traditional Filipino food preparations as potential media or carriers for indigenous probiotic microorganisms.

The inoculation of *tuba*-derived *Lactobacillus* into *lanot* was therefore necessary to determine whether the isolated bacteria could proliferate in a traditional rice-based matrix while maintaining a viable population prior to gastrointestinal exposure. Tantiado et al. (2016) demonstrated that *Lactobacillus* spp. isolated from *tuba* could proliferate in different beverage matrices, supporting the potential of *tuba*-derived strains to be incorporated into food-based carriers. However, the ability of a probiotic strain to grow in a carrier does not necessarily indicate that it can maintain sufficient viability during gastrointestinal transit.

Probiotic viability is influenced by the characteristics of both the bacterial strain and the surrounding food matrix, including factors such as pH and nutrient composition (Terpou et al., 2019). Therefore, assessing the CFU count of *tuba*-derived *Lactobacillus* after proliferation in *lanot* and subsequently after simulated gastrointestinal exposure provides a means of determining whether this traditional preparation can adequately support the bacterial population required for probiotic efficacy.

This is particularly relevant because the present study found that most samples did not reach the minimum CFU threshold even before gastrointestinal simulation, while the remaining viable counts declined substantially following simulation.

These ultimately indicate that, although *lanot* permitted measurable bacterial growth, its traditional preparation was insufficient to support the proliferation and subsequent gastrointestinal viability required of a probiotic carrier. As such, this study aims to assess the viability of *Lactobacillus* strains from locally sourced *tuba* (Filipino coconut wine) inoculated in rice am Medium.

MATERIALS AND METHODS

Sample Collection

Tuba (Filipino coconut wine) was obtained directly from a *tuba* seller in Barangay Talakitok, Dawis, Digos City. The *tuba* was produced through the traditional fermentation of freshly cut coconut sap to preserve its natural microbial profile. Jasmine rice used for the preparation of rice am (*lanot*) was purchased from the Digos City Public Market. Because no standardized preparation method for *lanot* was identified in the literature, the medium was prepared using a traditional method.

Isolation and Purification of *Lactobacillus* spp.

Isolation of *Lactobacillus* spp. from *tuba* was performed following the method of Tantiado et al. (2016). A 1-mL aliquot of *tuba* was transferred into 9 mL of sterile normal saline solution (NSS) to obtain a ten-fold dilution. Serial dilutions were subsequently prepared up to 10^{-10} using sterile NSS, with vortexing performed as necessary to ensure adequate mixing. From the prepared dilutions, 0.1-mL aliquots from the 10^{-2} , 10^{-4} , 10^{-6} , 10^{-8} , and 10^{-10} dilutions were spread onto Rogosa agar plates. The inoculated plates were placed in an anaerobic jar containing an atmosphere enriched with approximately 5–10% carbon dioxide and incubated at 37 °C for 72 h.

Following incubation, colonies were examined based on their observable morphological characteristics, including form, pigmentation or color, appearance, optical properties, and texture. Representative colonies exhibiting similar characteristics but differing in size were selected and subcultured for purification.

Phenotypic and Biochemical Identification

The purified isolates were characterized through Gram staining, catalase testing, and carbohydrate fermentation. For Gram staining, bacterial smears prepared from the isolates were subjected to the Gram staining procedure and examined microscopically for cellular morphology and Gram reaction. Catalase activity was assessed using the slide/drop method based on the protocol of the American Society for Microbiology. A portion of an isolated colony was transferred onto a sterile microscope slide and exposed to 3% hydrogen peroxide. Immediate bubble formation was interpreted as a positive catalase reaction, whereas the absence of bubbles was interpreted as negative.

Carbohydrate fermentation testing was conducted using phenol red carbohydrate broth containing starch, sorbitol, lactose, mannitol, sucrose, ribose, glucose, galactose,

maltose, fructose, and glycerol. Five milliliters of each medium were transferred into test tubes containing inverted Durham tubes for detection of gas production and subsequently sterilized. The media were aseptically inoculated and incubated at 35–37 °C for 18–24 h. Fermentation was determined based on the observed color change or transparency of the medium and the presence of gas within the Durham tube.

The morphological and biochemical characteristics obtained from the Gram staining, catalase, and carbohydrate fermentation tests were entered into the Advanced Bacterial Identification Software (ABIS) for species identification. The software compared the observed morphological, biochemical, and taxonomic characteristics with those contained in its reference database to generate the most probable bacterial identifications. Characteristics that were not assessed were entered as unknown.

Preparation of rice am (*lanot*)

Rice am (*lanot*) was prepared following the method of Rivera et al. (1983). Briefly, 300 g of Jasmine rice was washed twice using 500 mL of water. The washed rice was then boiled in 1,000 mL of distilled water for approximately 20 min. The resulting rice extract was separated from the cooked rice using a strainer and collected in a vessel. The collected rice am was sterilized at 121 °C for 15 min prior to use as the proliferation medium.

Preparation of *Lactobacillus* Inoculum and Proliferation in rice am

The isolated *Lactobacillus* cultures were first inoculated into 50 mL of nutrient broth and incubated at 37 °C for 72 h under anaerobic conditions following the method of Tantiado et al. (2016). Bacterial growth was assessed by comparison with a 0.5 McFarland standard before inoculation into rice am.

For proliferation, 50 mL of the prepared bacterial broth culture was mixed with 100 mL of sterilized rice am in a 250-mL media bottle. The inoculated medium was vortexed to facilitate homogeneous mixing and incubated at 37 °C for 72 h. Three experimental trials with two replicates each were prepared. Following incubation, samples were subjected to spread plating on MRS agar and incubated at 37 °C for 72 h. The resulting colonies were enumerated to determine the baseline viable bacterial count prior to gastrointestinal simulation.

Simulated Gastrointestinal Conditions

The gastrointestinal simulation was adapted from the in vitro gastrointestinal survival procedure of da Silva et al. (2021), which was based on the gastric simulation protocol described by Madureira et al. (2011), with modifications. The procedure was used to assess the ability of the isolated and proliferated *Lactobacillus* spp. to maintain viability following exposure to simulated gastrointestinal conditions. Unlike the reference procedure, in which bacterial counts were determined at individual stages of gastrointestinal transit, the present study determined the viable count after completion of the overall gastrointestinal simulation.

The simulation incorporated sequential changes in pH, exposure to digestive enzymes and bile salts, temperature, and agitation to approximate gastrointestinal conditions. Hydrochloric acid (HCl) and sodium bicarbonate (NaHCO₃) solutions were used for pH adjustment, while a shaker incubator maintained the temperature at 37 °C and provided controlled agitation. Because the initially prepared HCl concentration was insufficient to achieve the intended gastric pH, the HCl concentration was increased from 0.1 M to 0.2 M during the experiment.

During the initial phase, the sample pH was adjusted to 6.9 and maintained for 2 min at 200 rpm. For the gastric phase, pepsin prepared in 0.1 M HCl was added at 75 mg/mL, with the enzyme solution incorporated at 0.05 mL/mL. The pH was progressively adjusted to simulate gastric acidification and maintained at the specified stages before proceeding to the intestinal phases.

For the duodenal phase, pancreatin and bovine bile salts prepared in 0.1 M NaHCO₃ were added to the sample, and the pH was adjusted to 5.0. The sample was incubated at 37 °C with agitation at 45 rpm for 20 min. For the ileal phase, the pH was subsequently increased to 6.5 using 0.1 M NaHCO₃, followed by incubation at 37 °C and 90 rpm for 90 min.

Enumeration of Viable *Lactobacillus* Cells

Viable *Lactobacillus* counts were determined following the proliferation stage and after completion of the gastrointestinal simulation. Serial dilutions were prepared using the same dilution procedure employed during bacterial isolation. A 0.1-mL aliquot of each dilution was spread onto MRS agar and incubated at 37 °C for 72 h. The plate containing the first countable number of colonies, defined in the original procedure as 30–300 CFU, was selected for enumeration using a colony counter.

The viable bacterial population was expressed as colony-forming units per milliliter (CFU/mL) based on the dilution factor and plated volume. Samples that yielded colony numbers below the countable range were recorded as too few to count (TFTC). CFU/mL values obtained before and after gastrointestinal simulation were compared descriptively to assess changes in bacterial viability and were evaluated against the study's minimum probiotic viability criterion of 1×10^6 CFU/mL. The experimental dataset consisted of three trials with two replicates per trial.

Data Analysis

The viability of the isolated *Lactobacillus* spp. was assessed based on the colony-forming unit (CFU) counts obtained after the proliferation stage and following simulated gastrointestinal conditions. Colony counts were converted to CFU/mL using the dilution factor and plated sample volume, and the resulting values were expressed as both CFU/mL and log₁₀ CFU/mL to facilitate comparison across samples.

For each experimental trial, CFU/mL values from the two replicates were recorded separately. Descriptive statistics, including the mean and standard deviation, were calculated for groups with quantifiable CFU values. The proportion of samples meeting the minimum probiotic viability criterion of 1×10^6 CFU/mL was also determined before and

after gastrointestinal simulation. Samples reported as too few to count (TFTC) were retained as qualitative observations and were not assigned an arbitrary numerical value for statistical computation.

The change in bacterial viability following gastrointestinal simulation was evaluated by comparing the pre- and post-simulation log₁₀ CFU/mL values for samples with quantifiable counts at both time points. Log reduction was calculated as the difference between the pre-simulation and post-simulation log₁₀ CFU/mL values:

Log reduction = log₁₀(CFU/mL before simulation) – log₁₀(CFU/mL after simulation)

Because most post-simulation samples were reported as TFTC and only two replicates yielded quantifiable post-simulation counts, inferential statistical testing was not performed. Instead, the results were interpreted descriptively based on changes in viable counts and compliance with the predefined minimum probiotic threshold. This approach avoided assigning numerical values to censored TFTC observations and prevented inappropriate statistical inference from the limited number of quantifiable paired observations. All data were tabulated and analyzed using Google Sheets. Results were presented as CFU/mL, log CFU/mL, and descriptive summaries, as appropriate.

Ethical Considerations

This study was submitted to the Research Ethics Committee of the University of the Immaculate Conception under the protocol code UG-EX-01-25-0139. A certificate of exemption was issued. Furthermore, all methods used in the study followed standard operating procedures and policies in compliance with biosafety guidelines. Biological samples were handled using appropriate personal protective equipment, and procedures involving bacterial dilution, isolation, inoculation, and identification were conducted in a biosafety cabinet in the Pharmaceutical Microbiology Laboratory. Bacterial samples were appropriately stored and monitored until disposal, while inoculated laboratory apparatus were decontaminated following experimentation. The study was also documented as having a Certificate of Exemption from Research Ethics Committee review

RESULTS

As for the examination of physical attributes, the plated colonies isolated from *tuba* were small, circular, smooth, and white to off-white, with moist, slightly raised surfaces. Moreover, these colonies grew in an anaerobic jar containing 5%-10% carbon dioxide, suggesting that the isolated strains were anaerobic bacteria. Further morphological characteristics are reported in Table 1.

Table 1. Morphologic Examination Result

Test	Result/Observation
A. Physical Examination	Shape: Round
	Texture: Smooth

	Color: White to off-white in color
	Elevation: slightly raised surfaces
B. Gram Stain	Color: Purple (Gram-positive)
	Shape: Rod-shaped bacilli
C. Catalase Test	Catalase-negative (No formation of bubble)

Table 2 presents the carbohydrate fermentation results. Based on this table, the isolated colonies from *tuba* were fermenters of polysaccharides like starch and monosaccharides like fructose, galactose, and glucose. Furthermore, the colonies were able to ferment sugar alcohols like sorbitol and mannitol.

Table 2. Carbohydrate Fermentation Results

Carbohydrates	Trial 1	Trial 2	Trial 3
Starch	+	+	+
Sorbitol	+	+	+
Lactose	+	+	+
Mannitol	+	+	+
Sucrose	+	+	+
Ribose	-	-	-
Glucose	+	+	-
Galactose	+	+	+
Maltose	+	+	+
Fructose	+	+	+
Glycerol	+	+	+

Note: (+) indicates positive fermentation having observed acid/gas production; (-) indicates negative fermentation

Of the six samples representing three trials with two replicates each, only two samples reached the minimum target of 1,000,000 CFU/mL before gastrointestinal (GI) simulation. Trial 1 Replicate 2 (T1R2) yielded 2,880,000 CFU/mL, while Trial 2 Replicate 2 (T2R2) yielded more than 5,000,000 CFU/mL. Thus, these two samples were the only

samples that met the minimum pre-simulation viability criterion and could be directly compared with their corresponding post-GI simulation counts to evaluate changes in bacterial viability.

Although bacterial growth was observed after plating in the majority of the samples, their counts remained below the minimum standard of 1,000,000 CFU/mL. Differences were also observed in the physical characteristics of the inoculated media after three days of incubation. Specifically, the media from T1R2 and T3R1 developed a pink coloration, while the other samples produced a putrid odor resembling spoiled rice. These variations may be associated with differences in microbial activity or growth among the samples. The observed inconsistencies may also reflect the absence of a standardized formulation for *lanot*, which could result in variations in the composition and suitability of the medium for bacterial proliferation.

Table 3. CFU/mL counts of *Lactobacillus* in *tuba* inoculated in *lanot* before and after Gastrointestinal Simulation.

	CFU/mL		
	Before Simulation	After Simulation	Remarks
Replicate 1			
Trial 1	4.87 log CFU/mL (74,000)	TFTC	Marked reduction in bacterial growth
Trial 2	5.60 log CFU/mL (400,000)	TFTC	Marked reduction in bacterial growth
Trial 3	6.00 log CFU/mL (990,000)	3.73 log CFU/mL (5,400)	Marked reduction in bacterial growth
Replicate 2			
Trial 1	6.46 log CFU/mL (2,880,000)	TFTC	Marked reduction in bacterial growth
Trial 2	6.70 log CFU/mL (5,000,000)	3.79 log CFU/mL (6,100)	Marked reduction in bacterial growth
Trial 3	4.52 log CFU/mL (33,000)	TFTC	Marked reduction in bacterial growth

CFU/mL = Colony Forming Units per milliliter, TFTC = Too Few to Count

The ability of *Lactobacillus* isolated from *tuba* and cultured in rice am medium to meet the probiotic colony-forming unit (CFU) standard was assessed before and after gastrointestinal fluid simulation. Based on the data gathered, the initial CFU counts of most samples did not reach the accepted minimum of 1 million CFU/mL required for probiotics, with only two replicates (T1 R2 and T2 R2) exceeding this threshold. The remaining samples recorded counts as low as 33,000 CFU/mL (4.52 log CFU/mL). After gastrointestinal simulation, survival was markedly lower. Most replicates were classified as TFTC (Too Few To Count), while only two (T2 R2 and T3 R1) yielded measurable but markedly reduced CFU levels — 3.79 log CFU/mL (6,100 CFU) and 3.73 log CFU/mL (5,400 CFU), respectively. These values fall far below the 6 log CFU/mL (1 million CFU) benchmark considered necessary for a probiotic to reach the ileum and provide health benefits.

DISCUSSION

Morphologic Examination

The physical characteristics of the colonies were consistent with the typical *Lactobacillus* characteristics described by Goldstein et al. (2015) and Tantiado et al. (2016). Their studies described *Lactobacillus* spp. as having a colonial morphology of round, off-white to creamy color, shiny, and slightly raised when plated on an agar medium. Moreover, Goldstein (2015) further established that *Lactobacillus* is considered a facultative anaerobe. Hence, the results, compared with these standards, indicate that the isolated bacteria from *tuba* could be a *Lactobacillus* species.

On the other hand, microscopic examination and Gram staining were used to highlight their morphological characteristics. The examination revealed that the isolated colonies are rod-shaped and purple (Gram-positive) upon Gram staining and subsequent microscopic examination under oil immersion. As per Goldstein et al. (2015), the shapes of *Lactobacillus* species can vary from either short, plump rods or long, slender rods that are in chains or palisades. Tripathi and Sapra (2023) also noted that lactic acid bacteria are gram-positive bacteria. These established characteristics, compared with those of the isolates observed in this study, are indicative of *Lactobacillus* species.

Moreover, after the catalase test, the isolated bacteria from *tuba* were found to be incapable of producing bubbles when hydrogen peroxide was added. The conduct of 3 test trials resulted in no bubble formation, indicating that the tested bacterium is catalase negative. According to Sun et al. (2022) and textbooks, *Lactobacillus* species are catalase-negative bacteria, indicating that the bacteria isolated from *tuba* are potential *Lactobacillus* species. The carbohydrate fermentation test was conducted with 3 trials each containing 11 carbohydrates. All trials yielded consistent results: 10 fermented carbohydrates (positive) and 1 non-fermented carbohydrate (negative).

Characterization of fermentable carbohydrates, inclusion of catalase, and morphological examination results led to the identification of 2 distinct *Lactobacillus* species. These species were identified using the Advanced Bacterial Identification Software (ABIS), incorporating results from the Morphological examination and biochemical tests, with the identified species being *Schleiferilactobacillus harbinensis* and *Lactobacillus enshiensis*. Between the two species, *Schleiferilactobacillus harbinensis* was identified as the most probable bacterium isolated from the *tuba*, with a 93.4% probability, compared to *Levilactobacillus enshiensis*, which had a low probability of 6.1%.

Carbohydrate Fermentation Results

Schleiferilactobacillus harbinensis

Previously described as a novel lactic acid bacterium, *Lactobacillus harbinensis*, was originally isolated from Chinese fermented vegetables (“Suan cai”) and can be found in various fermented foods and vegetable materials (Zheng et al., 2021). Strains of its species are reported to survive high-pH environments, particularly at pH values close to 2.5, have good tolerance to bile salts, and they can inhibit the growth of pathogenic bacteria commonly present in the intestinal microbiota (Jung et al., 2021). Probiotic potential is correlated with its antimicrobial effect through adhesion to intestinal epithelial cells and its auto-aggregating capabilities, which prevent colonization of the mucosa by pathogens or other microorganisms (Colares et al., 2021).

Levilactobacillus enshiensis

A novel lactic acid bacterium, previously named *Lactobacillus enshiensis*, was originally isolated and identified from Zha-Chili in Enshi Autonomous Prefecture, which

served as the basis for its name. The bacterium demonstrates resistance to arsenic, and has the ability to produce important flavor compounds (Zhang, 2020). Its probiotic effects are demonstrated by its antimicrobial activity, which inhibits foodborne pathogens that can invade the intestinal mucosa. Additionally, the strain can promote antioxidant activity equal to that of ascorbic acid (Kwun et al., 2023).

CFU/mL counts of *Lactobacillus* in the tuba inoculated in lanot before and after Gastrointestinal Simulation

On the other hand, after gastrointestinal fluid simulation, a marked reduction of the initial CFU/mL counts was observed, with each trial falling way below the standard. These results inferred that the *Lactobacillus* strains used in this study did not meet the minimum requirement of 1 million CFU before and, more so, after gastrointestinal simulation. The researchers attribute this to a deviation from the standard protocol used in the related study by da Silva et al. In their study, peptone was used as the proliferation medium, whereas in the current experiment, *lanot* was used instead. In da Silva et al.'s experiment, *Lactobacillus* was able to survive the gastrointestinal simulation and maintain a viable count exceeding the minimum threshold required for probiotic efficacy.

The study by da Silva et al. reported survival rates exceeding 8 log CFU/g (equivalent to 100 million CFU) after simulation. Additionally, other samples maintained sufficient viability post-simulation, with CFU counts above 6 log CFU/g (1 million CFU). Literature indicates that for a probiotic to confer health benefits, it must reach the ileum portion of the small intestine at a minimum concentration of 6 log CFU/g. The simulation results indicate that the use of *lanot* as a proliferation medium may have affected the survival and growth of *Lactobacillus*, resulting in lower CFU counts prior to the simulation.

CFU compliance with the Standard of *Lactobacillus* after Simulation

This lack of survivability may be linked to modifications to the original protocol used by da Silva et al. (2021). The researchers used peptone as the proliferation medium and reported survival rates exceeding 8.23 log CFU/g (approximately 1.70×10^8 CFU) after the simulation. In da Silva et al. (2024) GI simulation, although the pH of the peptone water medium is unspecified, it must be noted that peptone water generally has a pH of 7.2 ± 0.2 . This value falls within the range of favorable growth for *Lactobacillus* species, which are typically cultivated in peptone water. However, some researchers noted that the optimal pH for *Lactobacillus plantarum* is between 5.0 and 6.5, where growth and lactic acid production are at their maximum. (Fu & Matthews, 1999; Vélez et al., 2007). On the other hand, in the present study, *lanot*, an unconventional rice-based medium, was used, which may have altered the physiological stress tolerance of *Lactobacillus* during simulated gastric exposure. The buffering capacity of *lanot* limited the initial pH reduction to the intended pH of 2.8 using 0.1 N HCL, requiring the use of 0.2 N HCL to achieve the intended value. The inability to acidify is likely to compromise the bacteria's stress adaptation and possibly contribute to decreased viability post-simulation.

Choosing the right nutrient source is crucial for scaling the production of probiotics such as *Lactobacillus* at a lower cost. As one recent study has shown, fermented rice extract is a cost-effective and efficient carbon source for growing probiotics because it enhances cell growth, metabolism, and survival (Sasikumar et al, 2025). Starch-rich rice is particularly the type of probiotic substrate needed because its starches are converted to sugars and organic acids during fermentation, which *Lactobacillus* can thrive on.

Another crucial factor in growing *Lactobacillus*, especially for probiotic use. Initial acidification of *Lactobacillus* is important in the probiotic industry, as it helps develop membrane strength under stress, which probiotics encounter in the gastrointestinal tract from acid and enzymes, as well as mechanical stress induced by peristalsis.

In this research, including *lanot* during the proliferation stage may not have been the proper method, since *lanot* is made from rice with high buffering capacity. Rice contains proteins, amino acids, and carbohydrates that slow down acidification by resisting pH change (García-Molina et al., 2021). The buffering capacity of *lanot* affects the supposed initial acidification in the proliferation stage. If the *lanot* buffers too much, it may have prevented the media from reaching the optimal pH level *Lactobacillus* needs for better growth and membrane health.

The issue of Initial Acidification also affects how probiotics survive GI tract simulation, as those cultivated under acidic conditions tend to be better equipped to withstand gastric acid. The conditions under which the lactobacilli proliferate greatly dictate how well they develop stress response mechanisms, and in this process, their physiological states are greatly influenced. Therefore, if the proliferation media (*lanot*) contains great buffering capacity, the proliferation stage becomes too neutral that the *Lactobacillus* may not achieve sufficient acid resistance, resulting in a decreased survival rate during GI simulation (Sasikumar et al., 2025).

In summary, *lanot* is an effective and affordable medium for cultivating *Lactobacillus*; however, careful management of acidification and buffering capacity during the proliferation phase is essential. Achieving the proper balance promotes healthy bacterial growth and enhances their survival prospects following gastrointestinal simulation. Consequently, fermented rice emerges as a promising candidate for aquaculture and other probiotic uses.

Overall, the results indicate that the *Lactobacillus* strains isolated from *tuba* and cultured in rice am (*lanot*) medium did not meet the minimum CFC count required for probiotic efficacy after gastrointestinal fluid simulation. The finding highlights the importance of the bacterial proliferation stage to ensure viability prior to gastrointestinal exposure. The use of rice am, which deviated from the original protocol of da Silva et al., suggests that preparing the bacteria for survival under the harsh conditions of the gastrointestinal tract could enhance their survival during simulation. However, it can be concluded that it is more beneficial to ensure that the CFU count before gastrointestinal transit reaches the recommended 8 log CFU, or at least the minimum standard of 6 log CFU, so that enough *Lactobacillus* CFU remain after GI passage to exert probiotic health effects.

CONCLUSION

The findings of this study suggest that *tuba* contains *Lactobacillus* species, as evidenced by phenotypic characterization. The distinct species identified exhibit morphologic and biochemical characteristics consistent with those described in the literature. Although measurable colonies were observed in *lanot* during the pre-simulation phase, the bacterial growth did not meet the minimum CFU/mL threshold required for a viable probiotic.

Following gastrointestinal simulation, a significant reduction in bacterial count was observed, with values falling well below the acceptable standard. These findings indicate that the traditional method of *lanot* preparation is inadequate for supporting

Lactobacillus proliferation and providing sufficient protection against gastrointestinal conditions. Failure to meet the minimum CFU requirement before gastrointestinal simulation led to failure to retain the minimum CFU after simulation. Additionally, the probiotics from *tuba* were not subjected to initial acidification, which could have improved their survival during the GI simulation. Overall, this study suggests that (a) *tuba* contains identifiable *Lactobacillus* strains with probiotic potential, and (b) the proliferation media used in this study did not appear to support the development of stress response mechanisms in the isolated probiotic strains, as related studies utilizing peptone or other stress-promoting media showed improved survivability outcomes.

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