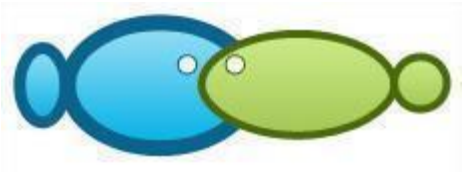


Probiotic potential of gut-derived lactic acid bacteria from *Eleotris melanosoma* Bleeker, 1853 in the Agusan River, Philippines

[1]Angelie C. Navarez, [1,2]Maria E. Q. Won

[1] Graduate School, Caraga State University, Ampayon, Butuan, Philippines; [2] Department of Biology, College of Mathematics and Natural Sciences, Caraga State University, Ampayon, Butuan, Philippines. Corresponding author: M. E. Q. Won, mqwon@carsu.edu.ph

Abstract. Lactic acid bacteria (LAB) are widely recognized as beneficial probiotics in aquaculture because they enhance host immunity, improve nutrient utilization, and help maintain gut microbial balance. Despite their importance, data on indigenous LAB associated with fish species from the Agusan River, Philippines, are scarce. The present study isolated, characterized, and evaluated the probiotic potential of LAB from the gut of the broadhead sleeper, *Eleotris melanosoma* Bleeker, 1853, locally known as "burod." Three representative isolates (ISO1, ISO5, and ISO8) underwent detailed characterization and were identified as *Lactiplantibacillus plantarum* through 16S rRNA gene sequencing. All isolates exhibited optimal growth at 25-37°C and tolerated moderately acidic conditions (pH 4.0-5.0), but did not grow at highly acidic pH (2.0-3.0). ISO5 and ISO8 demonstrated strong bile salt tolerance (0.5-1.5%), suggesting their potential to survive gastrointestinal conditions. Antimicrobial susceptibility testing showed high sensitivity to chloramphenicol and erythromycin, but resistance to vancomycin and amikacin. This study establishes baseline data supporting the potential of indigenous *L. plantarum* from *E. melanosoma* in the Agusan River as a probiotic candidate for freshwater aquaculture.
Keywords: broadhead sleeper, fish gut microbiome, freshwater aquaculture, *Lactiplantibacillus plantarum*.

Introduction. Probiotics, such as *Lactobacillus*, are beneficial microorganisms that promote gut health by maintaining microbial balance and inhibiting harmful pathogens, boosting their demand as a popular dietary supplement (Sadaqat 2024). They function as bioactive agents that improve digestion in animals and have been shown to enhance growth, immunity, and disease resistance in aquaculture species, including freshwater fish (Lingga et al 2023). Probiotics exert their beneficial effects through several mechanisms, as described by Latif et al (2023). These include competitive exclusion, in which they outcompete pathogens for nutrients and adhesion sites, and intestinal barrier enhancement, which strengthens epithelial integrity and mucus production. They also modulate immune response and may influence gut-brain communication through neurotransmitter regulation. However, it is important to note that each probiotic strain exhibits distinct characteristics, underscoring the need for strain-specific research on LAB, which holds significant value in the food industry (Terpou et al 2019). For their potential application as probiotics, LAB strains must undergo rigorous evaluation, including assessments of their survival in the host's gastrointestinal tract, tolerance to acidic pH and bile salts, antimicrobial activity, and overall safety (Sadiq 2022). These characteristics are essential in determining their functional efficacy and suitability for probiotic use.

Aquaculture, recognized as one of the fastest-growing food-producing sectors globally, faces numerous challenges, including disease management and the development of efficient feed pellets and feeding practices (Kong et al 2020). Thus, the use of probiotics is increasingly regarded as a promising alternative to antibiotics for minimizing the risk of infections caused by harmful bacteria in freshwater fishes (Rahayu et al 2024). Moreover, incorporating probiotics into aquafeeds can help alleviate digestive tract stress, promote

the development of healthier villi and microvilli, and reduce the risk of pathogen colonization (Lingga et al 2023). Various studies have demonstrated the successful isolation of LAB with potential probiotic characteristics from different fish species. For example, Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), has been recognized as a source of *Pediococcus acidilactic*i, *Pediococcus pentosaceus*, and *Lactiplantibacillus plantarum* strains that inhibit pathogenic microorganisms and withstand simulated gastrointestinal conditions (Coulibaly et al 2023). Likewise, LAB strains, similar to the genus *Lactobacillus,* isolated from *Anguilla bicolor,* have shown notable probiotic traits, including the ability to grow at acidic pH and in the presence of bile salts. These strains also exhibited antagonistic effects against harmful bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Peristiwati et al 2019). Furthermore, milkfish, *Chanos chanos* (Fabricius, 1775)*, has* been reported to host a variety of LAB species, some of which possess the potential to reduce mercury (Hg) concentration, indicating additional functional benefits (Dwyana et al 2018).

Altogether, these findings underscore the potential of fish species as promising sources of novel probiotic LAB with diverse beneficial properties. Although numerous LAB strains have already been discovered and documented, the ongoing discovery and detailed characterization of novel strains with targeted beneficial properties remain vital, especially in addressing emerging issues in aquaculture and food safety. Despite global research highlighting the probiotic potential of LAB isolated from various fish species, there remains a notable lack of data on the functional traits and applications of LAB in local Philippine aquaculture systems. In particular, limited studies have examined indigenous LAB strains from freshwater fish native to areas such as the Agusan River, underscoring the need for localized investigations to enhance aquaculture practices in the region. To address this gap, this study aimed to isolate and characterize LAB from broadhead sleeper, *Eleotris melanosoma* Bleeker, 1853, in the Agusan River and identify species using 16S rRNA gene sequencing, providing foundational data for probiotic applications in local aquaculture.

Material and Method

Sampling area. The sampling site was located along the Agusan River in Butuan City, at approximately 8.942536° N and 125.545610° E. The coordinates were determined using Google Maps (https://www.google.com/maps) and are presented in Figure 1. Sample collection was carried out on August 11, 2025.

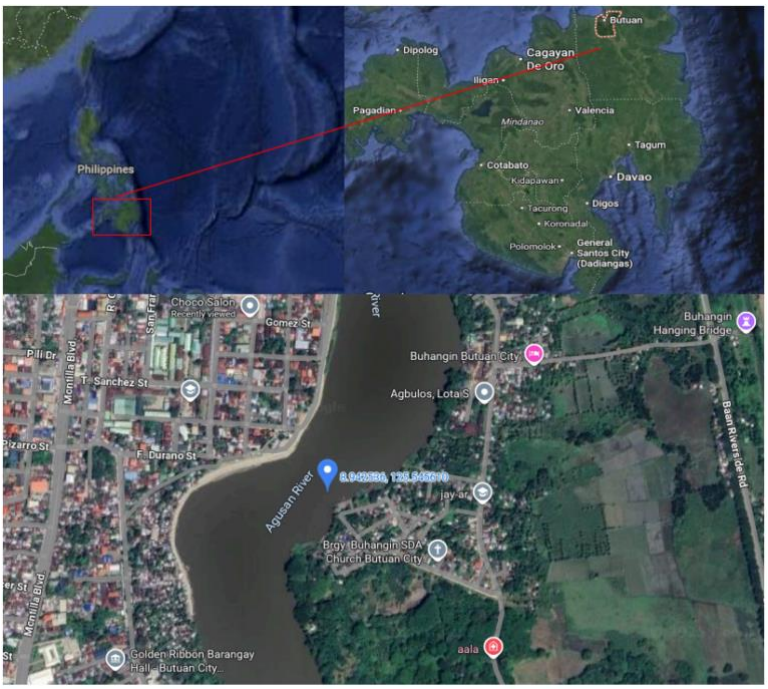


Figure 1. Map of the Philippines highlighting (A) Mindanao Island, (B) map showing Butuan City within Mindanao, and (C) detailed map of the sampling site along the Agusan River.

Sample collection. Fish samples (Figure 2) were collected from the Agusan River in Butuan City using a fishing net. A total of eleven (11) specimens of *E. melanosoma* were collected. The specimens had an average total length of approximately 13.3 cm and an average body weight of 31.73 g, and were of comparable size and developmental stage. Morphological identification of the collected specimens was performed and confirmed by the Bureau of Fisheries and Aquatic Resources (BFAR) Caraga Regional Office.

Immediately after capture, the fish were carefully handled to preserve freshness and the integrity of their gastrointestinal microbiota. Specimens were stored in an ice box and transported to the Department of Biology at Caraga State University for laboratory analysis. This standardized handling protocol minimized post-capture degradation and prevented the proliferation of opportunistic non-gut microorganisms.

Figure 2. *Eleotris melanosoma* from Agusan River, Butuan City.

Isolation of lactic acid bacteria (LAB). To isolate LAB from the gut contents of fish samples, the collected gut material was first pooled to form a composite sample. Five grams of this composite were inoculated into 50 mL of de Man, Rogosa, and Sharpe (MRS) broth supplemented with 25 g L^{-1} of NaCl and incubated at 37°C for 24 to 48 hours under anaerobic conditions. After incubation, serial dilutions were performed to 10^{-6} and 10^{-7}, streaked onto MRS agar plates, and incubated under anaerobic conditions at 37°C for 1 to 3 days to obtain well-isolated colonies. Distinct colonies exhibiting varied morphologies were carefully selected and isolated.

Morphological characterization and catalase test. The bacterial isolates were identified based on morphological features, including shape, edge, elevation, margin, color, and surface appearance. Gram staining was subsequently performed according to standard protocols to confirm their gram-positive nature. Isolates determined to be Gram-positive and exhibiting rod- or cocci-shaped morphology were classified as LAB.

The catalase activity was tested using 3% hydrogen peroxide (H_2O_2) solution. The appearance of bubbles indicated a positive result, while the absence of bubbles indicated a negative result. Isolates that tested negative for catalase were classified as LAB

Temperature tolerance test. To evaluate the ability of bacterial isolates to grow at varying temperatures, a standardized LAB culture was added to test tubes containing MRS broth. These tubes were then incubated at four temperatures: 15°C (low), 27°C (room temperature), 37°C (optimal incubation), and 40 °C (high). The incubation lasted 48 hours, after which bacterial growth was assessed by measuring optical density (OD) with a spectrophotometer.

Acid and bile resistance test. Acid and bile tolerance were evaluated according to the method described by Khushboo et al (2023). The acid tolerance of the isolates was assessed in MRS broth adjusted to pH 2-5 and bile tolerance in MRS broth containing 0.5-2% bile salts. Growth was quantified by OD measurements after 24-48 h incubation.

Carbohydrate fermentation test. To determine the carbohydrate fermentation profile of the LAB isolates, fermentation tests were conducted in phenol red broth. This pH indicator-based medium was individually supplemented with different carbohydrates at 5 mg mL^{-1}.

The sugars tested included glucose, lactose, and sucrose. Each carbohydrate was prepared in a separate test tube containing the phenol red broth. A small amount of actively growing LAB culture was aseptically inoculated into each carbohydrate-containing tube. Following inoculation, the test tubes were incubated at 37°C for up to 48 h. Observations were made at 24 and 48 hours. A color change from red to yellow indicated acid production due to fermentation of the corresponding sugar, while no color change signified no fermentation (Das et al 2019).

Antagonistic test. The experiment was carried out by adding 15 mL of Nutrient Agar (NA) to a Petri dish and allowing it to solidify. A sterile inoculating loop was used to uniformly streak the agar surface with *S. aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Klebsiella pneumoniae*. Wells were then aseptically created in the agar using a sterile cork borer. Each well was filled with a culture of LAB. The Petri dishes were incubated at 30 °C for 48 hours. After incubation, the presence of a clear zone around each well was measured to assess the antimicrobial activity of the LAB. The diameter of the inhibition zone was recorded as an indicator of the isolates' antibacterial activity.

Antibiotic susceptibility test. The antibiotic susceptibility of LAB isolates was assessed using the disc diffusion method, as described by Huligere et al (2022). The isolates were tested against several types of antibiotic discs, including chloramphenicol (30 µg $disc^{-1}$), vancomycin (30 µg $disc^{-1}$), erythromycin (15 µg $disc^{-1}$), and Amikacin (30 µg $disc^{-1}$). The results were interpreted as resistant (R), susceptible (S), or moderately susceptible (MS) based on the measured inhibition zone diameters.

Molecular identification of lactic acid bacteria (LAB) isolates. The molecular identification of the LAB isolates was performed by sending the bacterial cultures directly to Macrogen, Inc. for 16S rRNA gene sequencing. First, pure colonies of the LAB isolates were grown overnight on MRS agar to obtain sufficient bacterial biomass. The cultures were then preserved and stored appropriately until shipment. The isolates were packaged securely and sent with the necessary documentation required for the molecular identification process. A final report from Macrogen, Inc. was provided, detailing the precise identification of each LAB isolate and confirming their identity.

Multiple sequence alignment and phylogenetic analysis. The study employed a comprehensive approach to analyze the evolutionary relationships among a set of genetic sequences sent by Macrogen, Inc. Initially, the study used the Staden package to evaluate the quality of the raw sequence data and conducted essential trimming to eliminate low-quality bases, adapters, and potential contaminants using Pregap4 and Trev. Subsequently, Gap4 was used to assemble the trimmed sequences into contigs (overlapping segments).

The 16S rRNA gene sequences were identified via Basic Local Alignment Search Tool (BLAST) matches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) in the NCBI database. Reference sequences used for phylogenetic analysis were downloaded from the NCBI's GenBank sequence database. The 16S rRNA gene sequences were aligned using the ClustalW algorithm and manually adjusted to allow maximum sequence similarity. The alignment file was analyzed using the neighbor-joining criterion in Molecular Evolutionary Genetic Analysis (MEGA) software, version 10.2.26. The stability of the phylogenetic tree topology was assessed using the bootstrap method with 1,000 replicates. A distance matrix was generated using Kimura's two-parameter model.

Data analysis. All experimental measurements, including growth kinetics (OD_{600}) and antibiotic susceptibility (zone of inhibition), were performed in triplicate (n = 3) to ensure reproducibility and accuracy of the results. The collected data were expressed as the mean ± standard deviation (SD). Descriptive statistics were used to summarize the phenotypic characteristics and stress tolerance of the isolates.

Results

Isolation and characterization of lactic acid bacteria (LAB). A total of ten (10) presumptive LAB isolates were obtained from the gastrointestinal tract of *E. melanosoma*. The isolates exhibited heterogeneous colony morphologies, predominantly white, circular colonies with entire margins, although some yellow-pigmented colonies were also observed. Colony sizes ranged from small to moderately sized.

Gram staining revealed that eight (8) isolates were Gram-positive and rod-shaped, displaying both short and elongated morphologies. Based on phenotypic variation, three representative isolates (ISO1, ISO5, and ISO8) were selected for further characterization (Figure 3). All selected isolates were catalase-negative, consistent with typical LAB characteristics.

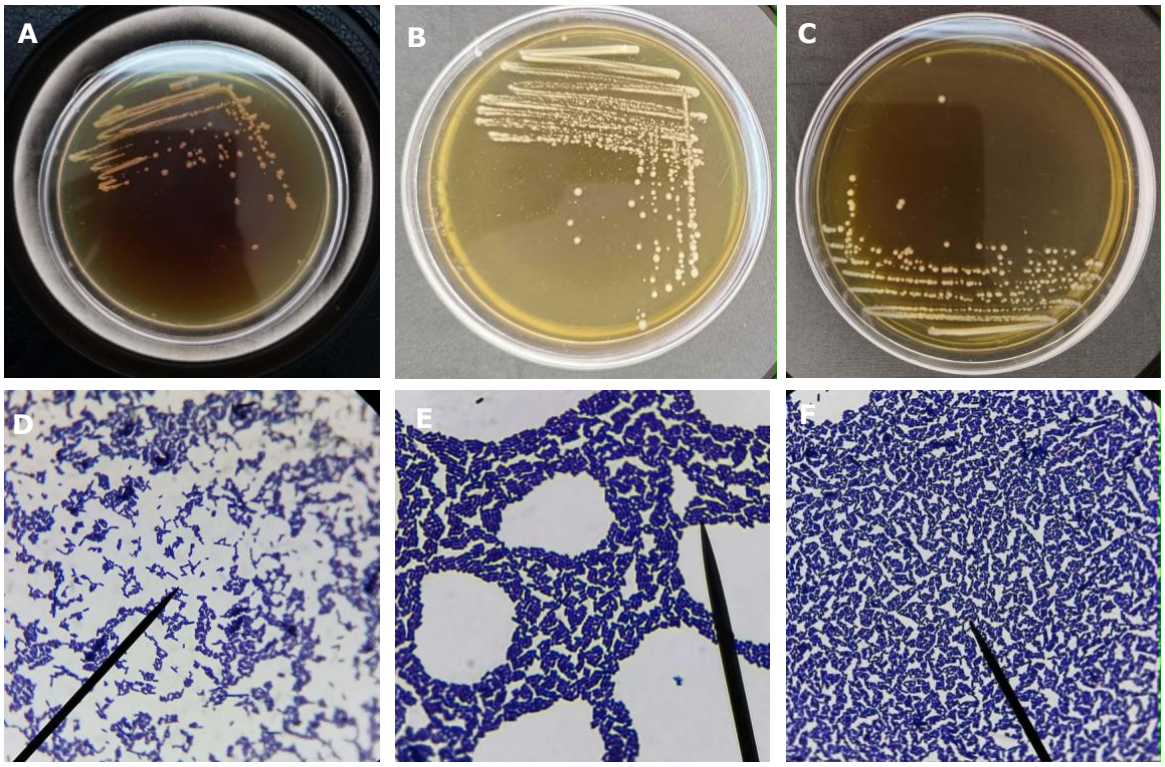


Figure 3. Colony (A. ISO1; B. ISO5; C. ISO8) and Gram stain (D. ISO1; E. ISO5; F. ISO8) morphology of LAB isolates from the gut of *Eleotris melanosoma*.

Temperature tolerance. All isolates demonstrated growth across the tested temperature range (5°C, 25°C, 37°C, and 40°C), except ISO5 at 15°C, which showed no detectable growth (Figure 4A). Optimal growth was observed between 25 and 37°C, with ISO5 reaching the spectrophotometric detection limit at both temperatures. In contrast, growth was reduced at 40°C for all isolates. Specific peaks were observed at 25°C for ISO1 and 37°C for ISO8.

Bile salt tolerance. Exposure to bile salts resulted in reduced growth across all isolates relative to the control. ISO5 and ISO8 demonstrated tolerance at concentrations ranging from 0.5 to 1.5%, as indicated by OD increases exceeding the threshold defined by Safi et al (2023). In contrast, ISO1 exhibited minimal growth and failed to meet the tolerance criterion at all tested concentrations. At a 2% bile salt concentration, all isolates showed markedly reduced growth, indicating a loss of tolerance to high bile stress.

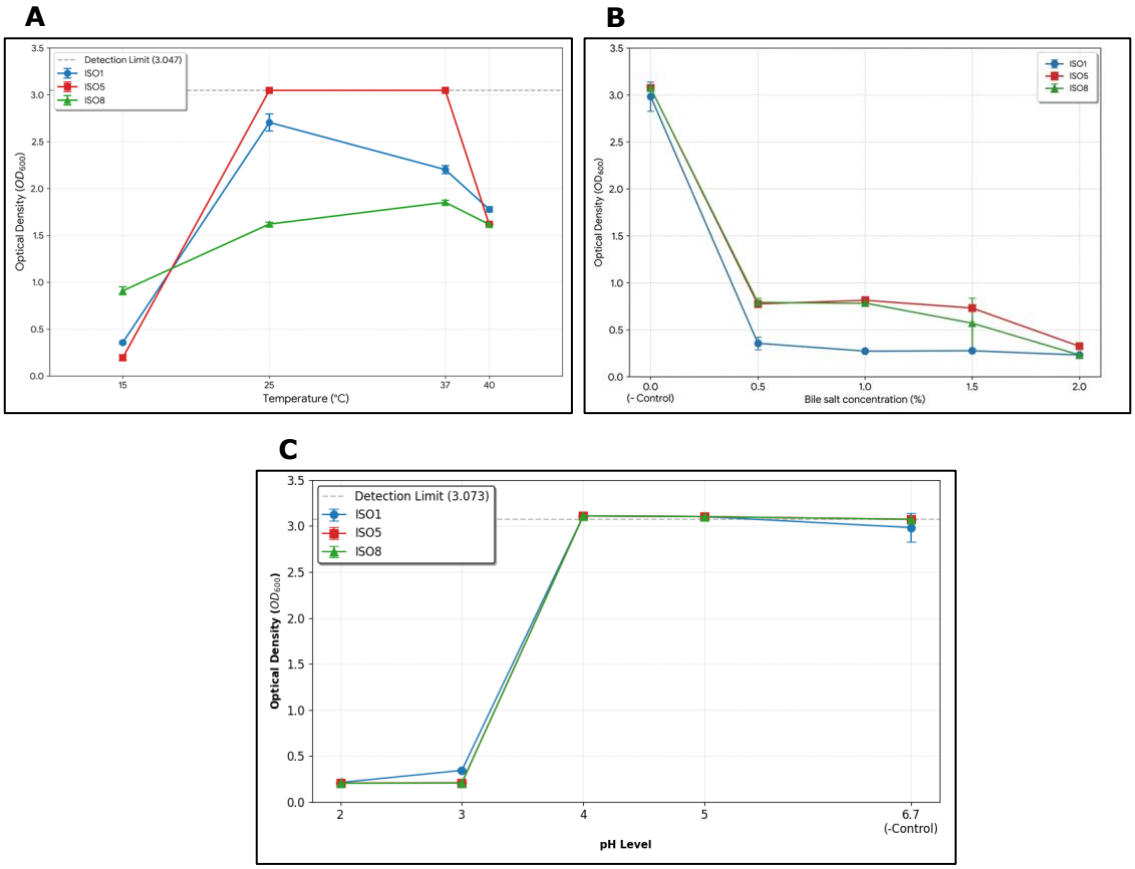


Figure 4. Growth response of bacterial isolates (ISO1, ISO5, and ISO8) under varying environmental conditions. (A) Effect of temperature on bacterial growth; (B) Growth response of isolates across different bile salt concentrations; (C) Growth response at different pH levels. Growth is measured by Optical Density (OD_{600}), and the dashed line indicates the detection limit.

Acid tolerance. All isolates exhibited inhibited growth under highly acidic conditions (pH 2 and 3). However, at pH 4 and 5, substantial growth was observed, with OD values approaching the detection limit. These results indicate tolerance to moderately acidic conditions. This observation is consistent with Safi et al (2023), who defined resistance as an increase in OD after incubation.

Antagonistic activity. None of the isolates demonstrated inhibitory activity against the tested pathogenic strains, as no zones of inhibition were observed. In contrast, positive controls produced distinct inhibition zones, confirming the assay's validity.

Antibiotic susceptibility. All isolates were susceptible to Chloramphenicol and Erythromycin but exhibited resistance to Amikacin, Cefixime, and Vancomycin. ISO5 displayed intermediate susceptibility to Cefixime. The interpretation of resistance and susceptibility followed the criteria described by Agustina et al (2022).

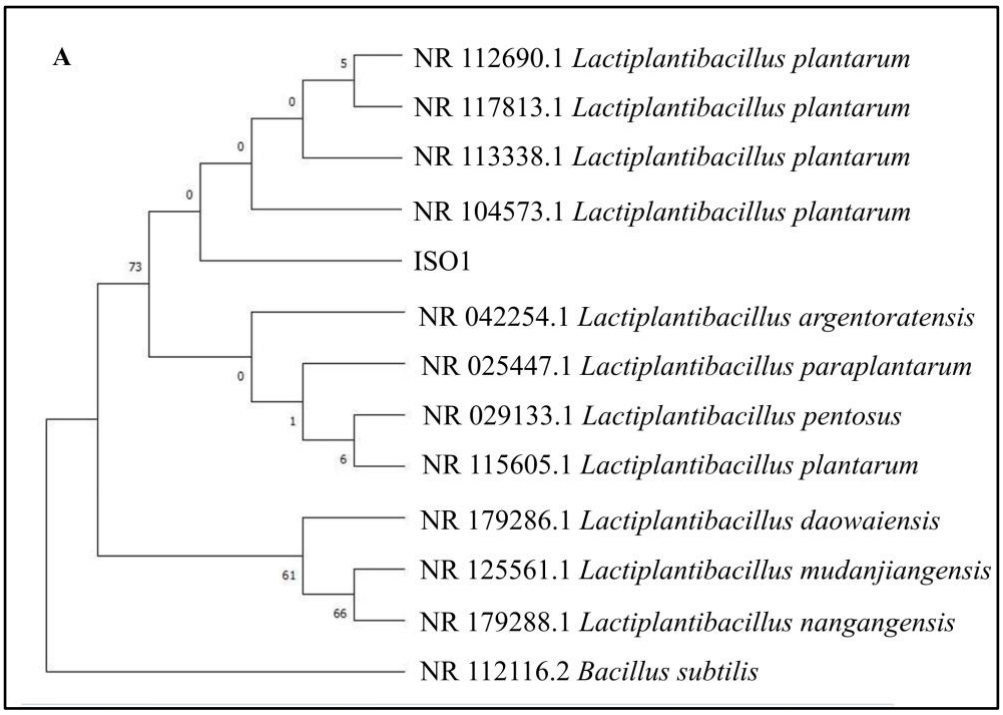
Table 1
Antibiotic susceptibility profiles of bacterial isolates against antibiotics (diameter zone of inhibition in mm)

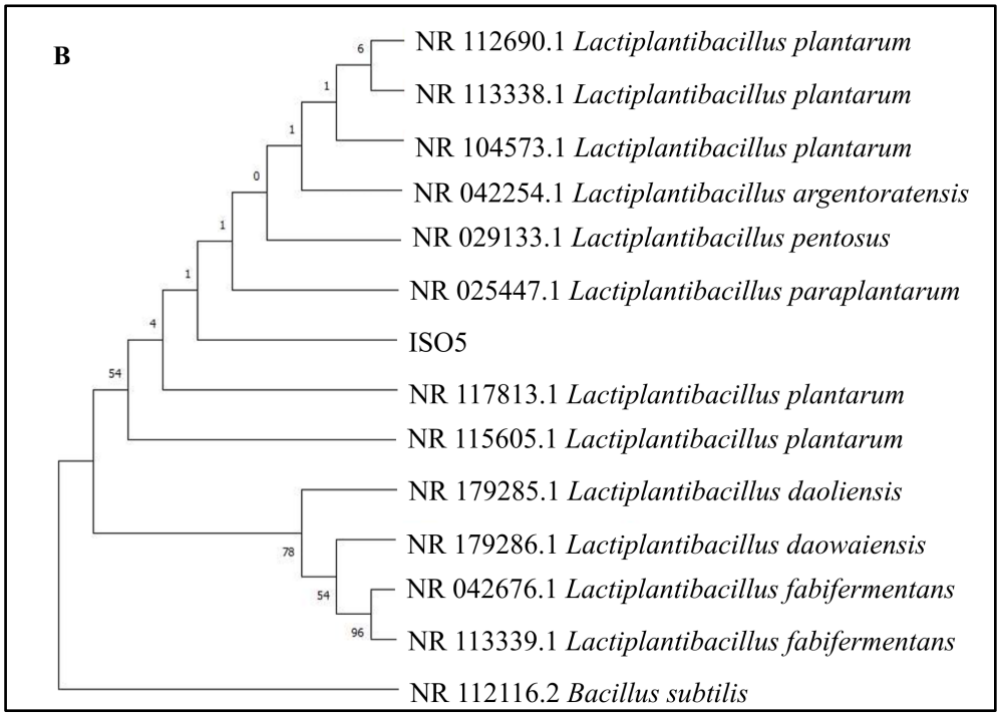
Isolate	*AK (30ug)*	*C (30ug)*	*E (15ug)*	*CFM (5ug)*	*VA (30ug)*
ISO1	10.4 (R)	28.7 (S)	27.1 (S)	12.8 (R)	6 (R)
ISO5	8.4 (R)	27.2 (S)	27.9 (S)	14.9 (I)	6 (R)
ISO8	9.3 (R)	25.5 (S)	25.3 (S)	11.5 (R)	6 (R)

Note: AK- Amikacin; C- Chloramphenicol; E- Erythromycin; CFM- Cefixime; VA- Vancomycin; R- Resistant; I- Intermediate; S- Susceptible.

Carbohydrate fermentation. All isolates were capable of fermenting glucose, lactose, and sucrose, as evidenced by the acid-induced color change in phenol red broth. This confirms their metabolic capacity to utilize multiple carbohydrate substrates, consistent with the findings of Das et al (2019).

Molecular identification. 16S rRNA gene sequencing identified all isolates (ISO1, ISO5, and ISO8) as *L. plantarum*, with sequence similarity ranging from 99 to 100%. According to Carvalho et al (2023), sequence identities ≥ 97% are considered reliable for species-level identification (Table 2). Phylogenetic analysis further confirmed clustering with reference *L. plantarum* strains (Figure 5).





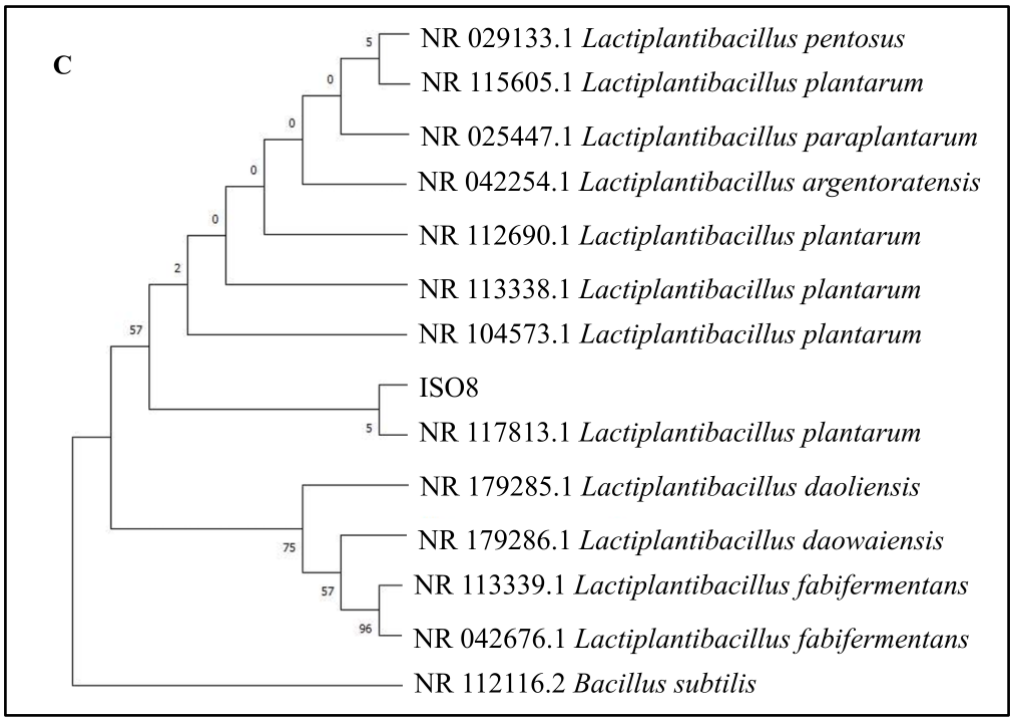


Figure 5. Phylogenetic analysis of the 16S rRNA gene sequences of the bacterial isolates (A. ISO1; B. ISO5; C. ISO8). The trees were constructed using the Maximum-likelihood method. Evolutionary distances were computed using the Kimura 2-parameter. *Bacillus subtilis* was used as the outgroup to root the trees.

Table 2

The analysis results of the test sequences used in the NCBI BLAST method

Description	*Isolate*	*Max score*	*Total score*	*Query cover (%)*	*E value*	*Per ident (%)*	*Acc. No*
Lactiplantibacillus	ISO1	1125	1125	97	0.0	100	NR_104573.1
plantarum strain	ISO5	1131	1131	96	0.0	99.52	NR_104573.1
CIP 103151 16S	ISO8	1125	1125	95	0.0	100	NR_104573.1
Lactiplantibacillus	ISO1	1125	1125	97	0.0	100	NR_11338.1
plantarum strain	ISO5	1131	1131	96	0.0	99.52	NR_11338.1
NBRC 15891 16S	ISO8	1125	1125	95	0.0	100	NR_11338.1
Lactiplantibacillus	ISO1	1125	1125	97	0.0	100	NR_025447.1
plantarum strain	ISO5	1127	1127	96	0.0	99.68	NR_025447.1
DSM 10667 16S	ISO8	1125	1125	95	0.0	100	NR_025447.1
Lactiplantibacillus	ISO1	1125	1125	97	0.0	100	NR_115605.1
plantarum strain	ISO5	1131	1131	96	0.0	99.52	NR_115605.1
JCM 1149 16S	ISO8	1125	1125	95	0.0	100	NR_115605.1

Discussion

Phenotypic and biochemical characterization. The successful isolation of Gram-positive, catalase-negative, rod-shaped bacteria confirms the presence of LAB in the gastrointestinal tract of *E. melanosoma*. The identification of all isolates as *L. plantarum* is consistent with previous reports of its occurrence in freshwater fish, including tilapia (Jiang et al 2022) and catfish (Kumaree et al 2014).

Stress tolerance. The temperature tolerance profile observed in this study indicates that the isolates are mesophilic, with optimal growth between 25 and 37°C. These findings are consistent with previous reports that *L. plantarum* grows optimally at 30 to 37°C but exhibits reduced growth at elevated temperatures (Kim et al 2019; Katiku et al 2022). Similarly, *Lactobacillus acidophilus* has been reported to exhibit optimal growth between 35 and 38°C and limited growth below 20°C (Gao et al 2022).

Tolerance to bile salts and acidic conditions is a critical determinant of probiotic potential. The ability of ISO5 and ISO8 to tolerate bile concentrations up to 1.5% suggests their potential to survive intestinal conditions. However, the absence of tolerance in ISO1 and the lack of tolerance at higher bile concentrations highlight strain-specific variability. The limited survival of the isolates under highly acidic conditions (pH 2-3) further suggests potential constraints during gastric transit. These findings align with reports indicating that LAB generally exhibits optimal growth within moderately acidic environments but reduced viability under extreme acidity (Yeboah et al 2023).

Antimicrobial activity. The absence of antagonistic activity observed in this study may be attributed to environmental conditions that were unfavorable for antimicrobial metabolite production. Research indicates that optimal growth conditions do not necessarily coincide with maximum production of antimicrobial compounds, and production can be significantly inhibited at pH levels below 6.4 (Lepecka et al 2021). Furthermore, while environmental stress can sometimes stimulate metabolite production (Nezhad et al 2015), the specific conditions in this study may not have triggered such a response.

Antibiotic profile and safety. The antibiotic resistance profiles observed in this study are consistent with previous findings. Resistance to Amikacin and Cefixime has been reported in various *Lactobacillus* strains (Anisimova et al 2022; Sharma et al 2022), while resistance to Vancomycin is frequently observed within this genus (Floris et al 2025; Sukmawinata et al 2025). Conversely, susceptibility to Chloramphenicol and Erythromycin is also well documented. Despite the generally recognized safety of LAB, the presence of antibiotic resistance genes raises concerns regarding potential horizontal gene transfer (Nandi & Mandal 2025; Sukmawinata et al 2025), emphasizing the need for careful safety evaluation.

Carbohydrate fermentation. The ability of the isolates to ferment multiple carbohydrates confirms their metabolic versatility, which is a defining characteristic of LAB (Gunkova et al 2021; Rhaiem et al 2016). Similar fermentation profiles have been reported for *L. acidophilus*, *Lactobacillus delbrueckii* (Khusboo et al 2023), and *L. plantarum* (Cui et al 2021).

Ecological relevance and innovation. Although all isolates were identified as *L. plantarum*, they exhibited notable differences in functional traits, including bile tolerance, acid resistance, and antimicrobial activity. This supports previous findings that strains within the same species can exhibit diverse physiological responses to environmental stress (Ferenci & Spira 2007). Therefore, strain-level characterization remains essential in accurately assessing probiotic potential.

Importantly, this study represents the first documented isolation of *L. plantarum* from *E. melanosoma* in the Agusan River. This finding contributes to the growing body of knowledge on host-associated LAB and provides new insights into the microbial diversity of freshwater fish in the Philippines.

Conclusions. This study reports the first isolation and characterization of gut-derived LAB from *E. melanosoma* in the Agusan River, Philippines. Three representative isolates (ISO1, ISO5, and ISO8) were identified as *L. plantarum* based on phenotypic, biochemical, and molecular analyses.

The isolates exhibited key LAB traits, including carbohydrate fermentation and growth at moderate temperatures (25-37°C) and pH (4-5). ISO5 and ISO8 demonstrated greater bile tolerance (up to 1.5%) compared to ISO1, indicating strain-dependent variability. However, the absence of antagonistic activity and limited survival under highly acidic conditions suggest constraints in their probiotic performance under gastrointestinal stress.

While susceptibility to Chloramphenicol and Erythromycin supports their safety profile, resistance to certain antibiotics highlights the need for further genomic evaluation. These findings expand knowledge on freshwater fish-associated LAB and emphasize the

importance of strain-level characterization. Further studies, including genomic analysis and in vivo validation, are recommended to fully assess their probiotic potential for aquaculture applications.

Acknowledgements. This research was supported by the Department of Science and Technology through the STRAND-N Scholarship Program, whose financial assistance is gratefully acknowledged. The author also extends sincere appreciation to the Bureau of Fisheries and Aquatic Resources, Caraga Region, for their assistance in fish identification and for providing essential biological support for this study.

Authors Contributions. Conceptualization: ACN; Formal analysis: ACN; Writing – original draft: ACN; Writing – review & editing: MEQW.

Conflict of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Funding. This study received external funding support from the Department of Science and Technology (DOST) through the thesis grant under the STRAND-N Scholarship Program.

References

Agustina A., Saptiani G., Hardi E., 2022 Isolation and identification of potential lactic acid bacteria as probiotics from the intestines of repang fish (*Puntioplites waandersi*). Aquaculture, Aquarium, Conservation and Legislation 15(1):24-33.

Anisimova E., Gorokhova I., Karimullina G., Yarullina D., 2022 Alarming antibiotic resistance of lactobacilli isolated from probiotic preparations and dietary supplements. Antibiotics 11(11):1557.

Carvalho L., Fernandes N., Silva B., Barron U., Cadavez V., 2023 Molecular identification and phylogenetic inference of lactic acid bacteria isolated from goat's raw milk cheese. Biology and Life Sciences Forum 26(1):39.

Coulibaly W. H., Kouadio N. R., Camara F., Camelia D., Matei F., 2023 Functional properties of lactic acid bacteria isolated from Tilapia (*Oreochromis niloticus*) in Ivory Coast. BMC Microbiology 23(1):152.

Cui Y., Wang M., Zheng Y., Miao K., Qu, X., 2021 The carbohydrate metabolism of *Lactiplantibacillus plantarum*. International Journal of Molecular Sciences 22(24):13452.

Das A. J, Das M. J, Miyaji T., Deka S. C., 2019 Growth and metabolic characterization of four lactic acid bacteria species isolated from rice beer prepared in Assam, India. Access Microbiology 1(4):e000028.

Dwyana Z., Priosambodo D., Haedar N., Erviani A. E., Djabura A. K., Sukma R., 2018 The potential for Probiotic Bacteria from milkfish intestine in reducing mercury metals in skimmed milk media. Journal of Physics: Conf Series 979(1):012002.

Ferenci T., Spira B., 2007 Variation in stress responses within a bacterial species and the indirect costs of stress resistance. Annals of the New York Academy of Sciences 1113(1):105-113.

Floris I., Battistini R., Tramuta C., Vozmediano A. G., Musolino, N., Scardino, G., Masotti C., Brusa B., Orusa R., Bianchi D. M., 2025 Antibiotic resistance in lactic acid bacteria from dairy products in Northern Italy. Antibiotics 14(4):375.

Gao H., Li X., Chen X., Hai D., Wei C., Zhang L., Li P., 2022. The functional roles of Lactobacillus acidophilus in different physiological and pathological processes. Journal of Microbiology and Biotechnology 32(10):1126-1233.

Gunkova P. I., Buchilina A. S., Maksimiuk N. N., Bazarnova Y. G., Girel K. S., 2021 Carbohydrate fermentation test of lactic acid starter cultures. Earth and Environmental Science 852(1):012035.

Huligere S., Kumari V., Alqadi T., Kumar S., Cull C., Amachawadi R., Ramu R., 2022 Isolation and characterization of lactic acid bacteria with potential probiotic activity and further investigation of their activity by α-amylase and α-glucosidase inhibitions of fermented batters. Frontiers in Microbiology 13:1042263.
Jiang J. H., Xin W. G., Zhang Q. L., Lin L. B., Deng X. Y., 2022 A novel bacteriocin against shigella flexneri from *Lactiplantibacillus plantarum* isolated from tilapia intestine: purification, antibacterial properties and antibiofilm activity. Frontiers in Microbiology 12:779315.
Katiku M. M., Matofari J. W., Nduko J. M., 2022 Preliminary evaluation of probiotic properties and safety profile of *Lactiplantibacillus plantarum* isolated from spontaneously fermented milk, *Amabere amaruranu*. Heliyon 8(8):e10342.
Khusboo, Karnwal A., Malik T., 2023 Characterization and selection of probiotic lactic acid bacteria from different dietary sources for development of functional foods. Frontiers in Microbiology 14:1170725.
Kim J. A., Bayo J., Cha J., Choi Y. J., Jung M. Y., Kim D. H., Kim Y., 2019 Investigating the probiotic characteristics of four microbial strains with potential application in feed industry. PLoS ONE 14(6):e0218922.
Kong W., Huang S., Yang Z., Shi F., Feng Y., Khatoon Z. 2020 Fish feed quality is a key factor in impacting aquaculture water environment: evidence from incubator experiments. Scientific Reports 10(1):187.
Kumaree K. K., Akbar A., Anal A. K., 2014 Bioencapsulation and application of *Lactobacillus plantarum* isolated from catfish gut as an antimicrobial agent and additive in fish feed pellets. Annals of Microbiology 65(3):1439-1445.
Latif A., Shehzad A., Niazi S., Zahid A., Ashraf W., Rehman A., Iqbal M., Riaz T., Aadil R., Khan I., Ozogul F., Rocha J., Esatbeyoglu T., Korma S., 2023 Probiotics: mechanism of action, health benefits and their application in food industries. Frontiers in Microbiology 14:1216674.
Lepecka A., Szymanski P., Rutkowska S., Iwanowska K., Krajewska D. K., 2021 The influence of environmental conditions on the antagonistic activity of lactic acid bacteria isolated from fermented meat products. Foods 10(10):2267.
Lingga R., Adibrata S., Roanisca O., Sipriyadi., Wibowo R., Arsyadi A., 2023 Probiotics potential of lactic acid bacteria isolated from Slender Walking Catfish (*Clarias nieuhofii)*. Biodiversitas 24(8):4572-4580.
Nandi S., Mandal., 2025 Probiotic potentiality, safety profiling and broad-spectrum antibacterial activity of lactic acid bacteria isolated from sour curd (Malda, India). The Microbe 7:100279.
Nezhad H., Hussain M., Britz M. L., 2015 Stress responses in probiotic *Lactobacillus casei*. Critical Reviews in Food Science and Nutrition 55(6):740-749.
Peristiwati, Sanjaya Y., Trinaya C., 2019 Isolation and identification of potential culturable probiotics bacteria from the intestine of Anguilla bicolor. Journal of Physics: Conference Series 1280(2):022005.
Rahayu S., Amoah K., Huang Y., Cai J., Wang B., Shija V., Jin X., Anokyewaa M., Jiang M., 2024 Probiotics application in aquaculture: its potential effects, current status in China and future prospects. Frontiers in Microbiology 11:1455905.
Rhaiem N., Chahboun N., Ineckach S., Ouhssine M., 2016 Identification and characterization of lactic acid bacteria isolated from cow milk and olive brine. Journal of Materials and Environmental Science 7(5):1505-1509.
Sadaqat M. H., 2024 Antibacterial, antiviral, antifungal, and immunomodulatory properties of probiotics. Afghanistan Journal of Basic Medical Sciences 1(2):111-120.
Sadiq M. B., 2022 Lactic acid bacteria as potential probiotics. Probiotics, prebiotics and synbiotics: technological advancements towards safety and industrial applications 57-72.
Safi E., Haddad M., Hasan M., Al-Dalain S., Proestos C., Siddiqui S., 2023 Characterization of potential probiotic activity of lactic acid bacteria isolated from camel colostrum by biochemical and molecular methods. Veterinary Medicine International 2023(1):8334152.

Sharma N., Singh P. K., Rawat S., Upadhyay A. K., Joshi R., Verma H., 2022 Antibiotic resistance profile of lactic acid bacteria isolated from raw milk of Badri cows. The Pharma Innovation Journal 11(8):1707-1709.
Sukmawinata E., Laila S. R., Pisestyani H., Noor S. M., Martindah E., Widiastuti R., Wahyuwardani S., Kusumaningtyas E., Andriani, Subangkit M., Endrawati D., Ariyanti T., Rachmawati F., 2025 Antibiotic susceptibility profiles and potential of lactic acid bacteria from dadih as antimicrobial producers and dangke whey fermenters. International Journal of Veterinary Science 14(1):1-7.
Terpou A., Papadaki A., Lappa I. K., Kachrimanidou V., Bosnea L. A., Kopsahelis N., 2019 Probiotics in food systems: significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. Nutrients 11(7):1591.
Yeboah P., Wijemanna N., Eddin A., Williams L., Ibrahim S., 2023 Lactic acid bacteria: review on the potential delivery system as an effective probiotic. Dairy Processing - From Basics to Advances. IntechOpen. pp. 1-29.

Received: 06 April 2026. Accepted: 08 May 2026. Published online: 23 June 2026.
Authors:
Angelie Colina Navarez (ACN), Graduate School, Caraga State University, Brgy. Ampayon, Butuan City, 8600 Agusan Del Norte, Philippines, email: angelieqc.navarez@gmail.com
Maria Elma Quiao Won (MEQW), Department of Biology, College of Mathematics and Natural Sciences, Caraga State University, Brgy. Ampayon, Butuan City, 8600 Agusan Del Norte, Philippines, email: mqwon@carsu.edu.ph

How to cite this article:
Navarez A. C., Won M. E. Q., 2026 Probiotic potential of gut-derived lactic acid bacteria from *Eleotris melanosoma* Bleeker, 1853 in the Agusan River, Philippines. AACL Bioflux 19(3):1401-1412.