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Indigenous Probiotics for African Catfish (*Clarias gariepinus*) Larviculture: Effects on Larval Growth Performance and Survival

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Abstract

High early larval mortality hinders African catfish (*Clarias gariepinus*) aquaculture. This study aimed to identify novel indigenous Ethiopian probiotics to enhance larval growth and survival. Lactic Acid Bacteria (LAB) were isolated from African catfish and Nile tilapia, then screened *in vitro* for probiotic potential. Selected strains were evaluated in a catfish larval feeding, and associated pathogens and their antibiotic resistance patterns were also assessed. *Lactobacillus* was the dominant probiotic genus. *In vitro*, strains showed excellent acid/bile tolerance and broad-spectrum antimicrobial activity. Crucially, host-specific *Lactobacillus* spp. from African catfish remarkably improved larval growth and survival (up to 78.3%) compared to the control (56.5%) and non-host-specific probiotics. However, widespread resistance to key antibiotics (e.g., penicillin G, kanamycin) was found in both LAB and pathogenic isolates, including *Staphylococcus aureus*. These findings underscore the considerable potential of indigenous, host-specific probiotics for sustainable African catfish aquaculture, while highlighting the critical need to address emerging antibiotic resistance.

Introduction

Aquaculture is a rapidly expanding sector crucial for global food security. Its share of total aquatic production surged from around 5% in the 1950s to 70s to 49.2% by 2020, reaching a historic 51% (130.9 million tonnes) in 2022 and surpassing capture fisheries for the first time (FAO, 2020, 2022, 2024). This growth is driven by rising demand, as annual per capita consumption of aquatic food increased from 9.1 kg in 1961 to 20.7 kg in 2022. Regionally, production in Africa is also expanding, reaching 2.9 million tonnes (USD 7.3 billion) in 2022. This occurred despite variations, such as in 2020 when a 14.5% output expansion in other African nations compensated for a decline in Egypt, the continent's primary producer (FAO, 2022, 2024).

In 2022, inland aquaculture constituted 62.6% of global farmed aquatic animal production, primarily composed of carps (15.7%), tilapias (10.0%), and salmonids (8.4%). By contrast, African finfish aquaculture exhibits limited species diversity, with production dominated by Nile tilapia (82.8%) and the less frequently farmed African catfish (10.1%) (FAO, 2022, 2024). Despite its smaller share, African catfish (*Clarias gariepinus*) is a widely cultivated pond species (Toko et al., 2007; Adewolu et al., 2008) due to its production and market advantages. These include resilience to high-density culture, disease, and harsh

conditions (Adewolu et al., 2010; Chor et al., 2013), rapid growth, efficient feed conversion, and excellent flesh quality (Hecht et al., 1996; Ali & Jauncey, 2005; Schram et al., 2010; Gbadamosi et al., 2017).

In Ethiopia, the African catfish is a widely distributed and commercially important species with high market value and consumer demand (Personal observations; Habteselassie, 2012; Getahun, 2017). However, its aquaculture potential is constrained by an unreliable seed supply, a direct result of high early larval mortality and underdeveloped rearing techniques (Brummett et al., 2008; Rothuis et al., 2012). Managing this mortality is therefore critical for establishing sustainable African catfish aquaculture.

Probiotics are an innovative and sustainable alternative to antibiotics for enhancing fish health and immunity in aquaculture (Hill et al., 2014; Wang et al., 2019; Subedi & Shrestha, 2021). These living microorganisms confer host benefits such as improved feed utilization, disease resistance, and water quality (Neven et al., 2010; Dawood et al., 2016; Calcagnile et al., 2024). Lactic acid bacteria (LAB) are particularly notable for their safety and ability to enhance growth, feed conversion, and immune function (Gildberg et al., 1997; Akhter et al., 2015; Wang et al., 2019; Hasan & Banerjee, 2020). Thus investigating probiotic supplementation to resolve African catfish production obstacles is warranted.

Despite their recognized potential, the specific effects of probiotics on African catfish larviculture, particularly from indigenous sources within a regional context like Ethiopia, remain understudied in indoor hatchery systems. This study directly addresses this critical knowledge gap by uniquely focusing on the screening and characterization of indigenous Ethiopian probiotics and associated fish pathogens. Subsequently, it evaluates the effects of these selected, locally-sourced probiotics on larval growth and survival under controlled conditions. Our primary aim is to contribute to more sustainable African catfish seed production, both in Ethiopia and globally, through the application of regionally adapted probiotic solutions.

Materials and Methods

This study was conducted in Jimma, Ethiopia (353 km southwest of Addis Ababa, 1780 m altitude), an area with a warm climate characterized by mean annual temperatures of 14–30°C and rainfall of 1138–1690 mm (Alemu et al., 2011). Local artisanal aquaculture of Nile tilapia (*Oreochromis niloticus*) is common but constrained by practices like single-species culture.

African catfish (*Clarias gariepinus*) and Nile tilapia were collected from the local Boye River for probiotic isolation. Probiotic candidates were isolated from the fish gut and surface mucus following methods by Wanka et al. (2018) and Thomas & Amaresan (2023). After surface sterilization with 70% ethanol, gut tissue was aseptically dissected and homogenized. Both gut homogenates and body surface swabs were serially diluted (up to 10^{-6}) in peptone water, and 0.1 mL aliquots were spread-plated onto de Man, Rogosa, and Sharpe (MRS) agar. Plates were incubated anaerobically at 32°C for 24 hours, after which distinct colonies were purified by repeated sub-culturing and maintained on MRS agar slants at 4°C.

For characterization, well-isolated colonies were cultured in MRS broth and incubated at 32°C for 24 hours. Cultures were purified by sub-culturing and the resulting LAB isolates were characterized using established morphological (Gram staining, motility, endospore detection), biochemical (KOH, catalase, cytochrome oxidase, carbohydrate fermentation), and physiological (temperature, salt tolerance) tests (Schaeffer & Fulton, 1933; Kovacs, 1956; Gregerson, 1978; MacFaddin, 1980; Tambekar & Bhutada, 2010; Shields & Cathcart, 2011; Ayo-Omogie and Okorie, 2016; Hassan, 2018). Genus-level identification was based on Bergey's Manual of Systematic Bacteriology (Vos et al., 2009).

LAB isolates were evaluated in vitro for key probiotic properties. Acid and bile tolerance were assessed by inoculating cultures into MRS broth adjusted to pH 2 & 3 (using HCl or NaOH), or supplemented with 0.3% or 0.5% bile salts, respectively, and incubated anaerobically at 32°C for 24 h. Subsequently, cultures from both tests were streaked onto MRS agar and incubated, with survival confirmed by subsequent growth on MRS agar (Ayo-Omogie & Okorie, 2016; Kim et al., 2018).

Antimicrobial activity was evaluated using an agar well diffusion assay, where cell-free supernatants (CFS) from centrifuged LAB cultures were filter-sterilized and tested against reference pathogens*: Bacillus cereus*, *Staphylococcus aureus* subsp. *aureus*, *Salmonella enterica* subsp. *Enterica* Serovar *Typhimurium*, *Escherichia coli*, and *Candida albicans.* Following 24 hours of anaerobic incubation at 32°C, the diameter of the resulting inhibition zones was measured to quantify the effect (Balouiri et al., 2016). Finally, antibiotic susceptibility was determined using the disk diffusion method against chloramphenicol, ciprofloxacin, clindamycin, erythromycin, kanamycin, penicillin G, and streptomycin. Isolates were classified, based on zones of inhibition, as susceptible (≥21 mm), intermediate (16-20 mm), or resistant (≤15 mm), with intermediate results conservatively classified as resistant (Vlkova et al., 2006).

Based on these evaluations, LAB isolates demonstrating strong antimicrobial activity (≥15 mm), tolerance to pH 2 and 0.5% bile salt, broad temperature tolerance, and resistance to common antibiotics were selected for the in vivo larval trial (Kosin & Rakshit, 2006).

To provide a holistic microbial profile, common fish pathogens were also isolated from the same probiotic source fish. Gut contents and surface swabs were homogenized in Buffered Peptone Water (BPW) by

shaking at 250 rpm for 5 minutes and serially diluted to 10^{-6} for downstream analyses. Aliquots were spread-plated onto selective media: Mannitol Salt Agar for *Staphylococcus aureus*; Pseudomonas Agar Base for *Pseudomonas* spp. (Su et al., 2018); and Eosin Methylene Blue Agar for *Escherichia coli* (Su et al., 2018). For *Salmonella*, *Shigella*, and *Listeria* spp., samples underwent pre-enrichment, selective enrichment, and plating on their respective selective agars (Keelara et al., 2015; Ashrafudoulla et al., 2021). All specified bacterial groups were incubated at 37°C, with *Staphylococcus aureus*, *Pseudomonas spp.*, and *Escherichia coli* for 48 hours, while *Listeria*, *Salmonella*, and *Shigella spp.* underwent multi-step enrichment and incubation processes lasting 24 hours per stage. Presumptive colonies were confirmed through Gram staining and a panel of standard biochemical tests relevant to each genus (Rhaiem et al., 2016).

African catfish broodstock for the larval trial were selected based on morphological signs of sexual maturity (De Graaf et al., 1995; Gadissa & Devi, 2013; Natea et al., 2017) and disinfected with formalin (25 mL/L for 30 minutes) before acclimatization (Floyd, 1996; Natea et al., 2017).

Live feed for the larvae, consisting of zooplankton and phytoplankton, was cultured in earthen ponds fertilized with cow dung. Plankton was harvested with 20 µm mesh nets and identified using established keys (Prescott, 1954; Belcher & Swale, 1976; Fernando, 2002; Bowling, 2009; Kobayashi et al., 2009).

For induced spawning, broodstock were injected with pituitary gland extract. Pituitary extract from mature male African catfish was isolated, transferred into 0.9 g saline solution, and administered at a dose of 2 ml proportional to a 2 kg donor weight. Eggs were stripped from females into a dry container, and milt was harvested from euthanized males by mincing testes in physiological saline (0.9% NaCl). Fertilization was achieved by gently mixing eggs and milt (Richter et al., 1987). Fertilized eggs were incubated in a 15 L aerated basin at 27-30°C on a fine mesh substrate. Hatching commenced after approximately 19 hours and was complete within 26 hours. Hatched larvae were separated from dead eggs to prevent fungal infection (Graaf & Janssen, 1996). After yolk sac absorption (2-3 days), free-swimming larvae were transferred to a larger tank and fed live feed, then gradually weaned onto a powdered artificial diet over seven days before being moved to glass aquaria for a 20-day acclimation period (Hogendoorn & Vismans, 1980; Haylor, 1993; Hecht et al., 1996).

Due to resource limitations, the indoor aquarium trial employed a completely randomized design with two replicates per control and four probiotic treatment groups. While this provided a basis for comparison, we acknowledge that a higher number of replicates would have further strengthened the statistical robustness of our findings and better accounted for potential biological variability within the experimental units. This limitation has been considered when interpreting the statistical significance and generalizability of the results. Four pre-selected LAB isolates were used for the treatments: two *Lactobacillus* spp. (Lb) from African catfish (AFC), one from Nile tilapia (NT), and one from the Ethiopian honey wine (T) as:

Control: Basal feed only

Treatment 1: Basal feed+0.5 mL *Lb* sp.$^{(AFC1)}$ suspension 1×10^7 cfu/mL

Treatment 2: Basal feed+0.5 mL *Lb* sp.$^{(AFC2)}$ suspension 1×10^7 cfu/mL

Treatment 3: Basal feed+0.5 mL *Lb* sp.$^{(NT)}$ suspension 1×10^7 cfu/mL

Treatment 4: Basal feed+0.5 mL *Lb* sp.$^{(T)}$ suspension 1×10^7 cfu/mL

Thirty-day-old African catfish larvae (initial weight 0.04±0.00 g; length 1.12±0.12 cm) were stocked at 23 fish per 100 L aquarium. The basal diet, consistent across all groups, was administered daily at 10% of body weight. The probiotic treatments received the basal feed plus a 0.5 mL suspension (1×10^7 cfu/mL) of their respective activated probiotic, applied every three days for over one month (Fakhri et al., 2019; Masjudi et al., 2020). The probiotic concentrations used in this experiment (1×10^7 cfu/mL) were within the established theoretical range of 10^6 to 10^8 cfu/mL (Kechagia et al., 2013). Water quality was maintained by siphoning 50% of the water every two days and replenishing it. The entire process, encompassing broodstock collection, acclimatization, microbiological analysis, and the probiotic experiment, spanned 120 days. The probiotic test itself constituted only 4 weeks of this period.

Weekly samples of six fry per aquarium were measured for weight (g) and total length (cm). Growth performance was assessed by calculating Absolute Growth Rate (AGR), and Survival Rate (SR) using standard formulas (Fulton, 1902; Lauzon et al., 2010; Lugert et al., 2016).

Absolute Growth Rate (AGR): Mean Wait gain (g) /Duration of the Experiment (days) (Lugert et al., 2016).

Fulton Condition Factor (FCF): Mean weight (g)/TL^3*100 (Fulton, 1902).

Survival rate (SR): Final fish number/Initial fish number*100 (Lauzon et al., 2010).

Data were expressed as mean±standard deviation (SD). A one-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test was used to determine significant differences ($P<0.05$) among treatment groups using SPSS (version 26).

During the laboratory experiment, water quality parameters were monitored weekly using a Palintest (model MICRO 800) for temperature, dissolved oxygen (DO), electric conductivity (EC), and total dissolved solids (TDS), and an Adwa (model AD 8000) for pH. Ammonia, nitrite, and nitrate levels were measured bi-weekly using a Palintest test kit (model photometer 7500) due to reagent shortages.

Results

Of the 99 isolates obtained from the fish samples, 80.81% (n= 80) were LAB. These belonged primarily to the genus *Lactobacillus* (91.25%), with smaller proportions of *Lactococcus* (7.5%) and *Leuconostoc* (1.25%). *Lactobacillus* spp. were the most prevalent isolates. The detailed morphological and physiological characteristics of these LAB genera are presented in Table 1.

The 80 LAB isolates were evaluated for probiotic potential, with a high proportion demonstrating tolerance to pH 3 (80%, n= 64) and 0.5% bile salt (93.75%, n= 75), while fewer were tolerant to pH 2 (60%, n= 48) (Table 2). Among 43 *Lactobacillus* isolates selected for further testing based on their resilience, 16 (37.21%) exhibited inhibitory activity against four or more reference pathogens (Table 3). Notably, three isolates (AFG8, AFG10 and NTG8) displayed the most pronounced effects, with inhibition zones ≥15 mm against all five tested pathogens. Subsequent antibiotic susceptibility testing on 17 of these broad-spectrum isolates revealed that while most were susceptible to erythromycin (64.71%) and chloramphenicol (58.82%), all exhibited resistance to kanamycin, ciprofloxacin, streptomycin, and penicillin G (Table 4).

Of the screened pathogens, only *Staphylococcus aureus* and *Salmonella* spp. were detected; *Listeria*, *Shigella*, *Pseudomonas*, and *E. coli* were absent. *S. aureus* was highly prevalent, found on 100% of fish surfaces with an overall prevalence of 60.71%, whereas *Salmonella* spp. had an overall prevalence of 32.14% (Table 5). Susceptibility testing showed that all *S. aureus* isolates were resistant to Penicillin G (100%) but susceptible to ciprofloxacin, chloramphenicol, and erythromycin. *Salmonella* spp. isolates were broadly susceptible to chloramphenicol, with varying susceptibility to other antibiotics (Table 6). Multidrug resistance (MDR) was observed in both pathogens, with profiles varying by species and fish origin (Table 7).

African catfish larvae administered probiotics showed improved growth performance compared to the control group over the experimental period (Figure 1). Treatments with probiotics from African catfish gut (*Lactobacillus* spp. in T1 and T2: AFG8 and AFG10)

Table 1. Morphological and physiological characteristics of LAB isolated from African catfish and Nile tilapia gut and surface samples

Characteristics	Category		
Shape	Rod	Cocci	Cocci
Arrangement		Single	Pair or short chain
Gram reaction	Positive	Positive	Positive
Catalase Test	Negative	Negative	Negative
Motility test	Non-motile	Non-motile	Non-motile
Oxidase	Negative	Negative	Negative
Endospore	Negative	Negative	Negative
Fermentation	Homo/Hetero	Homo	Hetero
Growth Temperature			
15^{o}C	Growth/No growth	Growth	Growth
37^{o}C	Growth	Growth	Growth
45 ^{o}C	Growth/No growth	No growth	No growth
Tolerance to NaCl (%)			
2%	Growth	Growth	Growth
4.5%	Growth	Growth	Growth
6.50%	Growth/No growth	Growth/No growth	Growth
Identification	*Lactobacillus*	*Lactococcus*	*Leuconostoc*
Number and % of isolates	73 (91.25%)	6 (7.5%)	1(1.25%)

Table 2. Acid and Bile tolerance of LAB isolated from African catfish and Nile tilapia gut and surface samples (n= 48)

Source and Isolate Code	Category	Acid tolerance		Bile tolerance	
		pH2	pH3	0.30%	0.50%
AFG: 1,3,5,6,8,10,11,14,15, 20,30,38	*Lactobacillus* spp.	+	+	+	+
AFS: 3,6,7,11,12,13,14,15,18	*Lactobacillus* spp.	+	+	+	+
NTG: 1,4,6,7,8,9,10,14,15, 16, 17,19,20	*Lactobacillus* spp.	+	+	+	+
NTS: 1,2,3,4,5,7,8,9,10	*Lactobacillus* spp.	+	+	+	+
AFG: 26; AFS: 4,17; NTG: 18; NTS: 6	*Lactobacillus* spp.	+	+	+	-

AFG = African catfish gut isolate, AFS = African catfish surface isolate, NTG = Nile tilapia gut isolate, NTS = Nile tilapia surface isolate, "+ ", Tolerant, "–" Non-tolerant

resulted in the highest final weight and length, with T1 being marginally higher (T1: 1.28±0.78 g, 4.70±0.92 cm; T2: 1.20±0.38 g; 4.65±0.68 cm). These were followed, respectively, by the groups treated with probiotics isolated from Nile tilapia gut (*Lactobacillus* sp. in T3: *NTG8*) and the local beverage, Tej (*Lactobacillus* sp. in T4) (T3: 0.90±0.83 g, 4.30±1.08 cm; T4: 0.51±0.25 3.80±0.67 cm). Analysis of variance indicated a significant difference in final larvae weight and length among treatment groups (P<0.05); however, a post-hoc

Table 3. Antimicrobial activity of LAB isolated from African catfish and Nile tilapia gut and body surface samples against reference pathogens (≥4)

Code	*Bacillus cereus*	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella enterica* Serovar Typhimurium	*Candida albicans*
AFG6	13.5±0.71^{d}	13±1.4^{efg}	14±0^{bcd}	16±0^{bc}	0±0
AFG8	16±1.41^{abc}	15.5±0.7^{abcd}	17±0^{a}	16±0^{bc}	15.5±0.71^{b}
AFG10	17±0.71^{a}	16±0^{abc}	16.5±0.71^{a}	16.5±0.7^{b}	16±0^{ab}
AFG20	14±1.41^{cd}	13.5±0.7^{defg}	13±0^{d}	14±1.4^{d}	0±0
AFG24	14±1.41^{cd}	15±0^{bcde}	13.5±0.71^{cd}	14±0^{d}	14.5±0.7^{c}
AFG30	15±1.41^{abcd}	14.5±0.7^{cdef}	15±0^{bc}	13±1.4^{de}	0±0
AFS4	15±0^{abcd}	17±0^{ab}	15.5±0.71^{ab}	18.5±0.7^{a}	0±0
AFS14	14±0^{cd}	16±1.4^{abc}	15.5±0.71^{ab}	13±0de	15.5±0.7^{b}
AFS15	14±0^{cd}	13.5±0.7^{defg}	15.5±0.71^{bcd}	16±0^{bc}	0±0
AFS18	14.5±0.71^{bcd}	14.5±0.7^{cdef}	15±0^{ab}	17±1.4^{ab}	0±0
NTG4	14±0^{cd}	17.5±0.7^{a}	0±0	16±0^{bc}	17.5±0.7^{a}
NTG7	14.5±0.71^{bcd}	14±0^{cdefg}	15.5±0.71^{ab}	14.5±0.7^{cd}	0±0
NTG8	16.5±0.71^{ab}	16.5±2.1^{ab}	15.5±0.71^{bc}	15±0^{e}	15.5±0.71^{b}
NTG15	14.5±0.71^{bcd}	0±0.0	14±1.41^{bcd}	0±0	0±0
NTG19	16±1.41^{abc}	14±0^{cdefg}	13±1.41^{d}	11.5±0.7^{e}	0±0
NTS6	17±0^{a}	12±0^{g}	15±1.41^{bc}	13.5±0.7^{d}	0±0
NTS7	14.5±0.71^{bcd}	13±1.4^{efg}	13.5±0.71^{cd}	14.5±0.7^{cd}	0±0

AFG= African catfish gut isolate, AFS= African catfish surface isolate, NTG= Nile tilapia gut isolate, NTS= Nile tilapia surface isolate. Within each column, values with the same lowercase superscript letter are not significantly different (P>0.05), while values with different letters are significantly different (P<0.05).

Table 4. Antibiotic sensitivity patterns of LAB isolated from African catfish and Nile tilapia gut and surface samples against different commercial antibiotics

Source		Isolate Code	Ery	Chlo	Clind	Kana	Cipro	Strept	Pen
African catfish	Gut	AFG10	21S	23S	9R	R	R	R	R
		AFG24	25S	22S	10R	R	R	R	R
		AFG8	26S	27S	10R	R	R	R	R
		AFG20	R	R	R	R	R	R	R
		AFG30	R	R	R	R	R	R	R
	Surface	AFG6	R	R	R	R	R	R	R
		AFS4	R	R	R	R	R	R	R
		AFS15	25S	25S	9R	R	R	R	R
		AFS18	R	R	R	R	R	R	R
		AFS14	23S	22S	9R	R	R	R	R
Nile tilapia	Gut	NTG4	26S	29S	26S	R	R	R	R
		NTG8	23S	22S	24S	R	R	R	R
		NTG7	23S	24S	23S	R	R	R	R
		NTG19	23S	19R	R	R	R	R	R
		NTG15	21S	22S	R	R	R	R	R
	Surface	NTS6	24S	25S	11R	R	R	R	R
		NTS7	R	R	R	R	R	R	R

Ery= Erythromycin, Chlo= Chloramphenicol, Clind= Clindamycin, Kana= Kanamycin, Cipro= Ciprofloxacin, Strept= Streptomycin, Pen= Penicillin G; AFG= African catfish gut isolate, AFS= African catfish surface isolate, NTG= Nile tilapia gut isolate, NTS= Nile tilapia surface isolate, R= Resistant, S = Susceptible; the numbers preceding the R and S represent the zone of inhibition in mm.

Table 5. Prevalence of common fish pathogens from African catfish and Nile tilapia gut and surface samples

Source		Frequency (%) of pathogens	
		S. aureus	*Salmonella* spp.
African catfish	Gut	1 (14.29)	1(14.29)
	Surface	7 (100)	3 (42.85)
Nile tilapia	Gut	2 (28.57)	1(14.29)
	Surface	7 (100)	4 (57.14)
Total		17 (60.71)	9 (32.14)

test revealed this was primarily driven by the significant difference between T1 and the control (P<0.05). Other growth parameters, including weight gain and absolute growth rate, and FCF followed a similar trend (Table 8). All probiotic treatments had higher survival rates compared to the control (56.5%), with T1 achieving the highest survival at 78.3% (Table 9). During the probiotic experiments, water temperature remained consistent across all groups, averaging around 28.14°C. Dissolved oxygen (DO) levels showed significant differences

Table 6. Antibiotic susceptibility patterns of *S. aureus and Salmonella* spp. isolated from fish samples

Pathogens	Source		Antibiotic pattern	Antibiotics (disc potency, µg/mL) Ery (15) Freq. (%)	Clin (2) Freq. (%)	Kana (30) Freq. (%)	Cipro (5) Freq. (%)	Pen (10) Freq. (%)	Strept (10) Freq. (%)	Chlo (30) Freq. (%)
S. aureus	African catfish	Surface	Resistant	4 (57.14)	5 (71.43)	1 (14.29)	2 (28.57)	7 (100)	-	1 (14.29)
			Sensitive	3 (42.86)	2 (28.57)	6 (85.71)	5 (71.43)	-	7 (100)	6 (85.71)
		Gut	Resistant	-	1 (100)	-	-	1 (100)	1 (100)	-
			Sensitive	1(100)	-	1(100)	1(100)	-	-	1(100)
	Nile tilapia	Surface	Resistant	5 (71.43)	6 (85.71)	3 (42.86)	1 (14.29)	7 (100)	4 (57.14)	6 (85.71)
			Sensitive	2 (28.57)	1 (14.29)	4 (57.14)	6 (85.71)	-	3 (42.86)	1 (14.29)
		Gut	Resistant	-	1 (100)	1 (100)	-	1 (100)	1 (100)	-
			Sensitive	1 (100)	-	-	1 (100)	-	-	1 (100)
Salmonella spp.	African catfish	Surface	Resistant	ND	3 (100)	2 (75)	1 (25)	ND	2 (75)	-
			Sensitive	ND	-	1 (25)	2 (75)	ND	1 (25)	3 (100)
		Gut	Resistant	ND	1 (100)	-	-	ND	-	1 (100)
			Sensitive	ND	-	1 (100)	1 (100)	ND	1 (100)	-
	Nile tilapia	Surface	Resistant	ND	3 (75)	2 (50)	1 (25)	ND	1 (25)	-
			Sensitive	ND	1 (25)	2 (50)	3 (75)	ND	3 (75)	4 (100)
		Gut	Resistant	ND	1 (100)	1 (100)	-	ND	1 (100)	-
			Sensitive	ND	-	-	1 (100)	ND	-	1 (100)

Ery= Erythromycin, Clin= Clindamycin, Kana= Kanamycin, Cipro= Ciprofloxacin, Pen= Penicillin G, Strept= Streptomycin, Chlo= Chloramphenicol; ND= Not determined; "– " = none of the isolates.

Table 7. MDR patterns of *S. aureus* and *Salmonella* spp isolated from fish samples

Source fish	*S. aureus* No. of antimicrobial resistance	Antimicrobial resistance pattern	No. of isolates (%)	Total(%)	*Salmonella* spp. No. of antimicrobial resistance	Antimicrobial resistance pattern	No. of isolates %	Total(%)
African catfish	One	P	1 (20)	1(20)	Two	CD/E	1 (25)	2(50)
	Two	C/P	1 (20)	1(20)		CD/C	1 (25)	
	Three	CD/S/P	1 (20)	1(20)	Four	E/CD/K/S	1 (25)	1(25)
	Four	E/CD/CIP/P	1 (20)	1(20)				
	Five	E/CD/CIP/P/K	1 (20)	1(20)	Five	E/CD/K/S/CIP	1 (25)	1(25)
Nile tilapia	Two	K/P	1(8.3)	2(16.7)	One	E	1 (20)	1(20)
		CD/P	1(8.3)		Two	E/CD	1 (20)	1(20)
	Three	S/C/P	1(8.3)	2(16.7)	Three	E/CD/K	1 (20)	1(20)
		CD/P/CIP	1(8.3)					
	Four	K/P/S/C	1(8.3)	4(33.3)	Four	E/CD/K/S	1 (20)	1(20)
		E/CD/K/P	1(8.3)					
		E/CD/CIP/P	2(16.7)					
	Five	E/CD/K/S/P	1(8.3)	3(25)	Five	E/CD/CIP/S/K	1 (20)	1(20)
		CD/K/S/C/P	1(8.3)					
		E/CD/S/C/P	1(8.3)					
	Six	E/CD/K/S/P/C	1(8.3%)	1(8.3)				

CD= Clindamycin; P= Penicillin; E= Erythromycin; C= Chloramphenicol; K= kanamycin; S= Streptomycin; CIP= Ciprofloxacin

(P<0.05), with the control group exhibiting 4.45 mg/L, lower levels compared to treatments T1 (4.92 mg/L), T2 (4.79 mg/L), and T4 (4.65 mg/L). Electrical conductivity (EC), averaging 193.30 µS/cm; and total dissolved solids (TDS), averaging 124.97 mg/L, were stable, showing no notable variations among the control and treatment groups. pH also presented significant differences (P<0.05); the control group recorded a lower pH of 6.89 compared to treatments T1 (7.08) and T2 (7.06). Ammonia (NH3), averaging 12.06 mg/L; ammonium (NH_4+), averaging 12.56 mg/L; nitrite (NO_2), averaging 0.35 mg/L; and nitrate (NO_3), averaging 42.16 mg/L, concentrations did not vary significantly across the experimental groups (P>0.05). Overall, while certain probiotic treatments influenced DO and pH, most water quality parameters remained largely unaffected.

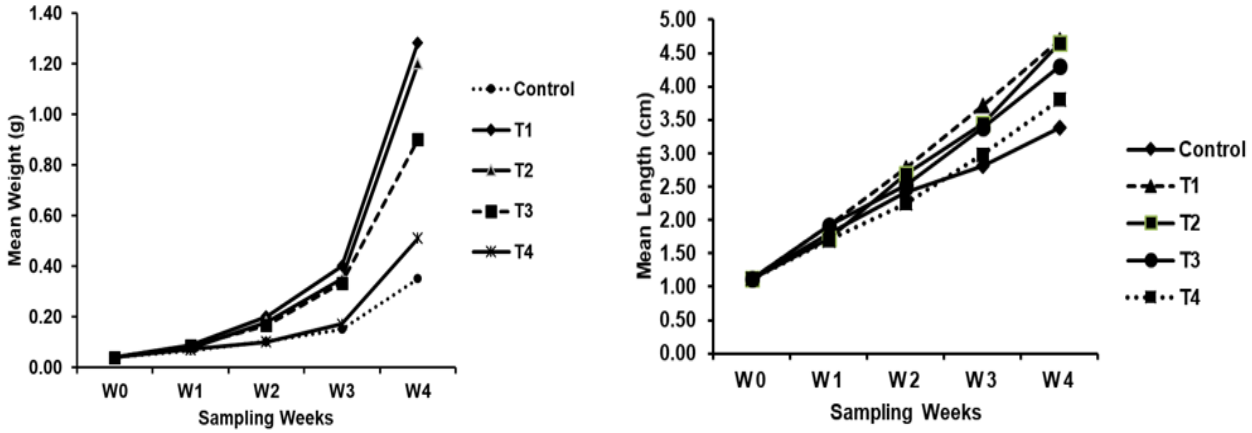

Figure 1. Weekly variation of growth patterns of the African catfish larvae during probiotic treatments.

Table 8. Growth parameters of African catfish larvae treated with different probiotic strains

Week	Treatment	Mean Weight (g)	Mean Total Length (cm)	Weight Gain (g)	FCF
0	-	0.04 ± 0.00	1.12 ± 0.12	-	-
1	Control	0.07 ± 0.01^{c}	1.80 ± 0.26^{a}	0.03 ± 0.01^{c}	1.22 ± 0.4
	T1	0.09 ± 0.01^{a}	1.92 ± 0.35^{a}	0.05 ± 0.01^{a}	0.69 ± 0.15
	T2	0.08 ± 0.01^{ab}	1.72 ± 0.35^{a}	0.04 ± 0.01^{bc}	0.63 ± 0.18
	T3	0.08 ± 0.01^{ab}	1.92 ± 0.26^{a}	0.05 ± 0.01^{ab}	0.89 ± 0.15
	T4	0.07 ± 0.01^{c}	1.70 ± 0.46^{a}	0.03 ± 0.01^{c}	1.48 ± 1.05
2	Control	0.10 ± 0.05^{c}	2.42 ± 0.35^{a}	0.07 ± 0.05^{a}	0.92 ± 0.27
	T1	0.2 ± 0.09^{a}	2.78 ± 0.56^{a}	0.16 ± 0.09^{a}	0.77 ± 0.20
	T2	0.18 ± 0.08^{ab}	2.68 ± 0.39^{a}	0.13 ± 0.08^{a}	1.11 ± 0.17
	T3	0.17 ± 0.08^{ab}	2.52 ± 0.52^{a}	0.06 ± 0.00^{a}	1.74 ± 0.88
	T4	0.10 ± 0.00^{c}	2.25 ± 0.19^{a}	0.03 ± 0.01^{a}	0.88 ± 0.20
3	Control	0.15 ± 0.08^{b}	2.82 ± 0.26^{c}	0.11 ± 0.08^{a}	0.87 ± 0.25
	T1	0.40 ± 0.15^{a}	3.72 ± 0.29^{a}	0.36 ± 0.15^{a}	1.20 ± 0.31
	T2	0.35 ± 0.10^{a}	3.45 ± 0.39^{ab}	0.31 ± 0.10^{a}	1.28 ± 0.40
	T3	0.33 ± 0.19^{a}	3.38 ± 0.35^{ab}	0.29 ± 0.19^{a}	1.12 ± 0.58
	T4	0.17 ± 0.08^{b}	2.98 ± 0.37^{bc}	0.10 ± 0.05^{a}	0.82 ± 0.23
4	Control	0.35 ± 0.10^{b}	3.38 ± 0.29^{b}	0.31 ± 0.11^{b}	0.94 ± 0.24
	T1	1.28 ± 0.78^{a}	4.70 ± 0.92^{a}	1.24 ± 0.78^{a}	1.96 ± 1.33
	T2	1.20 ± 0.38^{a}	4.65 ± 0.68^{a}	1.16 ± 0.38^{ab}	0.92 ± 0.22
	T3	0.90 ± 0.83^{ab}	4.30 ± 1.08^{a}	0.86 ± 0.83^{ab}	0.62 ± 0.16
	T4	0.51 ± 0.25^{b}	3.80 ± 0.67^{b}	0.47 ± 0.25^{ab}	0.89± 0.17

T= Treatment; T1 & T2= *Lactobacillus spp*. isolated from African catfish gut; T3= *Lactobacillus* sp. isolated from Nile tilapia gut, T4= *Lactobacillus* sp. isolated from Tej (honey wine); Similar letters along the same column indicate lack of significant difference (P>0.05) whereas different letters indicate a significant difference (P<0.05).

Table 9. Survival rate (SR) of experimental fish during probiotic treatments

Group	Initial number stocked (n_i)	Final number survived (n_f)	SR (%)
Control	23	13	56.5
T1	23	18	78.3
T2	23	17	73.9
T3	23	17	73.9
T4	23	15	65.2

Discussion

This study confirms the remarkable potential of indigenous LAB to enhance African catfish larviculture by addressing the critical challenge of high early larval mortality, thereby contributing to more sustainable aquaculture practices (Subedi & Shrestha, 2021). This aligns with findings in other fish species such as Atlantic cod (Gadus morhua) where *Carnobacterium divergens* probiotics remarkably improved larval growth and survival (Puvanendran et al., 2021). The prevalence of *Lactobacillus* species (91.25%) among the isolates aligns with previous research identifying it as a dominant probiotic genus in the gut of freshwater fishes, suggesting the local fish microbiome is a rich source for probiotic candidates (Muthukumar & Kandeepan, 2015; Kato et al., 2016). The adaptability of these isolates, demonstrated by their tolerance to varied temperatures and salinity, further supports their environmental adaptability and suitability for aquaculture applications (Belicova et al., 2013; George et al., 2018).

A high proportion of the isolated LAB demonstrated crucial probiotic characteristics, including tolerance to physiologically relevant acid and bile concentrations, a prerequisite for survival and efficacy within the gastrointestinal tract (Ayo-Omogie & Okorie, 2016; Hussain et al., 2021; Olorunshola et al., 2025). Furthermore, a subset of these resilient isolates exhibited broad-spectrum antimicrobial activity against multiple fish pathogens. This inhibitory action is likely mediated by the production of metabolites such as bacteriocins and organic acids, which disrupt pathogen viability and supports the potential of these strains as biotherapeutic agents (Rattanachaikunsopon & Phumkhachorn, 2010; Amarantinia et al., 2019).

The study also identified the presence of *S. aureus* and *Salmonella* spp., particularly on fish surfaces, which contrasts with other regional findings and highlights a potential human health risk during fish handling, underscoring the need for stringent hygiene (Tesfaye et al., 2018; Olorunshola et al., 2025). The detection of *S. aureus* and *Salmonella* spp. on fish surfaces in this study underscores a profound public health risk, potentially contrasting with other regional findings (*Ibid*) primarily focused on gram-negative internal pathogens. Given the prevalence of unhygienic fish handling practices in Ethiopia, as highlighted in the provided literature, these findings necessitate stringent hygiene protocols from capture to consumption to prevent human foodborne illness. This emphasizes the need for a comprehensive approach to food safety, expanding beyond typical internal pathogens to surface contaminants, thereby enriching the understanding of fish-borne hazards in the region. A more critical finding was the widespread antibiotic resistance observed in both the beneficial LAB and the isolated pathogens. The high resistance of LAB to antibiotics like kanamycin and penicillin G raises concerns about the potential for horizontal gene transfer in aquatic environments (Davies & Davies, 2010). Similarly, the resistance of *S. aureus* to penicillin G, consistent with other local studies, confirms the circulation of resistance genes in the region (Beyene et al., 2017).

The in vivo trial demonstrated that probiotic supplementation considerably improved larval growth, condition, and survival. This enhancement is attributed to probiotics' ability to improve nutrient utilization, stimulate the immune system, and maintain water quality (El-Haroun et al., 2006; Wang et al., 2019; Ringo, 2020; Calcagnile et al., 2024). Critically, the study revealed the importance of host-specificity; probiotics isolated from African catfish gut (AFG10 and AFG8) conferred the greatest benefits, substantially outperforming the probiotic sourced from a non-aquatic environment. This supports the principle that host-derived probiotics are often more effective due to superior adaptation and colonization capabilities, a finding consistent with previous aquaculture studies (Nguyen et al., 2017; Putra et al., 2017; Masjudi et al., 2020). However, this study was limited by the lack of molecular identification of the most effective strains due to funding and resource constraints; therefore, a follow-up study addressing this limitation is recommended.

The observed stability in most water quality parameters, such as temperature, salinity, EC, TDS, and nitrogenous compounds (ammonia, ammonium, nitrite, and nitrate), across the control and probiotic-treated groups is a crucial finding. This indicates that the probiotic interventions did not negatively impact the fundamental physical and chemical characteristics of the water, which is essential for maintaining a healthy aquatic environment in aquaculture. The slight variations observed in dissolved oxygen (DO) and pH, with some probiotic treatments showing higher DO and pH than the control, suggest a subtle positive influence of the probiotics on these parameters. Elevated DO can indicate improved aerobic conditions, potentially linked to probiotic metabolic activity or reduced organic load, while a moderately higher pH could signify a more buffered system. These minor shifts, however, did not destabilize the overall water quality, reinforcing the safety and environmental compatibility of using these indigenous LAB as probiotics, aligning with the general discussion on their potential to enhance aquaculture practices without compromising environmental parameters.

Conclusion

In conclusion, this study successfully demonstrates that indigenous, host-specific LAB can remarkably enhance African catfish larviculture, offering a promising strategy to address the critical challenge of high early larval mortality and improve seed supply in Ethiopia and elsewhere. The research identified native *Lactobacillus* strains with robust probiotic properties that improved both larval growth and survival,

underscoring the value of the local fish microbiome as a source for developing sustainable aquaculture solutions. However, the findings also highlight the pressing issue of antibiotic resistance in both probiotic candidates and pathogenic bacteria, which warrants caution and further investigation.

To build upon these findings, future research should focus on molecular identification of the most promising LAB strains to understand their specific mechanisms of action. Further studies are needed to optimize application strategies, including dosage, delivery methods, and long-term effects under various production systems. Additionally, the high prevalence of surface pathogens like *S. aureus* and the patterns of antibiotic resistance demand focused investigation into contamination sources and the development of strategies to mitigate the spread of resistance in aquaculture environments (Tamminenm et al., 2011). Ultimately, this work provides a strong foundation for the development of effective, sustainable, and safe probiotic applications in African aquaculture.

Ethical Statement

All procedures involving animal subjects, including the collection of fish from the wild, induced spawning, and the larval feeding trial, were conducted in accordance with established guidelines for the care and use of animals in aquaculture research to minimize stress and ensure welfare. The euthanasia of male broodstock followed standard protocols for scientific purposes. Furthermore, all microbiological procedures, including the isolation, characterization, and handling of probiotic and pathogenic bacteria, were performed in strict adherence to the Ethiopian Biosafety Regulatory Frameworks of 2022 and 2023 and relevant institutional biosafety and biosecurity guidelines. The protocol was approved by the Research and Ethics Review Board of the College of Natural Sciences, Jimma University (Dated Feb. 10-13, 2020, Minute Number CNS/RERB/013/ 2020).

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Author Contribution

AT: Conceptualization and study design, Data Curation, Formal Analysis, Investigation, Methodology, Visualization and Writing-original draft.

UB: Conceptualization and study design, Data Curation, Formal Analysis, Investigation, Methodology, Visualization and Writing-original draft.

RN: Conceptualization and study design, Supervision, Writing -review and editing, Data Curation, Formal Analysis.

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Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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