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IDENTIFICATION AND ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM MINCED FLESH OF COMMON FRESHWATER FISH

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ABSTRACT

Lactic acid bacteria (LAB) associated with fish represent potential sources of beneficial microorganisms for sustainable aquaculture, particularly for developing alternative strategies to control bacterial pathogens. This study aimed to isolate LAB from minced freshwater fish, identify selected isolates using 16S rRNA gene sequencing, and evaluate their *in vitro* antibacterial activity against intestinal pathogens. A total of 21 fish samples were collected during 2025–2026, yielding 96 presumptive LAB isolates, of which 21 exhibiting comparatively strong antibacterial activity were selected for further characterization. Based on 16S rRNA gene sequence analysis, the selected isolates were assigned to four species: *Levilactobacillus brevis*, *L. casei*, *L. plantarum*, and *L. rhamnosus*. Across 84 isolate–pathogen combinations, inhibition-zone diameters ranged from 16 to 22 mm, with an overall descriptive mean of 18.93 mm. The largest inhibition zones were recorded for isolate KAA63 against *Shigella flexneri* PTCC 1234 (22 ± 1.0 mm) and KAA23 against *Yersinia enterocolitica* PTCC 1154 (22 ± 1.1 mm). Among the identified species groups, *L. plantarum* exhibited the highest overall mean inhibition zone (19.68 mm) and relatively consistent antibacterial activity against the four indicator pathogens. At the individual-isolate level, KAA23, KAA31, and KAA86 showed the highest mean inhibitory activity. These findings indicate that minced freshwater fish may serve as a source of LAB with promising antibacterial properties. In particular, *L. plantarum* isolates KAA23, KAA31, KAA44, and KAA86 warrant further investigation as potential probiotic candidates. However, their suitability as live microbial feed additives in aquaculture requires comprehensive safety assessment, antimicrobial resistance profiling, and controlled in vivo studies to establish efficacy and practical applicability.

***Keywords*:** Minced fish, Lactic acid bacteria, Identification, Antimicrobial activity.

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1. INTRODUCTION

Aquaculture has become an increasingly important part of global food production, supporting food security and the sustainable supply of animal protein. However, intensifying aquaculture systems, often characterized by high stocking densities and increased environmental pressures, can facilitate the transmission and persistence of infectious agents (Torres-Maravilla et al., 2024). Effective disease prevention and control are therefore essential for maintaining aquatic animal health, production efficiency, and environmental sustainability. Although antimicrobial agents remain important for treating bacterial infections when clinically justified, inappropriate or excessive use may promote antimicrobial resistance, contribute to drug residues in aquaculture products, and disrupt microbial communities in aquatic environments (Rahayu et al., 2024; Mohammed et al., 2025). Consequently, sustainable aquaculture management increasingly emphasizes preventive strategies, including improved husbandry practices, biosecurity, vaccination where available, and developing effective microbial interventions. Among these approaches, probiotics have attracted considerable attention because selected microorganisms can influence host-associated microbial communities, compete with potentially pathogenic bacteria, and produce antimicrobial metabolites. Nevertheless, these benefits are strain-dependent and may vary by host species, diet, and environmental conditions. Therefore, promising laboratory screening results should be regarded as preliminary evidence rather than confirmation of probiotic efficacy under aquaculture conditions (Chizhayeva et al., 2022; Calcagnile et al., 2024).

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Lactic acid bacteria (LAB) comprise a physiologically diverse group of predominantly Gram-positive, non-spore-forming bacteria that produce lactic acid as a major end product of carbohydrate fermentation (Ingham, 1991; Arief et al., 2026). These microorganisms have long been associated with fermented foods because they contribute to acidification, flavor development, preservation, and microbial stability. Beyond their established roles in food fermentation, researchers have investigated selected LAB strains for potential probiotic properties and antimicrobial activities (Abouloifa et al., 2021; Chen et al., 2021). LAB's inhibitory effects against other microorganisms may involve organic acid production, environmental acidification, and the synthesis of antimicrobial compounds, including hydrogen peroxide, diacetyl, and bacteriocins, depending on the strain and growth conditions. However, an inhibition zone in an *in vitro* antimicrobial assay does not establish the underlying inhibitory mechanism, particularly when non-neutralized culture supernatants contain multiple potentially active metabolites (Hossain et al., 2026). Accordingly, antimicrobial activity is an important preliminary screening characteristic, but selecting probiotic candidates requires additional evaluation of taxonomic identity, safety, stability, and functional performance in the intended host. This distinction is particularly important when extending the established applications of LAB in food systems to their potential use in aquaculture (Cavalcanti de Albuquerque et al., 2020; Pereira et al., 2022).

LAB are widely distributed across diverse ecological habitats, including fermented dairy products, plant surfaces (Liu et al., 2022; Sundari et al., 2026), fruits (Ngea et al., 2021; Sapsuha et al., 2025), vegetables (Linares-Morales et al., 2020; Nweke et al., 2026), animal mucosal surfaces, and the gastrointestinal tracts of humans and aquatic animals (Ekelemu et al., 2023; Adeniyi & Olorunshola, 2024). Their physiological and functional characteristics may differ considerably depending on the ecological origin of the isolates. For example, plant-associated LAB vary in carbohydrate utilization, enzymatic activity, and antimicrobial properties, reflecting differences in the nutritional and environmental conditions of their source habitats (Mirzayeva, 2020). Similarly, traditional dairy products harbor LAB strains with diverse antagonistic activities and probiotic-associated characteristics (Khanghah & Ganbarov, 2014). These observations suggest that an isolate's ecological origin may influence its recoverable phenotypic characteristics, but functional properties must ultimately be evaluated at the strain level. Several factors influence the composition of host-associated microbial communities in aquatic animals, including fish species, developmental stage, diet, water quality, and rearing conditions (Yulvizar et al., 2025). Consequently, both the gastrointestinal tract and fish-associated food matrices may represent potential sources of LAB with distinct physiological and antimicrobial characteristics (Luan et al., 2023; Lingga et al., 2023).

Minced fish is a potentially useful but comparatively underexplored matrix for isolating and characterizing LAB. Mechanical comminution increases the exposed surface area of fish tissues, redistributes moisture and nutrients, and facilitates the mixing of microorganisms originating from different tissue surfaces and handling environments. Previous investigations have demonstrated that mechanical separation and processing can influence microbial populations and their distribution within fish products (Ingham, 1991). Although researchers have generally examined these changes in relation to microbial spoilage, product quality, and food safety, minced fish may also contain indigenous or environmentally acquired LAB suitable for preliminary functional screening. Studies of marine and freshwater fish have shown the diversity of fish-associated LAB, and factors such as storage conditions and dietary composition may influence fish-associated microbial populations and product characteristics (Govindaraj et al., 2021; Ekelemu et al., 2023; Zhumanova et al., 2026). Therefore, investigating LAB recovered from minced freshwater fish may provide an opportunity to characterize locally occurring bacterial isolates and identify strains exhibiting potentially useful antimicrobial properties. However, recovering LAB from this matrix does not necessarily establish their origin in the fish intestinal microbiota, as microorganisms may also be introduced during processing and handling.

Previous aquaculture studies have demonstrated that selected LAB strains can influence growth performance, immune responses, intestinal microbial communities, and resistance to infectious diseases in fish. However, these effects vary across bacterial strains, host species, and experimental conditions. Reviews of probiotic applications in finfish have emphasized the importance of host specificity and strain-level validation when evaluating potential health benefits. In channel catfish, dietary supplementation with *Lactobacillus casei* YYL3 and *Lactobacillus plantarum* YYL5 altered growth performance, immune responses, and intestinal microbiota, highlighting the potential functional relevance of selected LAB strains under experimental conditions (Thompson et al., 2022). Recent investigations in freshwater fish have also identified LAB isolates with inhibitory activity against bacterial pathogens, supporting further characterization and host-specific evaluation (Zhang et al., 2024; Olorunshola et al., 2025). However, antimicrobial activity demonstrated under laboratory conditions does not necessarily translate into improved growth, disease resistance, or other beneficial outcomes in living fish. Therefore, comprehensive assessment of candidate strains should include taxonomic identification, safety evaluation, antimicrobial resistance profiling, stability testing, and controlled *in vivo* investigations before their application as probiotics in aquaculture (Hoseinifar et al., 2018; Das et al., 2024).

Despite increasing interest in fish-associated LAB, information regarding the isolation and antimicrobial characteristics of LAB recovered from minced freshwater fish in Kazakhstan remains limited. Investigating this source may help characterize locally occurring LAB and provide preliminary evidence for identifying isolates with

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potential antimicrobial applications. Accordingly, this study aimed to isolate LAB from minced freshwater fish, identify selected isolates, and evaluate their *in vitro* antagonistic activity against selected intestinal bacterial pathogens. The findings are expected to provide baseline information on the antimicrobial potential of fish-associated LAB and support the selection of promising isolates for subsequent safety assessment and functional evaluation.

2. MATERIALS AND METHODS

2.1. Preparation of Samples for Isolation of Lactic Acid Bacteria

Fresh minced flesh of *Rutilus brama*, *Abramis brama*, *Pelecus cultratus*, *Leuciscus aspius*, and *Carassius* was obtained from Amangeldy "Atyraubalyk" LLC, Republic of Kazakhstan. Twenty-one minced-fish samples were collected during 2025–2026 yielded 96 catalase-negative presumptive LAB isolates. A suspension was prepared by adding 10 g of minced fish to 100 mL of sterile physiological saline (0.9% NaCl). An aliquot of 0.1 mL was spread on agar MRS medium with the following composition (g/L): glucose, 20.0; peptone, 10.0; meat extract, 8.0; yeast extract, 4.0; diammonium citrate, 2.0; sodium acetate, 2.0; Tween 80, 1.0; K_2HPO_4, 2.0; $MgSO_4$, 2.0; $MnSO_4$, 0.04; and agar, 20.0; pH 5.6.

The inoculated Petri dishes were placed in an evacuated desiccator and incubated for 48 h at 30°C. Colonies growing on the medium were screened presumptively as LAB by the catalase test. For this purpose, 1–2 drops of 0.1 M H_2O_2 solution were applied to each colony; colonies that showed no foaming or bubble formation were considered catalase-negative. The resulting strains were then examined by Gram staining (Pérez-Sánchez et al., 2011).

2.2. The Investigation of Antimicrobial Activity of Isolated Strains of Lactic-Acid Bacteria

The in vitro antimicrobial activity of the LAB was evaluated by the agar well-diffusion method (Balouiri et al., 2016). Overnight LAB cultures were centrifuged, and the resulting supernatants were used as the test material. The indicator pathogen cultures were *Escherichia coli* PTCC 1329, *Klebsiella pneumoniae* PTCC 1290, *Shigella flexneri* PTCC 1234, and *Yersinia enterocolitica* PTCC 1154. One hundred microliters of a fresh 24-h suspension of each indicator strain was distributed aseptically over nutrient agar (Liofilchem, Italy). Wells 8 mm in diameter were prepared in the agar and filled with 200 µL of each LAB supernatant. The cultures were incubated at 37°C for 24 h. Antimicrobial activity was assessed by measuring, in millimetres, the diameter of the clear inhibition zone around each well.

2.3. The Molecular Genetic Identification of Isolated Strains

The strains of lactic acid bacteria showing high antibacterial activity were identified by Polymerase Chain Reaction (PCR) analysis. DNA isolation and purification were carried out according to the cited method (Atashpaz et al., 2010; Mutmainna et al., 2026). A Taq DNA polymerase kit and the primers 16 lacFS, 5′-AGAGTTTGATCMTGGTCAG-3′, and 16 lacRS, 5′-TACCTTGTTAGGACTTCACC-3′, were used to amplify the 16S rDNA region (Lane et al., 1985). Reactions were performed in a thermal cycler (Bio-Rad, Hercules, CA, USA) under the following conditions: initial denaturation at 94°C for 4 min; 32 cycles of 94°C for 50s, 59°C for 50s, and 72°C for 90s; and a final extension at 72°C for 10 min. The reaction mixture contained 0.02 M/L Tris-HCl (pH 8.4), 0.05 M/L KCl, 0.003 M/L $MgCl_2$, 0.05 M/L of each primer, and 1 µL of isolated DNA in a total volume of 50 µL; products were stored at 4°C. PCR products were separated by electrophoresis in a 1% agarose gel (15 × 20 cm) containing 1.5 µM/L ethidium bromide at 4°C for 16 h at 55 V DC. Electrophoresis was conducted in Tris-acetate-EDTA buffer using an EPS 301 device (USA). PCR products were purified with the QIAquick PCR Purification Kit/250 (Germany), and nucleotide sequences were determined using the "CRIBİ-BMR servizio segmenziamento DNA" system (Italy). Sequence homology was evaluated by comparison with records available through NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.4 Statistical Analysis

All experiments were conducted in quadruplicate (n=4) and results are expressed as mean ± standard error. Statistical significance was evaluated using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. A significance threshold of $P<0.05$ was applied to ensure reliability of the results.

3. RESULTS

3.1. Phenotypic Characterization and Molecular Identification of Selected Lactic Acid Bacteria

Preliminary antimicrobial screening identified 21 LAB isolates exhibiting comparatively high inhibitory activity against four intestinal indicator organisms. The selected isolates were subjected to phenotypic characterization based on carbohydrate utilization patterns and growth at 45°C, following the identification criteria described in *Bergey's Manual of Systematic Bacteriology* (Vos et al., 2009). Based on their physiological characteristics, the 21 isolates were classified into four distinct groups (Table 1).

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Table 1: Carbohydrate-utilization profiles of strains and growth at 45°C

Strains	Sugars																		45°C
	Amygdaline	Arabinose	Cellobiose	Galactose	Glucose	Lactose	Maltose	Mannose	Melibiose	Raffinose	Rhamnose	Ribose	Salicin	Sucrose	Sorbose	Tregalose	Fructose	Xylose	
Group 1																			
KAA 3	+	±	+	+	+	-	-	+	±	-	-	+	+	-	+	+	+	-	-
KAA 14	+	±	+	+	+	-	-	+	±	-	-	+	+	-	+	+	+	-	-
KAA 28	+	±	+	+	+	-	-	+	±	-	-	+	+	-	+	+	+	-	-
KAA 63	+	±	+	+	+	-	-	+	±	-	-	+	+	-	+	+	+	-	-
KAA 77	+	±	+	+	+	-	-	+	±	-	-	+	+	-	+	+	+	-	-
Group 2																			
KAA 2	+	±	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	±	-
KAA 8	+	±	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	±	-
KAA 17	+	±	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	±	-
KAA 23	+	±	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	±	-
KAA 31	+	±	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	±	-
KAA 44	+	±	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-±	-
KAA 86	+	±	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	±	-
Group 3																			
KAA 6	-	+	-	-	+	-	+	-	+	-	-	+	-	±	-	-	+	±	-
KAA 38	-	+	-	-	+	-	+	-	+	-	-	+	-	±	-	-	+	±	-
KAA 54	-	+	-	-	+	-	+	-	+	-	-	+	-	±	-	-	+	±	-
KAA 59	-	+	-	-	+	-	+	-	+	-	-	+	-	±	-	-	+	±-	-
KAA 67	-	+	-	-	+	-	+	-	+	-	-	+	-	±	-	-	+	±	-
KAA 76	-	+	-	-	+	-	+	-	+	-	-	+	-	±	-	-	+	-	-
Group 4																			
KAA 11	-	+	+	+	+	-	+	-	-	+	+	+	-	+	+	-	+	±	+
KAA 41	-	+	+	+	+	-	+	-	-	+	+	+	-	+	+	-	+	±	+
KAA 88	-	+	+	+	+	-	+	-	-	+	+	+	-	+	+	-	+	±	+

Note: "+", growth; "±", weak growth; "−", no growth.

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The first group comprised five isolates (KAA3, KAA14, KAA28, KAA63, and KAA77) that exhibited strong growth in the presence of amygdalin, cellobiose, galactose, glucose, mannose, ribose, salicin, sorbose, trehalose, and fructose. Arabinose and melibiose showed weak growth, whereas lactose, maltose, raffinose, rhamnose, sucrose, and xylose showed no growth.

The second group consisted of seven isolates (KAA2, KAA8, KAA17, KAA23, KAA31, KAA44, and KAA86). These isolates grew well on amygdalin, cellobiose, galactose, glucose, lactose, maltose, mannose, melibiose, raffinose, ribose, salicin, sucrose, sorbose, trehalose, and fructose. Arabinose and xylose showed weak growth, while rhamnose showed no growth.

The third group included six isolates (KAA6, KAA38, KAA54, KAA59, KAA67, and KAA76) that showed strong growth on arabinose, glucose, maltose, melibiose, ribose, and fructose. They showed weak growth on sucrose and xylose, and no growth on amygdalin, cellobiose, galactose, lactose, mannose, raffinose, rhamnose, salicin, sorbose, or trehalose.

The fourth group comprised three isolates (KAA11, KAA41, and KAA88). These isolates grew well on arabinose, cellobiose, galactose, glucose, maltose, raffinose, rhamnose, ribose, sucrose, sorbose, and fructose. Isolates showed weak growth on xylose, whereas no growth was detected on amygdalin, lactose, mannose, melibiose, salicin, or trehalose. Notably, isolates in the fourth group grew at 45°C, distinguishing them from isolates in the other three groups.

We performed molecular identification by amplifying and sequencing the 16S rRNA gene, then comparing the sequences against reference sequences in the National Center for Biotechnology Information (NCBI) BLAST database. Sequence comparisons indicated that isolates in groups 1, 2, 3, and 4 were most closely related to *Levilactobacillus brevis, L. plantarum, L. casei*, and *L. rhamnosus*, respectively, with 100% sequence similarity for each group (Fig. 1–4). These assignments reflect the current nomenclature established after the taxonomic reclassification of the former genus *Lactobacillus*.

3.2. Antibacterial Activity of Selected Isolates against Intestinal Pathogens

We evaluated the antibacterial activity of the 21 selected LAB isolates against four intestinal indicator organisms: *Yersinia enterocolitica*, *Klebsiella pneumoniae*, *Shigella flexneri*, and *Escherichia coli*. We examined 84 isolate–pathogen combinations, and Table 2 presents the inhibition-zone measurements.

Table 2: *In vitro* antimicrobial activity of lactic acid bacterial strains isolated from minced fish against intestinal pathogens

Lactic acid bacteria		Test cultures (pathogens)			
Species	Strains	Escherichia coli PTCC 1329	Klebsiella pneumoniae PTCC 1290	Shigella flexneri PTCC 1234	Yersinia enterocolitica PTCC 1154
Lactobacillus brevis	KAA3	16 ± 0.4	19 ± 0.8	19 ± 0.8	19 ± 0.7
	KAA14	16 ± 0.6	19 ± 0.8	19 ± 0.8	19 ± 0.7
	KAA28	16 ± 0.7	20 ± 0.6	20 ± 0.7	20 ± 0.8
	KAA63	16 ± 0.6	21 ± 0.9	22 ± 1,0	21 ± 0.8
	KAA77	16 ± 0.7	20 ± 0.6	21 ± 0.8	21 ± 0.7
Lactobacillus plantarum	KAA2	19 ± 0.6	19 ± 0.7	20 ± 0.8	18 ± 0.3
	KAA8	19 ± 0.8	19 ± 0.8	18 ± 0.4	18 ± 0.6
	KAA17	19 ± 0.7	20 ± 0.9	18 ± 0.5	18 ± 0.8
	KAA23	20 ± 0.8	21 ± 1,0	21 ± 0.8	22 ± 1,1
	KAA31	20 ± 0.6	21 ± 0.8	20 ± 0.7	21 ± 0.7
	KAA44	19 ± 0.8	19 ± 0.7	19 ± 0.6	20 ± 0.8
	KAA86	20 ± 0.8	20 ± 0.8	21 ± 1,0	21 ± 1.0
Lactobacillus casei	KAA6	18 ± 0.38	17 ± 0.7	17 ± 0.8	17 ± 0.7
	KAA38	17 ± 0.8	17 ± 0.8	18 ± 0.6	19 ± 0.8
	KAA54	17 ± 0.7	20 ± 0.9	19 ± 0.8	18 ± 0.7
	KAA59	18 ± 0.7	18 ± 0.7	17 ± 0.8	19 ± 0.9
	KAA67	17 ± 0.6	19 ± 0.8	18 ± 0.9	19 ± 0.8
	KAA76	18 ± 0.8	19 ± 0.8	18 ± 0.9	19 ± 0.8
Lactobacillus Rhamnosus	KAA11	16 ± 0.8	18 ± 0.8	18 ± 0.7	19 ± 0.9
	KAA41	16 ± 0.7	20 ± 0.7	21 ± 0.8	21 ± 0.9
	KAA88	16 ± 0.9	20 ± 0.8	21 ± 0.7	20 ± 0.6

The reported central inhibition-zone values ranged from 16 to 22 mm, with an overall descriptive mean of 18.93 mm. Of the 84 isolate–pathogen combinations, 32 exhibited inhibition zones of at least 20 mm, indicating variation in inhibitory activity among the tested isolates and indicator organisms.

Among the indicator organisms, *Y. enterocolitica* exhibited the highest mean inhibition zone (19.48 mm), followed by *K. pneumoniae* (19.33 mm) and *S. flexneri* (19.29 mm). In contrast, *E. coli* exhibited the lowest mean

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inhibition zone (17.62 mm), indicating comparatively lower susceptibility to the inhibitory activity observed under the experimental conditions.

At the species-group level, isolates assigned to *L. plantarum* exhibited the highest overall descriptive mean inhibition zone (19.68 mm), with organism-specific means ranging from 19.57 to 19.86 mm. The corresponding overall mean inhibition zones were 19.00 mm for *L. brevis*, 18.83 mm for *L. rhamnosus*, and 18.04 mm for *L. casei*. These findings indicate differences in antibacterial activity among the four LAB groups, with the L. plantarum group showing the highest average inhibition across the tested organisms.

At the individual isolate level, KAA23 exhibited the highest mean inhibition zone across the four indicator organisms (21.0 mm), followed by KAA31 and KAA86 (20.5 mm each). Isolate KAA63 exhibited a mean inhibition zone of 20.0 mm. The largest individual inhibition zones were recorded for KAA63 against *S. flexneri* (22 ± 1.0 mm) and KAA23 against *Y. enterocolitica* (22 mm).

Isolates KAA23, KAA31, and KAA86 consistently exhibited relatively high inhibitory activity across the four indicator organisms. In contrast, KAA63 showed a more variable inhibitory profile, with a smaller inhibition zone against *E. coli* (16 ± 0.6 mm) but larger inhibition zones against the remaining three organisms (21–22 mm). Collectively, these findings demonstrate variation in *in vitro* antibacterial activity among the selected LAB isolates and identify KAA23, KAA31, and KAA86 as promising candidates for further functional characterization.

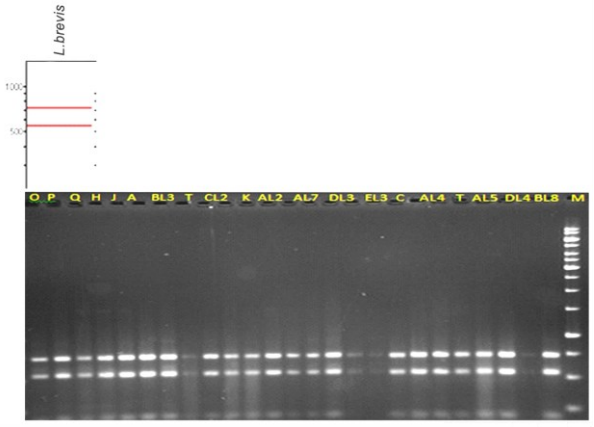

Fig. 1: Electrophoretic profile of 16S rDNA amplicons from *Lactobacillus brevis* strains.

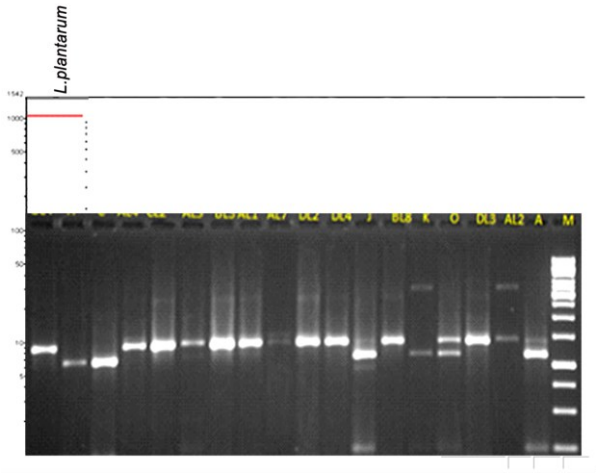

Fig. 2: Electrophoretic profile of 16S rDNA amplicons from *Lactobacillus plantarum* strains.

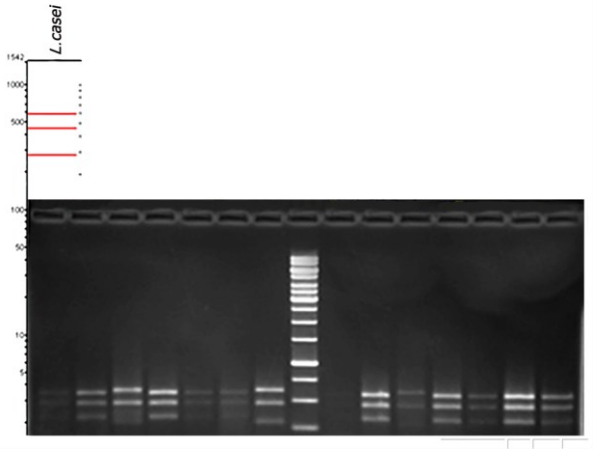

Fig. 3: Electrophoretic profile of 16S rDNA amplicons from *Lactobacillus casei* strains.

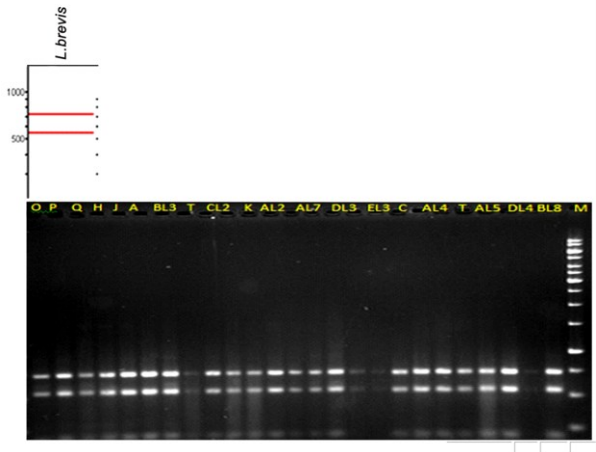

Fig. 4: Electrophoretic profile of 16S rDNA amplicons from *Lactobacillus rhamnosus* strains.

4. DISCUSSION

Lactic acid bacteria (LAB) associated with fish have attracted increasing attention as potential sources of antimicrobial agents and probiotic candidates for sustainable aquaculture. Their antagonistic activities may involve

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producing organic acids, bacteriocins, hydrogen peroxide, and other inhibitory metabolites, as well as competing with pathogenic microorganisms for nutrients and adhesion sites. However, these activities are highly strain-dependent and may vary by target microorganism, culture conditions, and experimental methods. Balcázar et al. (2008) investigated the probiotic-associated properties of fish-derived LAB, particularly their ability to inhibit pathogen adhesion to intestinal mucus and produce antimicrobial substances. Their findings showed that selected LAB strains could reduce adhesion of certain fish pathogens and inhibit bacterial growth, although the magnitude of these effects varied among isolates and indicator organisms. These observations emphasize the importance of evaluating antimicrobial characteristics at the strain level rather than assuming that all LAB exhibit comparable inhibitory properties. More recent investigations have also discussed the potential relevance of fish-associated microorganisms to aquaculture health management.

Govindaraj et al. (2021) investigated LAB recovered from the intestinal tract of freshwater fish to assess their potential applications as probiotics in aquaculture. Their findings highlighted the importance of screening fish-associated bacterial isolates for functional characteristics, particularly antagonistic activity against pathogenic microorganisms. These investigations support exploring indigenous LAB as potential components of biological disease-control strategies. Nevertheless, laboratory inhibition of bacterial pathogens does not necessarily establish their effectiveness as alternatives to conventional antimicrobial treatments. Similarly, Mazlumi et al. (2022) investigated the functional and probiotic-associated characteristics of LAB isolated from marine fish. They used phenotypic characterization and computational clustering to distinguish isolates based on functional properties. These findings demonstrate the value of combining complementary screening methods to identify promising bacterial candidates. Related investigations have further highlighted the diversity and potential functional significance of fish-associated microorganisms (Maratova et al., 2023).

Coulibaly et al. (2023) evaluated LAB isolated from the intestinal tract of Nile tilapia (*Oreochromis niloticus*) in Côte d'Ivoire. The recovered isolates, including representatives of the genera *Pediococcus* and *Lactobacillus*, exhibited antagonistic activity against several bacterial pathogens, including *Escherichia coli* and *Klebsiella pneumoniae*. In addition to antimicrobial activity, the study assessed several probiotic-associated characteristics, including tolerance to acidic conditions and bile salts, cell-surface properties, antioxidant activity, and antibiotic susceptibility. These findings illustrate the importance of integrating antimicrobial screening with broader functional and safety assessments when selecting potential probiotic strains. Likewise, Olorunshola et al. (2025) investigated the antibacterial activity of LAB isolated from the intestinal tract of African catfish (*Clarias gariepinus*) against multidrug-resistant bacterial pathogens. Their investigation highlighted the potential of fish-associated LAB as sources of inhibitory microorganisms and emphasized the relevance of exploring indigenous microbial communities in aquaculture systems. Additional studies have addressed the potential applications of beneficial microorganisms in aquatic animal health and microbial disease management (Paritova et al., 2024, 2025).

These findings align with the present study, in which 21 LAB isolates recovered from minced freshwater fish showed inhibitory activity against four intestinal indicator organisms. The observed inhibition zones ranged from 16 to 22 mm, with an overall descriptive mean of 18.93 mm, indicating variation in antibacterial activity among the selected isolates. Among the four identified LAB groups, isolates assigned to *Lactiplantibacillus plantarum* exhibited the highest overall mean inhibition zone (19.68 mm), followed by *L. brevis* (19.00 mm), *L. rhamnosus* (18.83 mm), and *L. casei* (18.04 mm). At the individual-isolate level, KAA23 showed the greatest mean inhibitory activity (21.0 mm), followed by KAA31 and KAA86 (20.5 mm each). These descriptive differences suggest that antibacterial activity varies among isolates, even within closely related bacterial groups. However, the absence of inferential statistical comparisons limits conclusions regarding significant differences in inhibitory effectiveness. Furthermore, variation in indicator-organism susceptibility suggests that observed antagonistic effects may depend on interactions between individual LAB isolates and their bacterial targets.

Collectively, the available evidence supports the potential of fish-associated LAB as sources of bacterial isolates with antimicrobial properties. However, differences in isolate identity, target microorganisms, growth media, antimicrobial assay procedures, and incubation conditions complicate direct comparisons of inhibition-zone measurements across studies. Moreover, inhibition zones obtained under laboratory conditions do not identify the specific antimicrobial mechanisms involved or establish probiotic efficacy in aquatic animals. Consequently, the antibacterial activity demonstrated by the selected isolates should be considered preliminary evidence supporting further investigation rather than confirmation of their suitability for aquaculture applications. Future studies should prioritize characterizing inhibitory metabolites, evaluating antibiotic resistance and safety profiles, assessing gastrointestinal survival and environmental stability, and conducting controlled *in vivo* experiments to determine whether the most promising isolates can provide measurable benefits to fish health and disease resistance.

5. CONCLUSION

This study recovered 96 presumptive lactic acid bacteria (LAB) isolates from 21 minced freshwater fish samples

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collected during 2025–2026, of which 21 exhibited comparatively strong *in vitro* antibacterial activity against four indicator pathogens. Based on 16S rRNA gene sequence analysis, we assigned the selected isolates to four bacterial species: *Levilactobacillus brevis*, *L. plantarum*, *L. casei*, and *L. rhamnosus*. Across 84 isolate–pathogen combinations, inhibition zones ranged from 16 to 22 mm, with an overall mean of 18.93 mm. The highest mean inhibition zone was recorded against *Yersinia enterocolitica* (19.48 mm), followed by *Klebsiella pneumoniae* (19.33 mm), *Shigella flexneri* (19.29 mm), and *Escherichia coli* (17.62 mm). Among the identified bacterial groups, *L. plantarum* exhibited the highest overall mean inhibition zone (19.68 mm) and relatively consistent antibacterial activity across the tested pathogens. These findings show that minced freshwater fish is a potential source of phenotypically diverse LAB with measurable antagonistic activity. In particular, *L. plantarum* isolates KAA23, KAA31, KAA44, and KAA86 warrant further investigation as potential probiotic candidates. However, incorporating them into aquaculture feeds requires comprehensive safety assessment, antimicrobial resistance profiling, evaluation of gastrointestinal tolerance and stability, and controlled *in vivo* studies to establish their efficacy in improving fish health and resistance to bacterial infections.

Declarations

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Data Availability: All the data are available in the article.

Ethics Statement: The ethical approved was granted by the Institutional Review Board of Kh. Dosmukhamedov Atyrau University, Republic of Kazakhstan (Approval No. 15-19/2509; dated: 21 October 2026). The study involved the use of minced muscle tissue samples from freshwater fish obtained from Amangeldy "Atyraubalyk" LLP, Republic of Kazakhstan. No experimental procedures or interventions involving live animals or human participants were performed. All procedures involving fish were conducted in accordance with standard aquaculture practices and applicable national legislation governing the use of aquatic biological resources and animal welfare in scientific research.

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