Antimicrobial and Probiotic Properties of Lactic Acid Bacteria Isolated from *Pha-ork*, a Traditional Fermented *Pangasius* Fish of Cambodia

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ABSTRACT

Cambodia's traditional fermented *Pangasius* fish paste, locally known as *Pha-ork*, is a central component of aquatic food systems. Fermentation preserves fish, enhances flavor and texture, and introduces beneficial microorganisms with high potential for food safety and health applications. This study isolated 15 lactic acid bacteria (LAB) strains from 153 samples of the fermented fish product across Kampong Thom, Siem Reap and Phnom Penh markets. The isolates comprised 5 species: *Lactobacillus pentosus* (n = 4), *Enterococcus faecalis* (n = 5), *Enterococcus hirae* (n = 2), *Lactococcus garvieae* (n = 2), and *Pediococcus pentosaceus* (n = 2). Most isolates exhibited moderate antibacterial activity against pathogenic *Aeromonas* and *Vibrio* species isolated from fresh *Pangasius* fish. Among the LAB isolates, *Pediococcus pentosaceus* (strain code 204020-10) demonstrated significant candidate probiotic potential, showing resilience to low pH (2.3), 0.6% bile salts, and 0.5g phenol. These findings highlight the prospective application of indigenous LAB strains as natural antimicrobials and probiotics, offering a sustainable approach to enhance food security and reduce antibiotic dependence.

Keyword: Aquatic Food System, Fermentation, Natural Antimicrobial, Food Preservation, Food Safety

INTRODUCTION

Cambodia's traditional, fermented fish is a pillar of national food, nutrition, and livelihood security. It serves as a secondary staple food after rice, providing key dietary protein and essential nutrients while supporting rural processing economies (Shern, 2023). Priced at 9-16 times higher than fresh fish, fermented products absorb surplus seasonal harvests and reduces post-harvest food loss. As an integral component of Khmer (Cambodian) cuisine and aquatic food systems, fermented fish plays a strategic role in national nutrition plans, including the National Strategy for Food Security and Nutrition (NSFSN 2024-2028).

Fish fermentation delivers both economic and nutritional values by improving organoleptic traits and introducing bioactive metabolites and beneficial microorganisms (Chrun et al., 2020; Rastogi et al., 2022; Khongthaw et al., 2026). Fermented foods often host Lactic Acid Bacteria (LAB), many of which hold Generally Recognized as Safe (GRAS) status and present functional probiotic characteristics (Nuraida, 2015). Probiotics are live microorganisms that confer health benefits to the host when administered in adequate

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amounts (Ayivi et al., 2020). LAB improve gastrointestinal wellness, modulate immunity, enhance nutrient digestibility, and suppress pathogenic microbes (LeGrand et al., 2020).

Despite these benefits, traditional fish fermentation in Cambodia relies largely on spontaneous microflora, which can yield inconsistent product quality, spoilage, or opportunistic contamination. Furthermore, certain bacterial genera associated with fermentation can occasionally harbor opportunistic pathogenicity. Characterizing indigenous LAB ensures controlled preservation by identifying strains that secrete organic acids, hydrogen peroxide, and bacteriocins. These metabolites rapidly decrease pH, inhibiting aquatic pathogens such as *Vibrio* spp. and *Aeromonas* spp. (Duk et al., 2024). Furthermore, evaluating acid, bile, and phenol tolerance identifies candidates capable of surviving gastrointestinal transit (Lars et al., 2024). This study aimed to isolate, identify, and evaluate the antimicrobial and probiotic properties of LAB strains isolated from Cambodian *Pha-ork*.

MATERIALS AND METHODS

Sample collection

A total of 153 samples of traditional fermented *Pangasius* fish (*Pha-ork*) (Figure 1) were collected from nine retail markets across three major target locations in Cambodia, comprising Kampong Thom Province (Kampong Thom, Stoung, and Romlong Markets), Siem Reap Province (Sot Nikum, Prasat Bakong, and Leu Thom Thmey Markets), and Phnom Penh (Prek Pnov, Chamkar Doung, and Deum Kor Markets). Seventeen samples were acquired per market from independent vendors, placed in sterile containers, and transported under cold chain conditions to the Microbiology Laboratory at the Faculty of Agro-Industry, Royal University of Agriculture, Phnom Penh.



Figure 1. Khmer traditional fermented *Pangasius* fish *(Pha-ork)*

As an indication of the presence and activity of LAB, pH of the samples was measured using a digital pH meter (Model: PH1100 Brand: HORIBA-Japan). Samples from Kampong Thom markets had a pH of 3.4-4.9; Siem Reap markets, 3.8-5.1; and Phnom Penh markets, 3.7-4.7.

Isolation and identification of LAB

LAB were isolated and characterized following the framework described by Haitham et al., (2017) (Figure 2). Homogenates were prepared by diluting 10 g of fish tissue into 90 mL of buffered peptone water (BPW) with 0.9% NaCl saline. Ten-fold serial dilutions (10^{-1} to 10^{-5}) were performed. Dilutions were inoculated into de Man, Rogosa, and Sharpe (MRS) broth (Merck-Darmstadt, Germany) and spread-plated on MRS agar, followed by anaerobic incubation at 30°C for 24-48 h.

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Isolates were screened using Gram staining and catalase reactivity to confirm Gram-positive, catalase-negative traits. Selected colonies underwent ethanol/formic acid extraction for identification via Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) using the Bruker BioTyper database (Neelja, et al., 2015). Phenotypic confirmation was conducted using API 50 CH and API 50 CHL test strips (bioMérieux) according to carbohydrate fermentation profiles.

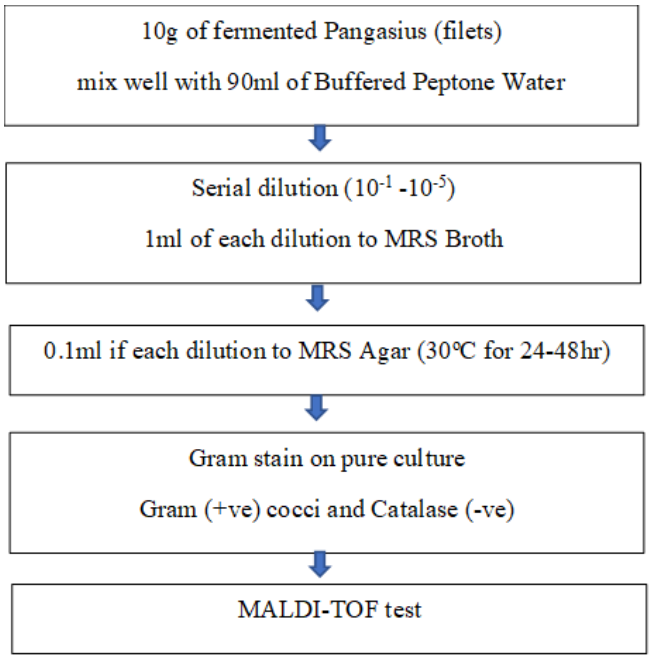


Figure 2. Isolation and identification of LAB (Haitham et al., 2017).

Antibacterial activity of LAB isolates

The antibacterial potential of the lactic acid bacteria (LAB) isolates was evaluated against indicator *Aeromonas* and *Vibrio* strains previously isolated from fresh *Pangasius* fish markets (Duk et al., 2024) using the agar well diffusion method described by Pratiksha (2023) (Figures 3). Briefly, indicator bacterial suspensions adjusted to 10^5 CFU/mL were seeded into Mueller-Hinton Agar (MHA) plates, and 6 mm diameter wells were created using a sterile metal cylinder. Aliquots (100 µL) of cell-free supernatant (CFS) from target LAB isolates were loaded into each well, allowed to pre-diffuse at 4ºC for 1 h, and subsequently incubated at 37ºC for 24 h. Diameters of the resulting inhibition zones were measured in millimeters and categorized based on activity level: weak (< 5 mm), moderate (5-10 mm), strong (11-20 mm), or very strong (> 20 mm) (Sanam et al., 2022).

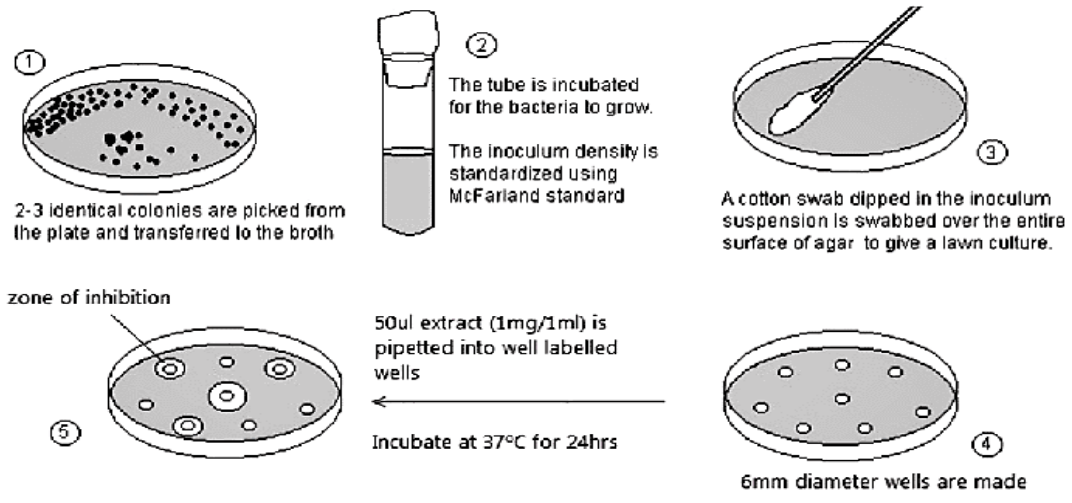


Figure 3. Antimicrobial screening by the agar well diffusion method (Pratiksha, 2023).

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In Vitro Probiotic Assessment

The *in vitro* probiotic potential of the LAB isolates was evaluated through assessments of acid, bile salt, and phenol tolerance. Acid tolerance was evaluated according to Charteris et al., (1998) by exposing isolates to MRS broth adjusted to pH 2.3 alongside a neutral pH 7.0 control, with survival determined by recording optical density (OD_{620nm}) before and after incubation. Bile salt tolerance was assessed following Matijasic and Rogelj (2000) by inoculating strains into MRS broth containing 0.1%, 0.3%, and 0.6% (w/v) oxgall bile salts and determining viability through spectrophotometric growth changes at 620 nm. Finally, phenol tolerance was performed per Aswathy et al. (2008) by incubating cultures in MRS broth supplemented with 0.2 g/L and 0.5 g/L phenol and measuring absorbance at 620 nm.

Statistical Analysis

Descriptive statistical metrics, specifically mean values, were computed to summarize the data, and boxplot visualizations were generated using IBM SPSS Statistics (Version 25) to illustrate differences in inhibition zone distributions across provinces (Field, 2018).

RESULTS AND DISCUSSION

Identification of LAB Isolates

Out of the 153 fermented fish samples analyzed, 15 yielded confirmed lactic acid bacteria (LAB) strains, representing an overall isolation rate of 9.80%. Subsequent MALDI-TOF MS analysis identified five distinct species across these 15 isolates: *Lactobacillus pentosus* (n = 4, 2.61%), *Enterococcus faecalis* (n = 5, 3.26%), *Enterococcus hirae* (n = 2, 1.30%), *Lactococcus garvieae* (n = 2, 1.30%), and *Pediococcus pentosaceus (*n = 2, 1.30%) (Table 1).

Table 1. LAB isolated from Cambodian *Pha-ork* identified via MALDI-TOF MS

No.	Sample Code	Lab Species	Location Source
1	204018-2	*Lactobacillus pentosus*	KPT, Kampong Thom
2	204018-3	*L. pentosus*	KPT, Kampong Thom
3	204018-4	*L. pentosus*	KPT, Kampong Thom
4	204018-5	*Enterococcus hirae*	ST, Kampong Thom
5	204018-6	*E. hirae*	ST, Kampong Thom
6	204018-7	*Enterococcus faecalis*	RL, Kampong Thom
7	204018-8	*L. pentosus*	RL, Kampong Thom
8	204011-9	*E. faecalis*	LTT, Siem Reap
9	204020-1	*Lactococcus garvieae*	PP, Phnom Penh
10	204020-2	*L. garvieae*	PP, Phnom Penh
11	204020-5	*E. faecalis*	CD, Phnom Penh
12	204020-7	*E. faecalis*	CD, Phnom Penh

13	204020-8	*E. faecalis*	CD, Phnom Penh
14	204020-9	*Pediococcus pentosaceus*	DK, Phnom Penh
15	204020-10	*P. pentosaceus*	DK, Phnom Penh

KPT - Kampong Thom, ST - Stoung, RL - Romlong, LTT - Leu Thom Thmey, PP - Prek Pnov, CD - Chamkar Doung, DK - Deum Kor markets.

The traditional fermentation of fish products typically relies on spontaneous anaerobic processes driven by complex successional dynamics of indigenous microflora, particularly lactic acid bacteria (LAB). Research demonstrates that LAB are an integral part of the natural gastrointestinal and tissue microflora of fresh fish, encompassing genera such as *Streptococcus*, *Leuconostoc*, *Pediococcus,* and *Lactobacillus* (Nair and Surendran, 2005; Quach et al., 2023; 2024). During the initial stages of fermentation, acid-producing LAB proliferate rapidly, consuming available carbohydrate substrates and accumulating lactic acid and other organic acids. This metabolic activity significantly lowers pH, creating an acidic environment that suppresses competing spoilage and pathogenic microorganisms while allowing acid tolerant LAB to reach maximum cell density (Nair and Surendran, 2005).

The structural composition and dominant species of LAB within aquatic foods vary considerably based on geographic locations, product types, and processing conditions, aligning with the regional shifts observed in this study as well as across traditional fermented fish products throughout Southeast Asia. For example, in Indonesian *Bekasam* (a fermented freshwater product including *Pangasius* species), LAB comprise the vast majority of isolated microflora encompassing species of *Lactobacillus*, *Aerococcus*, *Streptococcus*, *Pediococcus*, *Staphylococcus*, *Erysipelothrix*, and *Gemella* (Desniar et al., 2013). In *Rusip*, another Indonesian fermented product, a distinct microbial succession is observed, with *Streptococcus* dominating early fermentation, *Lactococcus* prevailing during mid-fermentation, and *Leuconostoc* becoming predominant at the terminal stage (Yuliana et al., 2018). Similarly, Thai *Plaa-som* features a diverse consortium including *Lactococcus garvieae*, *Weissella cibaria*, *Pediococcus pentosaceus*, *Streptococcus bovis*, *L. plantarum*, and *L. fermentum* (Kopermsub and Yunchalard, 2010), whereas Malaysian *Pekasam* is predominantly driven by *L. plantarum* and *L. pentosus* (Muryany et al., 2017). The isolation of *L. pentosus*, *E. faecalis*, *E. hirae*, *L. garvieae*, and *P. pentosaceus* in this study aligns with these regional microflora profiles, illustrating how localized environmental factors shape specific microbial colonization (Kopermsub and Yunchalard, 2010; Desniar et al., 2013; Muryany et al., 2017; Yuliana et al., 2018; Setiarto et al., 2024).

Antimicrobial activity of LAB isolates

When testing the 15 LAB isolates across five species (*L. pentosus*, *E. faecalis*, *E. hirae*, *L. garvieae*, and *P. pentosaceus*) against pathogenic *Vibrio* and *Aeromonas* species isolated from fresh market *Pangasius* fish (Duk et al., 2024), most LAB isolates exhibited moderate antibacterial activity (5-10 mm zone of inhibition), as supported by boxplot distributions showing consistent inhibition between 7 and 10 mm (Figure 4). However, specific isolates showed variable efficacy: *L. pentosus* (204018-8) was inactive against *V. parahaemolyticus* and all four *Aeromonas* species; *E. faecalis* (204011-9) and *L. garvieae* (204020-1, 204020-2) were active solely against *V. cholerae*; *E. faecalis* isolates 204020-7 and 204020-8 lacked activity against *V. cholerae* while exhibiting weak inhibition (< 5 mm) against *A. hydrophila*; and *P. pentosaceus* (204020-10) showed weak activity against *A. hydrophila* alongside an absence of activity against *V. parahaemolyticus*.

Table 2. Antibacterial activity (inhibition zone, mm) of LAB isolates against *Aeromonas* and *Vibrio* species isolated from Kampong Thom markets

Sample Code	LAB Isolate	Average Diameter of Inhibition Zone (mm)						
		V. cholerae	*V. fluvialis*	*V. parahaemolyticus*	*A. hydrophila/ caviae*	*A. caviae*	*A. sobria*	*A. hydrophila*
204018-2	*Lactobacillus pentosus*	9.5	9.0	9.0	8.0	8.0	8.0	8.0
204018-3	*L. pentosus*	9.5	8.0	8.5	9.0	8.0	8.0	8.0

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204018-4	*L. pentosus*	9.0	8.0	8.0	8.0	8.0	8.0	8.0
204018-8	*L. pentosus*	8.0	7.0	weak	weak	weak	weak	weak
204018-7	*Enterococcus faecalis*	8.0	8.0	8.0	8.0	8.0	8.0	7.0
204011-9	*E. faecalis*	7.0	weak	weak	weak	weak	weak	weak
204020-5	*E. faecalis*	8.0	8.0	8.0	8.0	8.0	8.0	7.0
204020-7	*E. faecalis*	weak	7.0	7.0	7.0	7.0	7.0	3.5
204020-8	*E. faecalis*	weak	7.0	7.0	7.0	7.0	7.0	3.5
204018-5	*Enterococcus hirae*	9.5	7.0	7.0	8.0	8.0	8.0	8.0
204018-6	*E. hirae*	8.5	8.5	8.5	7.5	7.5	7.0	7.0
204020-1	*Lactococcus garvieae*	7.5	weak	weak	weak	weak	weak	weak
204020-2	*L. garvieae*	7.0	weak	weak	weak	weak	weak	weak
204020-9	*Pediococcus pentosaceus*	7.0	7.0	7.0	7.0	7.0	8.0	7.0
204020-10	*P. pentosaceus*	7.0	7.0	-	7.0	7.0	7.0	weak

Note: Inhibition zone interpretation: weak (<5 mm), moderate (5-10 mm) , strong (11-20 mm), very strong (>20 mm). (-) indicates no detectable zone of inhibition.

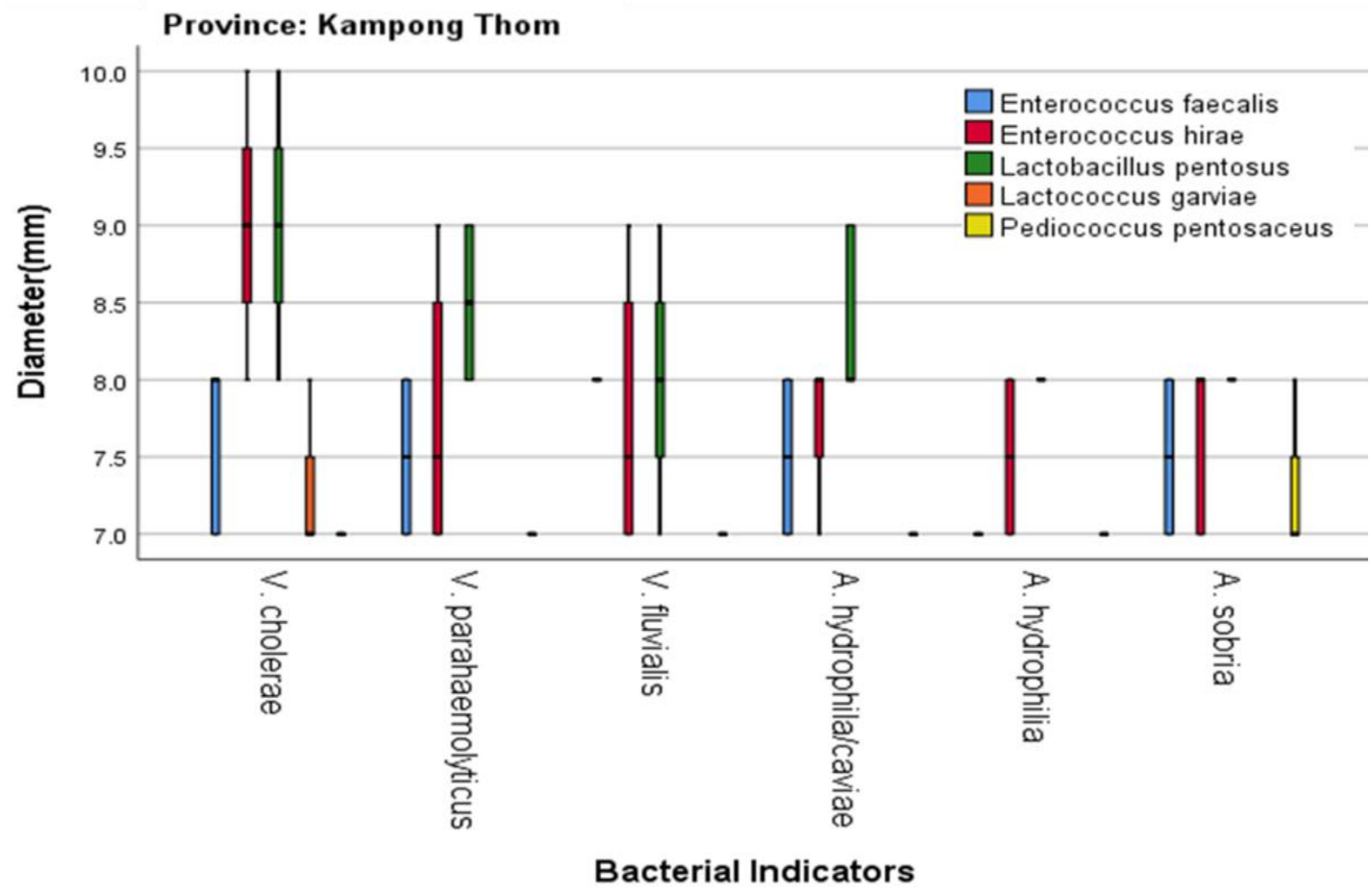


Figure 4. Boxplot of antibacterial activity of 5 LAB species against *Aeromonas* and *Vibrio* species isolated from Kampong Thom markets.

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The antagonistic activities of the 15 LAB isolates against *Vibrio* and *Aeromonas* species isolated from Siem Reap markets are summarized in Table 3. Overall, the majority of LAB isolates exhibited moderate antibacterial activity (5-10 mm zone of inhibition). However, notable strong inhibitory responses (11-20mm) were identified in specific strains: *Lactobacillus pentosus* isolate 204018-2 (from KPT Market) exerted strong inhibition against both *Aeromonas hydrophila*/*caviae* (14.5 mm) and *A. hydrophila* (17.0 mm). Similarly, strong antibacterial activity against *Vibrio alginolyticus* was recorded for *Enterococcus faecalis* isolate 204018-7 (15.0 mm, from RL market) and *Lactococcus garvieae* isolate 204020-2 (16.0 mm, from PP market. Conversely, *L. garvieae* isolate 204020-2 exhibited complete inactivity against *V. cholerae* and *V. parahaemolyticus.* Boxplot distribution analysis (Figure 5) clearly illustrates the pronounced inhibitory capacity (12-17 mm) of *L. pentosus* and *L. garvieae*, whereas the activity profile for *E. faecalis* demonstrated lower variance. The remaining LAB species yielded inhibition zones spanning 7 mm (moderate) to 10.5 mm (borderline strong).

Table 3. Antibacterial activity of LAB isolates against *Aeromonas* and *Vibrio* species isolated from Siem Reap markets

Sample Code	LAB Isolate	Average diameter of inhibition zone (mm)				
		V. cholerae	*V. parahaemolyticus*	*V. aglinolyticus*	*A. hydrophila/caviae*	*A. hydrophila*
204018-2	*Lactobacillus pentosus*	9.0	9.0	9.0	14.5	17.0
204018-3	*L. pentosus*	9.0	7.5	9.0	10.0	9.5
204018-4	*L. pentosus*	9.5	8.0	8.0	8.0	8.0
204018-8	*L. pentosus*	8.0	7.0	7.0	7.0	7.0
204018-7	*Enterococcus faecalis*	8.0	8.0	15.0	8.0	8.0
204011-9	*E. faecalis*	8.0	8.0	8.0	8.0	8.0
204020-5	*E. faecalis*	7.0	7.5	7.0	7.5	7.0
204020-7	*E. faecalis*	8.0	7.5	7.5	8.0	7.5
204020-8	*E. faecalis*	8.5	8.0	7.0	9.5	9.0
204018-5	*Enterococcus hirae*	9.5	8.0	8.0	8.0	8.0
204018-6	*E. hirae*	8.5	8.5	8.5	7.5	7.0
204020-1	*Lactococcus garvieae*	9.0	8.5	8.5	9.0	8.5
204020-2	*L. garvieae*	weak	weak	16.0	10.5	10.5
204020-9	*Pediococcus pentosaceus*	10.5	9.0	8.5	8.0	8.5
204020-10	*P. pentosaceus*	10.5	9.5	10.5	9.0	9.5

Note: Inhibition zone interpretation: weak (<5 mm), moderate (5-10 mm), strong (11-20 mm), very strong (>20 mm).

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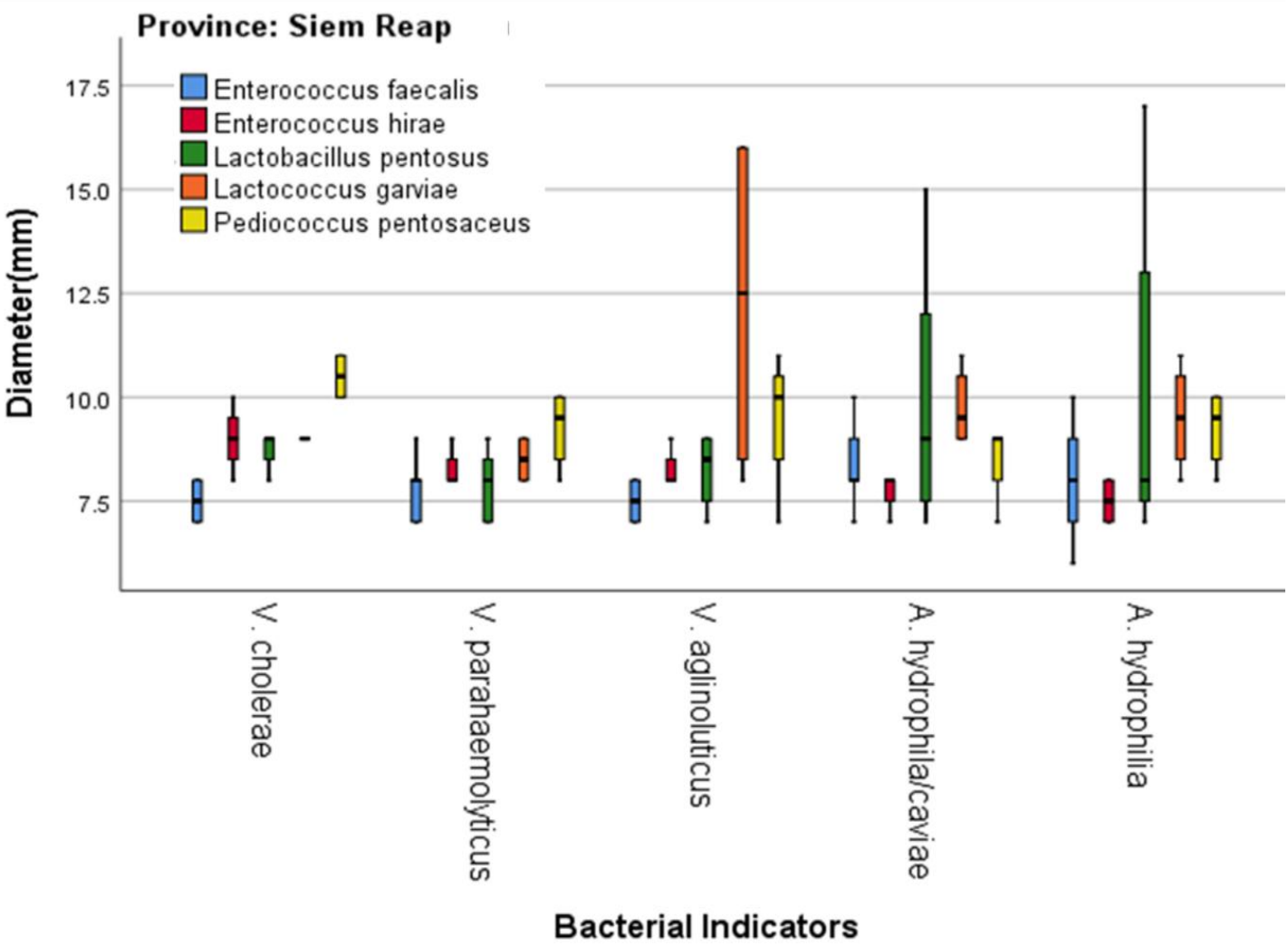


Figure 5. Boxplot of antibacterial activity of 5 LAB species against *Aeromonas* and *Vibrio* species isolated from Siem Reap markets.

Testing against the pathogenic strains isolated from Phnom Penh markets revealed predominantly moderate antibacterial profiles across all LAB isolates (Table 4). The singular exception to this overall susceptibility was *E. faecalis* isolate 204018-7, which failed to inhibit any of the tested indicator pathogens. However, *Pediococcus pentosaceus* isolate 204020-10 displayed distinct strong antibacterial activity again all three indicator strains: *V. cholerae* (11.5 mm), *V. parahaemolyticus* (11.0 mm), and *A. hydrophila*/*caviae* (11.0 mm). This strain-specific efficacy was highlighted in the comparative boxplot distribution across the five LAB species (Figure 6).

Table 4. Antibacterial activity of LAB isolates against *Aeromonas* and *Vibrio* species isolated from Phnom Penh markets

Sample Code	LAB Isolate	Average Diameter of Zone of Inhibition (mm)		
		V. cholerae	*V. parahaemolyticus*	*A. hydrophila/caviae*
204018-2	*Lactobacillus pentosus*	8.0	8.5	8.5
204018-3	*L. pentosus*	8.0	8.5	8.0
204018-4	*L. pentosus*	7.5	8.0	8.0
204018-8	*L. pentosus*	8.0	7.0	7.0
204018-7	*Enterococcus faecalis*	weak	weak	weak
204011-9	*E. faecalis*	9.0	8.0	9.0
204020-5	*E. faecalis*	9.5	10.5	10.0
204020-7	*E. faecalis*	8.0	8.0	8.5

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204020-8	*E. faecalis*	9.0	9.5	10.0
204018-5	*Enterococcus hirae*	9.5	8.5	9.0
204018-6	*E. hirae*	8.5	10.0	8.0
204020-1	*Lactococcus garvieae*	9.0	9.0	8.0
204020-2	*L. garvieae*	8.5	9.0	8.0
204020-9	*Pediococcus pentosaceus*	8.0	9.5	9.0
204020-10	*P. pentosaceus*	11.5	11.0	11.0

Note: Inhibition zone interpretation: weak (<5 mm), moderate (5-10 mm), strong (11-20 mm), very strong (>20 mm).

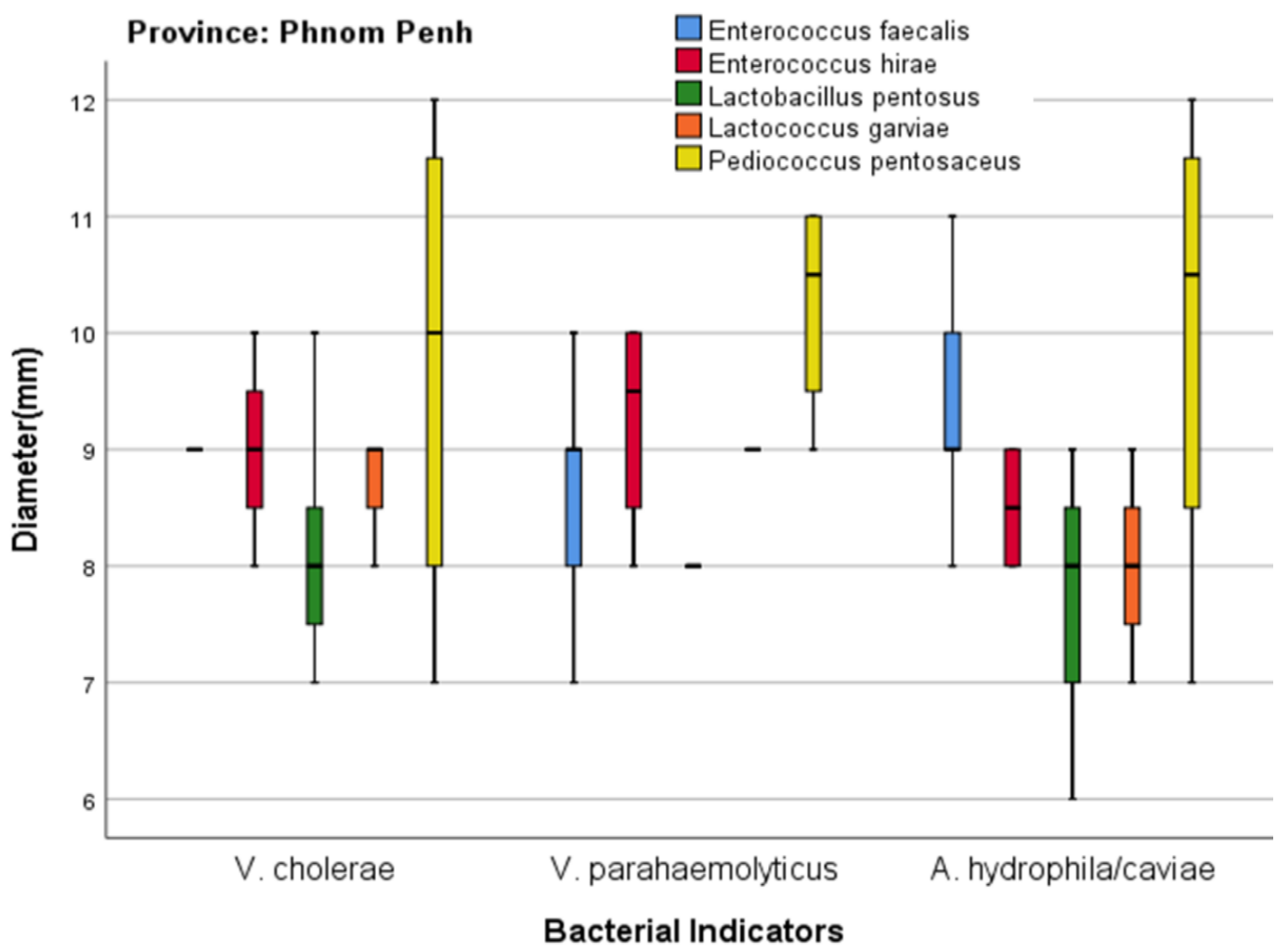


Figure 6. Boxplot of antibacterial activity of 5 LAB species against *Aeromonas* and *Vibrio* species isolated from Phnom Penh markets.

The broad-spectrum, although variable, antibacterial activity exhibited by these fermented fish LAB isolates against pathogenic *Aeromonas* and *Vibrio* species aligns with established literature emphasizing the biopreservative functionality of LAB (Gao et al., 2019; Ibrahim et al., 2021). The antagonistic mechanisms of LAB are multifaceted, primarily driven by organic acid production (such as lactic and acetic acids) that causes rapid medium acidification, hydrogen peroxide generation, carbon dioxide accumulation, and synthesis of antimicrobial peptides including bacteriocins and defensins. Furthermore, LAB suppress enteric pathogens via competitive exclusion, surface adhesion blockade, and maintenance of mucosal epithelial barrier integrity.

Intraspecific and strain-dependent variations in antimicrobial performance, where specific isolates within the same species demonstrated contrasting strong versus negligible activity, can be attributed to differential expression of antimicrobial gene clusters, localized micro-environmental adaptations, and target pathogen heterogeneity (Pessoa et al., 2019; Loo et al., 2022). Pathogenic *Aeromonas* and *Vibrio* strains possess diverse

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virulence profiles, membrane lipid configurations, toxin expression mechanisms that differentially dictate susceptibility to metabolic bio-inhibitors.

Probiotic Properties of LAB Isolates

Evaluation of survival under extreme acidic stress (pH 2.3) revealed that out of 15 candidate strains, only *P. pentosaceus* isolate 204020-10 maintained viability and proliferation. This was confirmed by an increase in optical density (OD_{620nm}) post-incubation (1.198) relative to baseline controls (Table 5). The remaining 14 LAB isolates failed to survive, exhibiting optical density reductions or zero values. At neutral pH (pH 7.0), positive cellular growth was confirmed for all strains.

Table 5. Spectrophometric growth assessment (OD_{620nm}) of LAB isolates under acidic (pH 2.3) and neutral (pH 7.0) conditions

No.	Sample Code	Supernatant	Baseline OD_{620nm}	Post-Incubation OD_{620nm}		Viability Status	
				pH 2.3	pH 7	pH 2.3	pH 7
1	204018-2	*Lactobacillus pentosus*	0.899	0.000	1.990	-	+
2	204018-3	*L. pentosus*	0.944	0.132	1.845	-	+
3	204018-4	*L. pentosus*	0.824	0.096	2.617	-	+
4	204018-8	*L. pentosus*	0.967	0.000	1.221	-	+
5	204018-5	*Enterococcus hirae*	0.992	0.127	2.444	-	+
6	204018-6	*E. hirae*	0.912	0.000	1.991	-	+
7	204018-7	*Enterococcus faecalis*	0.972	0.000	1.889	-	+
8	204011-9	*E. faecalis*	0.899	0.000	1.138	-	+
9	204020-1	*Lactococcus garveiae*	0.900	0.000	1.232	-	+
10	204020-2	*L. garvieae*	0.902	0.000	1.199	-	+
11	204020-5	*E. faecalis*	0.899	0.000	1.110	-	+
12	204020-7	*E. faecalis*	0.879	0.000	1.200	-	+
13	204020-8	*E. faecalis*	0.992	0.000	1.181	-	+
14	204020-9	*Pediococcus pentosaceus*	0.952	0.000	1.160	-	+
15	204020-10	*P. pentosaceus*	0.886	1.198	2.090	+	+

Note: Growth status: (+) growth/viable ($OD_{post} > OD_{pre}$), (-) non-viable ($OD_{post} \leq OD_{pre}$)

With the exception of *Enterococcus hirae* isolate 204018-6, all evaluated LAB strains demonstrated tolerance to 0.1% w/v oxgall bile salts (Table 6). Increasing the physiological stress to 0.3% w/v reduced overall tolerance, with only four strains sustaining growth: *E. faecalis* (204011-9 and 204020-5) and *P. pentosaceus* (204020-9 and 204020-10). At the stringent threshold of 0.6% w/v bile concentration, survival was strictly limited to the two *P. pentosaceus* isolates (204020-9 and 204020-10), both maintaining absorbance levels above baseline (OD_{620nm} = 1.221 and 1.199, respectively).

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Table 6. Survial and growth (OD_{620nm}) of LAB isolates exposed to gradient concentrations (0.1%, 0.3%, and 0.6% w/v) of oxgall bile salts

No.	Sample Code	Supernatant	Baseline OD_{620nm}	Post-Incubation OD_{620nm}		
				0.1% Oxgall	0.3% Oxgall	0.6% Oxgall
1	204018-2	*Lactobacillus pentosus*	0.886	1.318	0.811	0.664
2	204018-3	*L. pentosus*	0.944	1.455	0.712	0.551
3	204018-4	*L. pentosus*	0.824	1.551	0.772	0.442
4	204018-8	*L. pentosus*	0.992	1.122	0.789	0.456
5	204018-5	*Enterococcus hirae*	0.991	0.887	0.881	0.511
6	204018-6	*E. hirae*	0.999	1.221	0.812	0.565
7	204018-7	*Enterococcus faecalis*	0.899	1.323	0.891	0.575
8	204011-9	*E. faecalis*	0.889	1.112	0.981	0.757
9	204020-1	*Lactococcus garvieae*	0.992	1.331	0.891	0.551
10	204020-2	*L. garvieae*	0.991	0.999	0.981	0.887
11	204020-5	*E. faecalis*	0.899	0.992	0.901	0.665
12	204020-7	*E. faecalis*	0.871	0.877	0.811	0.771
13	204020-8	*E. faecalis*	0.881	0.999	0.881	0.773
14	204020-9	*Pediococcus pentosaceus*	0.897	1.331	1.122	1.221
15	204020-10	*P. pentosaceus*	0.879	1.318	1.208	1.199

Resistance to toxic phenolic compounds varied significantly among isolates (Table 7). *Pediococcus pentosaceus* isolate 204020-10 and three *L. pentosus* isolates (204018-2, 204018-3, and 204018-4) demonstrated growth at both 0.2 g/L and 0.5 g/L phenol concentrations. *Lactococcus garvieae* isolate 204020-2 exhibited moderate resistance, surviving at 0.2 g/L phenol (OD_{620nm} = 0.980) but succumbing at 0.5 g/L. All remaining isolates were susceptible to phenolic stress.

Table 7. Phenol tolerance screening (OD_{620nm}) for LAB isolates at 0.2 g/L and 0.5 g/L concentration thresholds

No.	Sample Code	Supernatant	Baseline (OD_{620nm})	Post-Incubation (OD_{620nm})	
				0.2 g/L Phenol	0.5 g/L Phenol
1	204018-2	*Lactobacillus pentosus*	0.119	0.787	0.222
2	204018-3	*L. pentosus*	0.111	0.481	0.229
3	204018-4	*L. pentosus*	0.214	2.010	0.245
4	204018-8	*L. pentosus*	0.114	2.213	0.365

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5	204018-5	*Enterococcus hirae*	0.121	0.100	0.067
6	204018-6	*E. hirae*	0.133	0.100	0.058
7	204018-7	*Enterococcus faecalis*	0.131	0.099	0.077
8	204011-9	*E. faecalis*	0.129	0.089	0.069
9	204020-1	*Lactococcus garvieae*	0.122	0.099	0.000
10	204020-2	*L. garvieae*	0.123	0.980	0.000
11	204020-5	*E. faecalis*	0.118	0.079	0.000
12	204020-7	*E. faecalis*	0.115	0.099	0.000
13	204020-8	*E. faecalis*	0.101	0.077	0.050
14	204020-9	*Pediococcus pentosaceus*	0.113	0.076	0.000
15	204020-10	*P. pentosaceus*	0.114	2.213	0.365

To serve as functional probiotics, candidate bacterial strains must withstand the physiological chemical barriers of the human gastrointestinal tract (Ahire et al., 2021). Gastric acidity in the range of pH 1.5 to 3.0 represents the initial biological barrier; hence, survival at pH 2.3 is a fundamental selection criterion (Nath et al., 2021). Subsequently, strains must survive physiological concentrations of bile salts (0.3% w/v threshold) in the duodenum, which function as biological detergents that disrupt bacterial cell membranes (Nath et al., 2021). Finally, tolerance to phenolic compounds, which are toxic metabolites produced during deamination of dietary proteins by indigenous gut microflora, is essential for sustained microbial colonization (Huligere et al., 2023).

While several isolates in this study displayed isolated resistance to bile salts or phenol stress, partial physiological resilience is insufficient to confirm probiotic status (Divyashree et al., 2024; Guney et al., 2025). Robust candidate strains must comprehensively fulfill all survival criteria alongside validated intestinal cell adhesion, bio-safety confirmation including the lack of hemolytic activity and transferable antibiotic resistance genes, and antagonistic efficacy. In the current investigation, *Pediococcus pentosaceus* isolate 204020-10 was the sole strain to meet all core screening metrics by exhibiting broad-spectrum antibacterial activity, strong acid endurance (pH 2.3), maximum bile resistance (0.6% w/v), and phenol tolerance (0.5 g/L). Therefore, *P. pentosaceus* 204020-10 represents a promising candidate strain for further functional probiotic and industrial biopreservative applications.

CONCLUSION

This study isolated 15 lactic acid bacteria (LAB) strains across five distinct species (*Lactobacillus pentosus*, *Enterococcus faecalis*, *Enterococcus hirae*, *Lactococcus garvieae*, and *Pediococcus pentosaceus*) from Cambodian fermented *Pangasius* fish paste (*Pha-ork*). Most isolates demonstrated moderate-to-strong antibacterial activity against pathogenic *Aeromonas* and *Vibrio* species isolated from regional fish markets. Among the tested isolates, *Pediococcus pentosaceus* isolate 204020-10 demonstrated strong probiotic characteristics, surviving gastric acid (pH 2.3), high bile salts (0.6%), and elevated phenol levels (0.5 g/L).

Future work should focus on whole-genome sequencing to characterize functional probiotic genes, confirm the absence of transferable antibiotic resistance mechanisms and virulence factors, and perform *in vivo* clinical validation to support application in food safety and functional probiotic formulations.

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Conflict Of Interest

The authors declare no potential conflict of interest.

Compliance With Ethical Standards

This article does not cover any studies with human contributors or animals performed by any of the author.

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