College of Natural Sciences

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Department of Biology

Isolation and Characterization of Probiotic Lactic Acid Bacteria from African Catfish (*Clarias gariepinus*) and Nile Tilapia (*Oreochromis niloticus*) for African Catfish Aquaculture Production

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A Thesis submitted to the Department of Biology, College of Natural Sciences, Jimma University, In Partial Fulfillment of the Requirement for the Degree of Master of Science in Applied Microbiology

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A Thesis Presented to the School of Graduate Studies, Jimma University, in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Biology (Applied Microbiology)

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Declaration

I, the Undersigned, declare that this thesis entitled **Isolation and Characterization of Probiotic lactic acid bacteria for African Catfish (Clarias gariepinus) and Nile Tilapia (Oreochromis niloticus) Aquaculture Production** is my original work and that all sources of materials used for the thesis have been correctly acknowledged.

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List of Acronyms

AFG	African catfish gut
AFS	African catfish surface
AGR	Average growth rates
BPW	Buffered Peptone Water
CFS	Cell Free Supernatant
DF	Dry Feed
EFSA	European Food Safety Authority
EMB	Eosin Methylene Blue
FAO	Food and Agriculture Organization
GIT	Gastrointestinal tract
LAB	Lactic acid bacteria
Lb	*Lactobacillus*
Lc	*Lactococcus*
LF	Live feed
MHA	Muller Hinton Agar
MRS	de Man Rogosa Sharpe
MSA	Mannitol Salt Agar
NT	Nile tilapia
NTG	Nile tilapia gut
NTS	Nile tilapia surface
SSA	Salmonella and Shigella agar
WG	Weight gain
WHO	World health organization

Abstract

African catfish (Clarias gariepinus) is one of the most commercially important fish species in Ethiopia known for its nutritional richness. However, it is usually affected by pathogens. The objective of this study was to evaluate the probiotic lactic acid bacteria isolated from African Catfish and Nile Tilapia for African Catfish aquaculture production. The study was conducted in Jimma city. A cross-sectional study design was employed. A total of 14 fish samples (7 each of African catfish and Nile tilapia) were collected from Jimma city, Boye River, and a purposive sampling technique was employed. Evaluation of probiotic properties of lactic acid bacteria, detection of pathogens from aquarium, surface, and gut of fish, and application of probiotics in larvae of African catfish was conducted following the standard procedures. Data were analyzed using SPSS software version 20.0. A total of 80 LAB isolates were obtained among which Lactobacillus spp were the dominant (91.25%) followed by Lactococcus (7.5%). From a total of 80 isolates of LAB, 64 (80%), and 48(60%) tolerated pH3 and pH2, respectively. On the other hand, all 80 (100%) of the isolates survived 0.3% bile salt while 75 (93.75%) survived 0.5% bile salts. A total of 43 isolates of LAB, which tolerated the pH2 and 0.5% bile salt, the highest antimicrobial activity (≥ 15mm diameter of zone of inhibition) in all pathogens was exhibited by isolates coded AFG8, AFG10, NTG8, and JUT89. Moreover, from 43 isolates, 17 (39.53%) showed antimicrobial activity against 4 to 5 pathogens. On the other hand, the majority of LAB isolates were susceptible to chloramphenicol and erythromycin (64.7% each) followed by clindamycin (52.9%). However, all isolates were highly resistant (100%) to penicillin G, streptomycin, ciprofloxacin, and kanamycin. Overall, 60.71% of fish samples were positive for S. aureus, and 32.14% for Salmonella spp. In both surface and gut of African catfish and Nile tilapia, Listeria, Shigella, Pseudomonas spp, and E. coli were not detected. All isolates of S. aureus isolated from the guts of African catfish and Nile tilapia were susceptible to ciprofloxacin, chloramphenicol, and erythromycin. Furthermore, all Salmonella spp isolated from the gut of African catfish were also susceptible to kanamycin, ciprofloxacin, and streptomycin. The pre and post-assessment of pathogens in an aquarium during application of probiotics showed that all aquaria had initially E. coli, Listeria spp, Salmonella spp, and Pseudomonas spp. After probiotics LAB were applied, an aquarium that had probiotic Lactobacillus spp (coded AFG10 and AFG8) inhibited all pathogens. Moreover, the aquarium which had Lactococcus spp NTG8, inhibited all pathogens except E. coli whereas Lactobacillus spp (coded JUT89) inhibited only Salmonella spp. The application of probiotics enhanced the weight and length of Africa catfish with the highest fish growth performance recorded in an aquarium that had AFG10 and AFG8, with slightly higher values for AFG10. In contrast, the least growth values were observed in the aquarium that had JUT89 followed by the control. The variation in the final mean weight between the control and the treatment groups is statistically significant (P=0.02). Generally, both fishes are good sources of probiotic lactic acid bacteria. The probiotics isolated from African catfish enhanced the growth performance of African catfish than Nile tilapia. and Tej. Besides optimization of the growth condition of probiotics isolated from African catfish for its better growth performance, a comprehensive evaluation of other sources of probiotics for wider application in the growth enhancement of Nile tilapia was recommended.

Keywords: *African catfish, Aquaculture, probiotic, pathogen*

1. Introduction

1.1. Background

Aquaculture is the farming of aquatic organisms in both coastal and inland areas involving interventions in the rearing process to enhance production (FAO, 2020). The global aquaculture production of fish has grown tremendously during the last seventy years from the production of less than a million tons in the early 1950s to 82 million tons with a value of USD 250 billion in 2018 **(**Bidika and Abhimanyu, 2021). The contribution of aquaculture to world fish production reached 46% in 2018, up from 25.7% in 2000 (FAO, 2020). Global food fish consumption increased at an average annual rate of 3.1% from 1961 to 2017, a rate almost twice that of annual world population growth that is 1.6% for the same period, and higher than that of all other animal protein foods (meat, dairy, milk, etc.), which increased by 2.1% every year (FAO, 2020). The world aquaculture grows at a faster rate with an average annual growth rate of 5.3% per year in the period 2001-2018 with global fish consumption increasing at a higher rate than other animal protein sources **(**Subedi and Shrestha, 2021).

Fish provide vital nutritional benefits such as protein, vitamins, minerals, and micro-nutrients (Akter *et al.,* 2019). Among these African catfish **is** an important species for aquaculture in Sub-Saharan Africa as well in areas of Europe and Asia where it has been introduced and cultured for its omnivorous feeding habit, high growth rate, and its resistance to handling and stress (Afia, *et al*, 2020). In Ethiopia, Africa catfish are widely distributed almost in all water bodies such as in the rift valley, Abay, Awash, Baro-Akobo, Omo-Gibe, Tekeze, and Wabishebele-Genale basins (Awoke, 2015). African catfish have the ability to adaptability to tropical environments, suitability for monoculture and polyculture with other freshwater fish species, tolerance to high stocking density, ability to withstand handling stress, disease resistance, high fecundity, high weight gain, palatability, and nutritional quality, and low production cost (Madubuike & Kennedy, 2016).

Another important fish in Ethiopia is Nile tilapia (*Oreochromis niloticus)* which is native to Central and North Africa and has been introduced to many parts of Asia, Europe, North America, and South America due to its suitability to aquaculture (Abebe, *et al.,* 2020). Nile tilapia is among the most productive and cultured fish species worldwide and has great commercial importance as the base of commercial fisheries in many lakes in Africa (Hyuha, *et al.,* 2017). It has spread extensively in the lakes and rivers of Ethiopia, contributing to more than 60% of the total annual landings in the country and approximately 65% for Lake Tana (Degsera, *et al.,* 2020). It is the most dominant and commercially important species in the

newly formed Tekeze Reservoir and accounts for about 82.4% of the total production of fish in the reservoir (Tsegay, *et al.*, 2018).

Both African catfish and Nile tilapia have an intensive use in the food industry, however, they are highly affected by an outbreak of viral, bacterial, and fungal diseases (Allameh, *et al.*, 2021). The common bacterial species that are isolated from African catfish and Nile tilapia are *Pseudomonas spp, Salmonella* spp*, Staphylococcus* spp, and *Listeria* spp (Isaac, 2019). Moreover, Negash, *et al*. (2017) reported that *E. coli, Shigella dysenteriae, Staphylococcus aureus, Listeria monocytogenes, Salmonella* spp, *Vibrio* spp, and *Aeromonas* species are among indigenous bacterial pathogens found naturally living in the fish habitat of Lake Tana, Ethiopia.

Antibiotics used either for animal therapy or growth promotion purposes can result in a transfer of resistant genes from animals to humans and thereby establish a reservoir of resistant microbes (Maripandi and Al-Salamah, 2010). Subsequently, fish contamination with antibiotic-resistant bacteria can be a major threat to public health, as it can be transferred to other bacteria of human clinical significance (O'Brien, 2002). The choices of antibiotics for the treatment of common infectious diseases in humans are becoming increasingly limited, expensive, and ineffective due to the emergence of antibiotic-resistant bacteria that are considered the main cause of high mortalities and economic losses among fish and fish farms (Weese *et al*., 2011). The presence of pathogens influences the general well-being of the fish, the digestive tract, and the body (Enyidi and Onuoha, 2016). Therefore, in recent decades, the use of probiotics as a new strategy is suggested to promote and improve the immunity system and feed utilization in fish (Wang, *et al.*, 2019).

Probiotics are live microorganisms administered in adequate amounts that confer health benefits to the host by enhancing growth, improving feed utilization, enhancing disease resistance and immune response, and improving the water quality in aquaculture (Subedi and Shrestha, 2021). Production of antagonistic compounds against pathogens, competition for nutrient chemicals energy sources, adhesive sites, enhancement of immune responses, improve water quality by interacting with phytoplankton's and zooplanktons, vitamin production, the release of short-chain fatty acids, and improving blood parameters are the possible modes of action in the aquaculture (Dahiya, *et al*., 2020)

Nowadays, there are commercial probiotic products prepared from the main important bacteria such as non-pathogenic *Bacillus spp, Lactobacillus spp, Enterococcus spp, Carnobacterium spp*, and the yeast *Saccharomyces cerevisiae* that their use depends on careful management of

recommendations (Allameh, *et al.*, 2021). According to the report by Muthukumar and Kandeepan (2015), *Lactobacillus spp, Bacillus spp, Lactococcus spp,* and *Micrococcus spp* are the predominant probiotic strains in the gut of Fresh Water Fishes.

Probiotics can be administered to the host or into its ambient environment in different ways: addition to the artificial diet, addition to the culture water, and bathing and addition through live food (Dahiya, *et al*., 2020). Among all the routes of probiotic administration in aquaculture, supplementation of rearing water is the best method that is applicable for all ages of fish (Jahangiri and Esteban, 2018). The administration via feeding (dry feed) has limitations during early larval stages due to immature digestive tracts of fish in that stage of development (Jahangiri and Esteban, 2018).

Probiotics play a vital role in maintaining the gut health of fish by modulation of microbial community structure, they proliferate independently of the host animal in response to diseases, in aquaculture, improve the water quality and growth performance (Fakhri, *et al.*, 2019). According to a report by Hasan and Banerjee (2020), The supplementation of *Lactobacillus plantarum* in tank water results in the highest survival rate of *C. gariepinus* juvenile by 96.22% also increasing the body's mass and length of the fish. Probiotics with a combination of live feed are efficient to adapt, colonize and grow within the gut of the host and develop beneficial stability of microorganisms to improve animals' health (Carbone & Faggio, 2016). Moreover, aquaculture probiotics could prevent diseases, promote growth and reduce the extensive use of antibiotics, the competitive exclusion to unwanted microbes, also boost up the immune response and secretion of mucosal enzymes to promote host growth and they do not cause secondary pollution problems (Austin & Austin, 2012; Xia *et al*., 2020).

Even though probiotics have numerous advantages in fish development and health, there is no scientific study conducted in Ethiopia on the application of probiotics from different sources (Fish and beverage) to enhance African catfish production. Hence, the present study aims to evaluate the probiotic potential of lactic acid bacteria isolated from aquatic and non-aquatic sources for African Catfish Aquaculture Production.

1.2. Statement of the Problem

African catfish (*Clarias gariepinus)* is one of the commercially important fish species in Ethiopia (Awoke, 2015)**.** Unlike in the past whereby the demand for this fish species was very low due to its appearance, currently both its demand and prices are high. However, the survival rate from juvenile to adult fish form is minimal making it hard to culture in the laboratory and pond to distribute to the people **(**Enyidi and Uchenna, 2016)**.** Moreover, due to the possible health hazard, the change in micro-flora (probiotic and pathogenic), both in the gut and external body parts of fish affects productivity (Awoke, 2015)**.**

Increased quantity of fish production can meet the protein demand of the increasing human population. In fish farming, however, sometimes pathogens significantly smash their yield (Rubenfeld *et al*., 2005) creating about 45% damage in fish farms (Kabata, 2008). These pathogens can be viruses, bacteria, fungi, and helminths. Diseases caused by bacteria are accountable for heavy loss in both cultured and wild fish. This disease is usually caused by pathogens such as *Staphylococcus aureus, Pseudomonas putida, Salmonella spp,* and *Pseudomonas aeruginosa* which cause large-scale commercial fatalities in fish farming (Hossain *et al*., 2006). Increased density of farmed fish is the source of more production, but often, increased density quickens countless parasitic diseases, such diseases escort to skin and gill scratches, where opportunistic microorganisms get invasion and cause the maximum mortality of fish and diminish their growth rate (Abd-El-Khalek *et al*., 2012). Escalation of pond fish production also increases infection agents and pathological conditions that posed severe consequences, especially under crowded conditions (Kabata, 2008).

Thus, the use of antimicrobial drugs in the treatment and prevention of diseases was chosen as a method to solve this problem (Okacha *et al*., 2018). The use of these drugs in aquaculture is important for successfully treating sick fish and maintaining the health and well-being of animals (Miranda *et al*., 2013). However, the excessive use of these drugs leads to the development of pathogen's resistance to antimicrobials. However, probiotics provide multifunctional roles such as inhibiting pathogens as well as enhancing the growth performance of the fish.

Providing adequate food for a rapidly increasing human population is one of the greatest challenges in the world. The problem is particularly acute in countries like Ethiopia where, population explosion, natural and man-made disasters have aggravated the problem. African Catfish is nowadays one of the most commercially important fish species in Ethiopia. This species is easy to culture because it feeds on various food sources, but the survival rate

from juvenile to adult fish form is minimal making it hard to culture in the laboratory and pond to distribute to the people. This calls for searching for means by which the survival rate of juveniles is extended or enhanced through management of loss due to microbial pathogens. Probiotic LAB is a promising candidate that enhances fish productivity. Moreover, so far in Ethiopia, there is no scientific research done using probiotics isolated from African catfish, Nile tilapia, and ***Tej*** to enhance the growth performance of African catfish.

1.3. Objectives

1.3.1. General objective

- To evaluate the probiotic lactic acid bacteria from aquatic and non-aquatic sources for African Catfish Aquaculture Production.

1.3.2. Specific objectives

- To characterize LAB isolated from African catfish and Nile tilapia
- To assess the probiotic potential of LAB and evaluate the growth performance of African catfish in aquaculture using promising probiotics isolated from African catfish, Nile tilapia, and fermented beverage (Tej)
- To determine the prevalence of pathogens on the body surface and in the gut of African catfish and Nile tilapia
- To evaluate the antimicrobial susceptibility of the pathogens isolated from body surface and gut of African catfish and Nile tilapia
- To determine the load and prevalence of pathogens in aquaria under conditions of pre-and-post probiotic administration

1.4. Significance of the study

The study initiates the concerned body to focus on African catfish and provides information about controlling disease-causing microorganisms by using probiotics. Although probiotics isolated from the same source have promising probiotic potential in suppressing disease-causing pathogens, the study also showed the application of probiotics in enhancing the growth performance of fish. Moreover, the findings also serve as baseline data for further research and help alleviate the shortage of fingerling supply in African catfish aquaculture practice, and thus it would eventually have a paramount contribution in improving food security via improved aquaculture practices and production.

2. Literature Review

2.1. Aquaculture production in Ethiopia

The term aquaculture covers all forms of farming of aquatic animals and plants in freshwater, brackish water, and saltwater (FAO, 2012). Aquaculture is employed for a variety of reasons: fish may be raised to stock public waters for sport fishing and commercial fishing; it may be used to save an endangered species, or it may be used to harvest a commercially valuable crop in ponds or coastal waters. In simple terms, aquaculture is an extension of agriculture (Lee and Connelly, 2006).

A potential for aquaculture production in Ethiopia exists, but it is not yet fully realized. Aquaculture in Ethiopia is still in its infancy despite favorable physical conditions available in the country and fish species documented. The high central plateau above 2,500 m (11% of total area) has potential for all-year-round farming of cold water species. Likewise, the surrounding and central highlands present temperature characteristics favorable for the breeding of a large number of species, from cold water to warm water fish (Lakew *et al*., 2016)

In addition, Ethiopia is endowed with 12 drainage basins (8 river basins, 1 lake basin, and 3 dry basins) with a mean annual flow estimated at 122 billion cubic meters and a total length estimated at 8065 km. Similarly, the country has also many lakes and reservoirs, small water bodies, and floodplain areas covering a total surface area of about 13,637 km2 (Gashaw and Wolff, 2014). The reservoirs under construction such as the Grand Ethiopian renaissance dam will undoubtedly increase the total inland water by over fifteen percent at the end of their completion (Lakew *et al*., 2016).

In the same way, Ethiopia has very diverse agro-ecological zones offering a favorable potential for developing fish culture both in terms of land/water and in its climatic system. Based on physical, socioeconomic, climatic, and infrastructure suitability indicators, as well as the biology of the selected fish species, a GIS analysis was carried out. According to the FAO Sub-regional Office for Eastern Africa report about 15158 km2 and 871731 km^2 of the total country's land, respectively, is highly and moderately suitable for Nile tilapia pond culture. Nevertheless, this is equivalent only to about 1% of the country's surface area, it has been indicated to be sufficient enough to produce a significant amount of fish in pond aquaculture (Wakjira *et al*., 2013). On the other hand, as the report of Habtesilassie (2012) shows the country has nearly 200 freshwater fish species in its lakes and rivers. However, candidate

species for aquaculture development in Ethiopia include mainly only Nile tilapia (*Oreochromis niloticus*) and a few the African catfish (*Clarias gariepinus*) (Yalew *et al*., 2015).

2.2. African catfish

The African Catfish (*Clarias gariepinus*) is an opportunistic and omnivorous feeder ingesting a wide variety of food items such as algae, macrophytes, zooplankton, insects, fish prey, detritus, Amphibians, and sand grains (Admasu *et al*., 2015). The diet composition may vary within a season and spatial conditions of the environments (Houlihan *et al*., 2001). In the same way, the diet composition may also vary depending upon the fish size, maturity, and habitat differences (Kamal *et al*., 2010). According to Alemayehu (2009) report, the African catfish feed on a variety of foods based on the environment in which they live. However, there is no compiled information on the food and feeding habit of African catfish in different water bodies, which give the general perception of the feeding of the fish in the country.

The African catfish is widely distributed in Africa freshwater and the Middle East (Tesfahun, 2018). In Ethiopia, it is widely distributed almost in all water bodies such as in the rift valley, Abay, Awash, Baro-Akobo, Omo-Gibe, Tekeze, and Wabishebele-Genale basins (Awoke, 2015). African catfish has sharp tooth catfish, is a large, eel-like fish, usually of dark gray or black coloration on the back, fading to a white belly. In Africa, this catfish has been reported as being second in size maximum length, and weight (Figure 1)

Figure 1. African catfish (*Clarias gariepinus*)

In developing countries, fish has played an important role in resolving the sustainability of people's food and livelihoods. About 2.6 billion people consume 20% of their animal protein from fish, and over 400 million people in Asia and Africa consume at least 50% of their animal protein from fish. However, only 13% of animal protein consumption is provided in developing countries (FAO, 2008). In the tropics, fish is one of the most essential sources of animal protein, and it has long been recognized as a source of high-quality protein and other essential nutrients (Gomma, 2005).

African Catfish are rich in nutritional value and highly valued by consumers by a high-protein source that contains essential amino acids. It also has a high concentration of omega-3 fatty acids and a variety of other nutrients, such as vitamins fat-soluble, and micro-elements. Africa can be a very nutritious part of the human diet; it contains most of the vitamins he requires, a wide range of minerals, and all of the necessary amino acids are properly proportioned in the proteins, lipids, carbohydrates, minerals, and vitamins, as do many other animal products (FAO,2001). Consumption of African catfish has increased rapidly in recent years as a result of its availability, consistency, and health benefits (Hoke *et al*., 2000).

The food and feeding habits of freshwater fish species are an issue of continuous research. This is because it makes up a basis for the development of a successful management program on fish capture and culture. Moreover, studies on the natural feeding of fish enable us to the identification of the trophic relationships present in aquatic ecosystems, identifying the feeding composition, structure, and stability of food webs in the ecosystem (Otieno *et al.,* 2014). Freshly hatched African catfish larvae do not have a fully developed digestive system. First feeding catfish have little or no gut microbiota community as other larvae. They are exposed to microbes in their water, and feed (Christos *et al*. 2014). The presence of microbes in the system would influence the general well-being of the fish, the digestive tract, and the body. The inclusion of exogenous microbes like the probiotics could control or enhance the microbial communities of the fish (Enyidi and Uchenna, 2016).

2.3. Nile tilapia

Nile tilapia (*Oreochromis niloticus)* is an important fish in the ecology of tropical and subtropical aquatic ecosystems. In Ethiopia, Nile tilapia is widely distributed in lakes, rivers, reservoirs, and swamps, and contributes about 60% of the total landings of fish, but currently reduced to 49% (Gashaw and Wolff, 2014), and in Lake Ziway particularly its contribution has declined from 89.3% in 1994 to 27% in 2014 (Abera, 2016). The body of Nile tilapia is compressed; caudal peduncle depth is equal to length. Scales cycloid. A knob-like

protuberance is absent on the dorsal surface of the snout, upper jaw length showing no sexual dimorphism, lateral line interrupted, have a dorsal fin with 16 - 17 spines and 11 to 15 soft rays, anal fin with 3 spines and 10-11 rays, caudal fin truncated, color in spawning season, pectoral, dorsal and caudal fins becoming reddish; caudal fin with numerous black bars (Figure 2).

Figure 2. Nile tilapia *(Oreochromis niloticus)*

As a result of the declining contribution of Nile tilapia in Lake Ziway, around 70% of the annual catch of the lake is covered by exotic fish species (Abera, 2016). Nile tilapia is the most popular fish in Ethiopia as well as around the reservoir due to its value as a commercial and subsistence fish for most of the inhabitants living around the reservoir. This is because it has fewer bones in its flesh compared to the Barbus species (Tsegay, *et al.* 2018).

2.4. Fermented beverages (Tej)

Traditional fermented beverages are those fermented products produced from varieties of locally available substrates following indigenous knowledge developed by the local people over years. Thus, their manufacturing processes rely on old-age techniques and locally available rudimentary equipment (Kebede *et al.*, 2002). Besides their cultural importance, traditional beverages constitute a significant portion of people's diet because of their nutritional properties (Mulaw & Tesfaye, 2017). In fermented beverages, the sources of dominant microbes such as lactic acid bacteria (LAB) and yeasts are usually the ingredients and utensils used for fermentation processes and during fermentation, these microbes contribute to the lowering of pH resulting in suppression of growth of some unwanted microbes, improvement in organoleptic properties of the fermenting mash, and produce beneficial compounds. Such changes make fermented beverages good sources of energy and ideal products for consumption by majorities of the population (Phiri *et al*., 2019).

Tej is a popular Ethiopian fermented beverage and it is home-processed honey wine, yellow in color, sweet, and produced from water, honey, and leaves of Gesho (*Rhamnus prenoides*) (Bahiru *et al*, 2001). Microbial, physicochemical, and proximate analysis of selected Ethiopian traditional fermented beverages showed that Lactic acid bacteria and yeast were the dominant microbes in Tej samples (Nemo and Bacha, 2020).

2.5. Probiotics

The term probiotic is defined with clarity and distinctiveness as 'live microorganisms that confer a health benefit to the host when administered in an adequate amount (Hill, *et al.* 2014). Over the past decades, scientific investigations keen on probiotics and their health benefits have rocketed sky-high. Regardless of the beneficial effects on human health as good bacteria, probiotics have shown high potential in clinical practice. There are reliable proofs that probiotic microbes can hamper various ailments and infections or be useful in their health, particularly in direct connection to numerous gastrointestinal disorders (Floch, *et al.* 2015).

LAB are considered Probiotics as they have probiotic properties, including the production of some inhibitory compounds. These are also considered normal flora in the gastrointestinal tract of aquaculture animals and mammals (Sayyed *et al*, 2012). The acid and bile tolerance and antibiotic sensitivity are the fundamental properties of probiotics (Muthukumar and Kandeepan, 2015). Many microorganisms could potentially function as probiotics, of which *Lactobacillus* and *Bifidobacterium* species are the most commonly used. In addition, nonpathogenic species belonging to the class of *Saccharomyce*s, *Streptococcus*, and *Lactococcus* are also used as probiotics (Hemaiswarya *et al*, 2013). Furthermore, modern molecular techniques should be applied to ensure the species of probiotics used in aquaculture for quality assurance as well as safety (Bermudez *et al.*, 2012).

2.5.1. Sources of Probiotic Strains

The main sources may emanate from human origins like the human large intestine, small intestine, or even breast milk. It can also be from animal origins, various food biotopes such as raw milk or fermented food products. Probiotic strains isolated from human microflora are well characterized by high adhesive levels to the human intestinal epithelial barrier than others and are more likely to be safe. Nevertheless, several probiotic dietary foods and supplements may carry different bacteria and microbes with no history of safe use in humans or other animals (Zommiti *et al.*, 2020).

The hypothetical first niche of the ancestral LAB is considered soil and plants and, subsequently, the gut of herbivorous animals (Morelli *et al*., 2012). The mammalian intestine is colonized by 100 trillion microorganisms that are essential for health (Kamada *et al*., 2013). Scholars are usually isolated probiotics from traditional fermented foods and beverages, from different organs of humans and other animals (Koirala and Anal, 2021).

2.5.2. Selection Criteria and Requirements for Probiotic Strains

Safety, functionality, and technological utility represent the crucial criteria for the selection of probiotic microbes. This drastic selection was set up according to the World Health Organization (WHO), Food and Drug Organization (FAO), and EFSA (the European Food Safety Authority). Hill and co-workers (Hill, *et al*., 2014) revealed that probiotic potential is directly connected to particular strains, not to the genus or species of a microorganism (Zommiti *et al.,* 2020). The selection criteria for protic are acid tolerance, bile tolerance, production of antimicrobials, resistance to common antibiotics, and safe for consumption (Kosin and Rakshit, 2006). As for the functional aspects, viability represents a prerequisite for probiotic functionality it enhances several mechanisms, including adherence to epithelial cells, reduction of mucosal gut permeability, immuno-modulatory effects, and this represents an industrial challenge (Zommiti *et al.,* 2020). The probiotic dose levels should be based on the ones found to be efficient in human clinical trials, minimum effective values that are generally accepted that probiotic products should have a minimum concentration of 10^6 CFU and a maximum 10^8 to 10^9 per milliliter or gram (Kechagia, *et al*., 2013).

2.5.3. Mechanism of action of probiotics

During the past decade, microbiologists, immunologists, and gastroenterologists have actively studied the mechanism by which commensal bacteria improve mucosal defenses of the gastrointestinal tract. Inside a human organism, probiotics are responsible for the development of the microflora residing in the gastrointestinal tract in the way of ensuring an appropriate microbial balance flanked by pathogens and the good bacteria, also known as homeostasis (Oelschlarger, 2010). These beneficial microbes, through this equilibrium, could restore natural microbiota after antibiotic therapy (Carter, *et al*., 2017). Another astounding role played by probiotics is counteracting the activity of pathogenic intestinal microbiota. Thus, probiotics have a salient potential in inhibiting the growth of sturdy pathogens encompassing *Clostridium perfringens, Campylobacter jejuni, Salmonella enteritidis, Escherichia coli, Shigella spp, Staphylococcus, Yersinia*, spp, and *Listeria* spp thus preventing food poisoning (Schoster, *et al*., 2013; Chingwaru, *et al*., 2017).

An investigation showed that the mechanism of action of probiotic microorganisms depends on several factors such as their resistance to colonization, stimulation of phagocytosis, production of antimicrobial compounds, anti-mutagenic effects, chemokines production, and impact on enzyme activity and enzyme delivery (Vemuri, *et al*., 2014). Furthermore, large-scale molecular, bioengineered, and genetic investigations permitted to unravel the basic concept of the beneficial effect of good bacteria so-called 'probiotics' with the direct involvement of four mechanisms: Microbial antagonism via the exertion of antimicrobial, compounds, competitively with pathogenic bacteria for adhesion to the epithelium and immune modulation of the host and inhibition of bacterial toxin production (Carter, *et al*., 2017).

2.5.4. Safety of Probiotics

Generally, probiotic microorganisms are well distinguished by their safe aspect with Generally Regarded as Safe (GRAS) by the World Health Organization (WHO). Safety for human health corresponds to the salient determinant for probiotic selection. Probiotic strains should be characterized by the absence of their virulent profile and their low resistance to antibiotics (Shanahan, 2012). These beneficial microbes have had a good safety record during history, primarily related to the use of *Lactobacilli* and *Bifidobacteria* strains (Patel, *et al.,* 2014). Experience and field trials with other microbial species used as probiotics are more limited (Shanahan, 2012).

The selection criteria of new potential probiotic microorganisms target new bacterial strains and even new genera with higher beneficial potential and/or with more particular properties, and this is not an easy task. The introduction of novel microbes needs acute investigations and assessment of their safety and the risk-to-benefit ratio. New probiotic bacteria must belong to genera and strains commonly found in the healthy human intestinal microbiota, and caution must be taken for bacteria belonging to the genus Bacillus or Enterococcus, in which pathogens or opportunistic pathogens have also been described (Hanchi, *et al.,* 2018).

The majority of probiotics are safe. The world of probiotics is continuously growing, not only by the increasing number of people who use probiotics but also by the variety of probiotic products and novel probiotic strains. Future investigations and scientific studies need to report a more detailed description of the tested probiotic microbe encompassing the genus, species, and strain level, additionally, to the daily dose and the duration of the treatment (Bull, *et al.,* 2013). Three major elements composed from the public, healthcare providers, and manufacturers have to win the challenges face to face to probiotics, in purpose to focus on

international regulations and standards and to provide guidance for strain-specific evidence-based therapy (Dinleyici, *et al.*, 2014).

2.5.5. Importance of Probiotics

2.5.5.1.Increase Nutritional value

Beneficial effects for finfish farmers exerted by probiotic applications encompass nutritional, metabolic, and health effects. This particularly includes increased growth performance and appetite, enhanced food conversion by an enzymatic contribution to digestion, improved feed value (macro and micronutrients made available by the probiotic), inhibition of pathogenic microorganisms (adherence and colonization), stimulation of the immune system, increased stress resistance, and improved general vigor (Wuertz *et al*., 2021) (Figure 3).

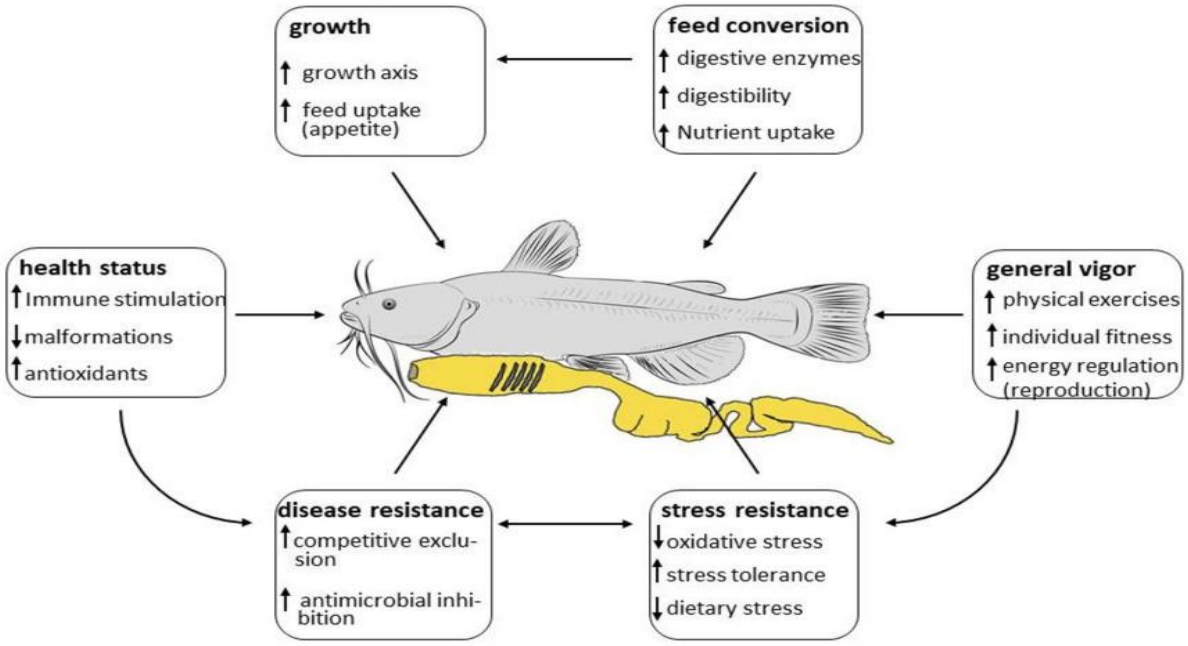

Figure 3. Stimulate fish immune system and enhance general vigor (active body or mental strength) (Wuertz *et al*., 2021).

2.5.5.2.Enhance growth (Height and weight)

Probiotics can act either directly by increasing appetite and growth regulation or indirectly via improved digestibility. In tilapia, weight can increase by up to 115.3%, but growth performance might have been linked to the better feed conversion reported. Nevertheless, probiotics stimulate the growth axis, increasing the transcription of insulin-like growth factor 1 and the growth hormone receptor (Yi *et al*., 2019). The use of an additive containing *Bacillus subtilis*, *Bifidobacterium bifidum*, *Enterococcus faecium*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus lactis*, *Lactobacillus plantarum,* and *Pediococcus acidilactici* reduced the

expression of myostatin, thereby enhancing the growth of white muscle. Still, in a study on *Lactobacillus acidophilus*, the food-intake-stimulating hormone ghrelin was downregulated (Hosseini *et al*., 2016).

Probiotics increase the growth performance and appetite, enhance feed conversion by secretion of microbial digestive enzymes or improve feed value (macro- and micronutrients), increase stress tolerance, improve disease resistance due to the inhibition of pathogenic microorganisms (adherence and colonization), improve health status by stimulating the fish's immune system. In tilapia, weight can increase by up to 115.3%, but growth performance might have been linked to the better feed conversion reported (El-Haroun *et al*., 2006).

2.5.5.3.Feed Conversion

There are several studies on probiotics that report increased feed conversion, but establishing a complex microbiota restored nutrient uptake, suggesting that the gut microbiota contributes substantially to the nutrient uptake and assimilation of the host. Probiotics also convert less degradable compounds into forms that can be easily digested by the host. Various microbial enzymes, such as lipases, phytases, amylases, cellulases, trypsin, and other proteases, can be involved (Santos *et al*., 2020; Zhang *et al*., 2021). In modern aqua-feeds, supplemented with high amounts of plant ingredients, specific probiotics may increase the digestion of feed components such as no starch hydrocarbons, cellulose, or chitin, which are indigestible for the fish host. In addition, probiotics are sources of vitamins (Eck and Friel, 2013).

2.6. Lactic Acid Bacteria

2.6.1. General characteristics and classification

Lactic acid bacteria are Gram-positive, non-spore-forming, non-respiring but aero-tolerant, which produce lactic acid as one of the key fermentation products by utilizing carbohydrates during fermentation. These bacteria produce lactic acid as an end product of carbohydrate catabolism and also make organic substances that contribute to the flavor, texture, and aroma that result in unique organoleptic characteristics (Sadishkumar and Jeevaratnam, 2017). Orla Jensen (1919) first published a monograph that laid the foundation for classifying lactic acid bacteria. This system of classification was linked to certain factors that entailed the following; glucose fermentation characteristics, cell morphology, capacity to utilize sugars, and optimum growth temperature range. This classification system thus recognized genera: *Lactobacillus, Pediococcus, Leucnostoc, and Streptococcus* (Quinto *et al*., 2014).

Lactic acid bacteria have also been classified into different genera/species based on their acid production characteristics by fermenting sugars and their growth at specific temperatures (Parvez *et al*., 2006). Additionally, the LAB can be classified as homofermentative or heterofermentative organisms based on their ability to ferment carbohydrates (Mokoena, 2017). The homofermentative lactic acid bacteria such as *Lactococcus* and *Streptococcus* yield two molecules of lactates from one glucose molecule whereas heterofermentative such as *Leucnostoc, Wiessella,* and some *Lactobacilli* generate lactate, ethanol, and carbon dioxide from one molecule of glucose (Mokoena, 2017). The conventional approach to LAB classification was based on physiological and biochemical characteristics; however, more recently, molecular characterization has become an important tool for classification.

2.6.2. Antimicrobial Activity of LAB

2.6.2.1.Organic acids

Organic acids, particularly lactic acids are generally thought to exert their antimicrobial effect by interfering with the maintenance of cell membrane potential, inhibiting active transport, reducing intracellular pH, and inhibiting a variety of metabolic functions (Rattanachaikunsopon & Phumkhachorn 2010). The production of lactic acid and reduction of pH are depended on species or strain, culture composition, and growth conditions (Olaoye & Onilude 2011). They have a very broad mode of action and inhibit both gram-positive and gram-negative bacteria as well as yeast and molds (Rattanachaikunsopon & Phumkhachorn 2010). At low pH, a large amount of lactic acid is in the un-dissociated form, and it is toxic to many bacteria, fungi, and yeasts. However, different microorganisms vary considerably in their sensitivity to lactic acid (Soomro *et al*., 2012).

2.6.2.2.Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is widely used in the fields of foods, pharmaceuticals, dental products, textiles, environmental protection, and it is also involved in advanced oxidation processes and various biochemical processes (Abbas et. al. 2010). H_2O_2 is also produced by LAB in the presence of oxygen. The antimicrobial effect of H_2O_2 may result from the oxidation of sulfhydryl groups causing denaturing of several enzymes, and from the peroxidation of membrane lipids thus the increased membrane permeability and also be as a precursor for the production of bactericidal free radicals such as superoxide (O^{-2}) and hydroxyl (OH-) radicals which can damage DNA (Sunil & Narayana 2008). H_2O_2 can have a strong oxidizing effect on membrane lipids and cellular proteins and is produced using such enzymes as the flavoprotein oxidoreductases, NADH peroxidase, NADH oxidase, and α-glycerophosphate

oxidase (Rattanachaikunsopon & Phumkhachorn 2010). The synthesized H_2O_2 can inhibit the growth of psychotropic and pathogenic microorganisms (Zalan *et. al*. 2005). Some foodborne pathogens, such as *Aeromonas hydrophila, Listeria monocytogenes, Yersinia enterocolitica*, and *Clostridium botulinum* can grow 5 °C (Abbas et. al. 2010).

2.6.2.3.Bacteriocins

Bacteriocins are heat-stable, ribosomal synthesized antimicrobial peptides. Both Gram-positive and Gram-negative bacteria and archaea release antimicrobial peptides extracellularly in the late-exponential to the early-stationary growth phases that attribute the antimicrobial activity against different bacteria, fungi, parasites, viruses, and even against natural resistant structures, such as bacterial biofilms (Martín-Escolano *et al*., 2019). Interestingly, the combination of bacteriocins and antibiotics has been proposed as novel therapeutic options for food-producing animals. The possibility of replacing the use of antibiotics is explored to avoid bacterial resistance. Various reports have also established LAB bacteriocins' advantages and synergistic actions with other biomolecules, such as nisin and citric acid, against *Staphylococcus aureus* and *Listeria monocytogenes* (Kumariya *et al*., 2019). It has also been documented that bacteria can develop resistance to bacteriocins. However, resistance to bacteriocins is minimal compared to conventional antibiotics. Since the frequency of spontaneous mutations in cells exposed to bacteriocins is low. This resistance is generally through modifications in the cell envelope, such as alterations in the charge and thickness (McBride and Sonenshein, 2011).

2.7. Common Pathogen of fish

Fish disease outbreaks adversely affect aquaculture production), and losses are particularly high in the tropics where mitigative interventions are limited (Leung and Bates, 2013). Although aquaculture is increasing in the East African region, the risk of losing profits due to diseases and parasites is already manifesting (Akoll and Mwanja, 2012). The presence of human pathogenic microorganisms in fish and fish products may be affected by various factors, including cultural practices, environmental conditions, processing, and distribution of products. The most important fish pathogens can be generally divided into two groups: those native to natural freshwater habitats and those associated with water pollution. The bacterial species include, *Shigella spp.*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella spp.*, *Yersinia spp., and Staphylococcus aureus* represent both groups of bacteria mentioned above native freshwater habitats and contaminants arising from different sources, including sewage and direct contamination by wild animals, livestock, and feed (Bottone *et al*. 2005; Adgamov *et al*. 2013).

Fish pathogens are very common and are one of the most difficult health problems to deal with. These bacteria are generally saprophytic and only become pathogenic when fishes are physiologically unbalanced, nutritionally deficient, or there are other stressors such as poor water quality, overstocking, which allow opportunistic bacterial infections to proceed (Sandeep *et al*., 2016). Bacterial diseases have been frequently encountered in eggs, fry, fingerlings of fish, causing heavy mortality. These microorganisms are essentially opportunistic pathogens that invade the tissues of a fish host rendered susceptible to infection by stress factors. The occurrence of bacterial diseases was not considered to be a serious problem in our country, as economic losses in fish culture are not known. Economic losses in many countries. Some of the important bacterial pathogens such as *Vibriosis* spp, *Streptococcal* spp, *E. coli, Salmonella* spp, and *Pseudomonas* spp are often being reported in carp culture (Mohanty and Sahoo, 2007). In general, there are four types of bacterial infections: fin rot - usually resulting from environmental stress, bacterial body ulcers-open, shallow to deep, lesions on the fish's body, through gills as the primary target and invade and cause damage to internal organs (Das, 2011).

2.8. Common disease caused by pathogens in fish

Staphylococcus spp

Two species of staphylococcus have been reported to cause staphylococcosis in fish, S. aureus and *S. epidermidis. Staphylococci* may be present in fish throughout the year, but the disease is induced by a sudden rise in water temperatures or other stress factors in the aquatic environment (Ibrahim, 2020). Disease caused by *Staphylococcus* spp showed the symptoms like a pronounced eye with the cornea becoming reddish, due to vascularization, and then opaque. Thereafter, there was degeneration of the eye tissues, leaving a hollow cup, the brain and optic nerves were affected (Austin & Austin, 2012).

Pseudomonas spp

Fish pathogenic *Pseudomonas* includes *P. anguilliseptica, P. baetica, P. chlororaphis, P. fl uorescens, P. koreensis, P. luteola, P. plecoglossicida, P. pseudoalcaligenes*, and *P. putida*, which are the causes of Sekiten byo (= red spot), a disease of wedge sole without external or internal disease signs, distended abdomen and hemorrhaging on the body surface, fin/tail rot with or without the presence of external hemorrhaging, hemorrhagic septicemia, bacterial hemorrhagic ascites of ayu, extensive skin lesions, and exophthalmia with external ulceration, respectively. Molecular diagnosis has been achieved with *P. anguilliseptica* (Austin & Austin, 2012).

***Salmonella* spp**

Salmonella is not a biological contaminant originally reported in fish, being introduced through contaminated water or improper handling (Sant'ana, 2012). Salmonella causes the septicaemic condition, eyes (corneas) displaying opacity and Lesions and congestions in the mucus membranes of the stomach and intestine (Austin & Austin, 2012).

***Bacillus* spp**

Diseased fish were characterised by weakness, lethargy, emaciation and generalized necrotising dermatitis, with death occurring in a few days. Blood tinged fluid was present in the peritoneal cavity. Petechia and focal necrosis was evident in the liver and kidney. The spleen was enlarged, soft and friable. The myocardium was described as soft and flabby. The stomach was hyperaemic (Austin & Austin, 2012).

Esherchia coli

Fish infected with pathogenic E.coli has the following infectious processes: bleeding in the pectoral fin, ulceration head above the eyes (Oliveira *et al*., 2014).

Candida albicans

Fish infected with candida albicans has the following symptoms: respiratory manifestations typified by rapid opercular movement, swimming near surface of water, gasping of atmospheric air, haemorrhagic patches scattered over the body especially at the base of pectoral and dorsal fins, uni- or bilateral exophthalmia, dark discolouration of skin with or without detachment of scales and sometimes button- like ulcers on the area of caudal peduncle (Zayed *et al.*, 2016)

2.9. Antibiotic Resistance of Pathogens

The use of antibiotics in food-growing facilities is responsible for resistant bacteria in the food chain. Many food animals and birds are the natural reservoirs of human pathogenic bacteria such as *Salmonella enterica*, Shiga toxin-producing *E. coli*, and *Campylobacter* (Heredia and García, 2018). The use of antimicrobials in food animals naturally leads to the development of resistance in these bacteria, which get easily transmitted to humans via the food. Also, since resistant bacteria reside in these food animals, their excreta can contaminate the environment and reach humans via water or food (Kumar *et al*., 2017).

Further, humans can acquire such bacteria through touch or contact with pet or livestock animals carrying resistant bacteria such as MRSA. When the resistant mechanisms are associated with plasmids and the transposons, they spread quickly among related and unrelated

bacteria. The magnitude of human health risk due to antibiotic-resistant bacteria has been highlighted by a study in which ready-to-eat shrimps from four countries were found to harbor multidrug-resistant bacteria (Duran, and Marshall, 2005). Forty-two percent of the isolates from shrimp were resistant to antimicrobial agents, including important human pathogens such as *E. coli*, *Enterococcus* spp., *Salmonella* spp., *Shigella flexneri*, *Staphylococcus* spp., and *Vibrio* spp. The presence of antibiotic-resistant bacteria in ready-to-eat products constitutes a significant human health hazard since the consumers are directly exposed to the risk of infection by these pathogens upon consumption (Singh *et al*., 2016). In a study in the United States, 105 Salmonella strains isolated from imported seafood during 2000-2005 were tested for antibiotic resistance, and two isolates belonging resistant to trimethoprim/sulfamethoxazole, sulfisoxazole, ampicillin, tetracycline, and chloramphenicol were found, which were isolated from seafood imported from India and Vietnam (Khan *et al*., 2009).

3. Materials and Methods

3.1. Description of the study area

The study was conducted in Oromia Regional State, Jimma zone, in Jimma city (Figure 1). Jimma city is located 353 km southwest of Addis Ababa. Jimma is mainly known for being an origin of coffee and its immense coffee production. It lies between 1,500 - 2,400 m above sea level and is considered ideal for agriculture as well as human settlement. The city is generally characterized by a warm climate with a mean annual maximum and minimum temperature of 30 and 14^{o}C, respectively. The annual rainfall ranges between 1138 to 1690

mm (Alemu *et al.*, 2011). Jimma city has sold several types of foods of plant and animal origin. From the animal origin, food milk, cattle fillet, sheep and goat fillets, and fish fillet are common.

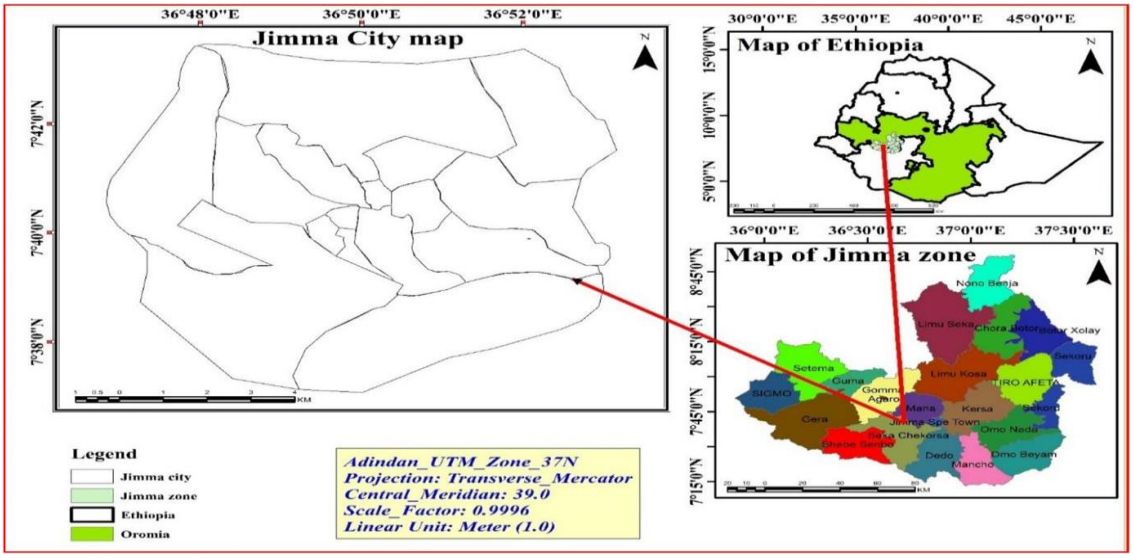

Figure 1. Map of the study area

3.2. Study design, sample size, and sampling technique

A cross-sectional study design was employed. A total of 14 fish samples were used for this study. A purposive sampling technique was used to collect fish samples.

3.3. Sample collection

A total of 14 fish samples (7 of each African catfish and Nile tilapia) were collected from Boye river, Jimma city, using a large plastic bucket (sterile) of 40 L capacity containing approximately 30 L of water from the river and transported to Research and Postgraduate Laboratory, Biology Department, Jimma University, for isolation and characterization of LAB, evaluation of probiotic properties of LAB isolates, and detection of pathogens. The samples were kept in a large plastic bucket containing water (from the source) until examined.

3.4. Sample preparation for isolation of LAB

From the fish body surfaces, a 4cm^2 area (prepared by cutting 2cm x 2cm square at the center of aluminum foil and placed on the body surface of fish) was swabbed and a tip of the swabbed cotton was cut using sterile tweezers and inoculated into 9 ml peptone water. Then, the sample was homogenized in a vortex mixer for 1 minute (Banu *et al*, 2015). The isolation of LAB from the gut was done based on the protocol of Liu *et al*. (2014). Briefly, the surface of fish samples was sterilized using 70% ethanol and the gut was aseptically removed by dissecting the fish using sterile tweezers and 10 g of it was ground by the grinder and homogenized in 90 ml of peptone water, followed by further homogenization for 5 ml at 250 rpm in a shaker. Then, 1 ml each of homogenized samples (from body surface and gut) was separately transferred to 9 ml of peptone water and serial dilution was done up to 10^{-6}. After then, 0.1 ml of an aliquot from appropriate dilution was spread plated in de Man Rogosa Sharpe (MRS) agar and incubated anaerobically at 32°C for 24 hrs. Then, the district colonies were further purified by repeated plating.

3.5. Characterization of LAB

A total of 5 to 10 distinct colonies were randomly picked from countable plates and aseptically transferred into a tube containing 5 ml MRS broth. The inoculated cultures were incubated anaerobically at 32°C for 24 hrs. Cultures were purified by sub-culturing and preserved on slants at 4°C for a month. Finally, the obtained LAB was characterized using morphological, biochemical, and physiological tests and tentatively identified to genus level using Bergey's Manual of Systematic Bacteriology Volume 3 (Vos *et al*., 2009).

3.5.1. Cell morphology

Gram staining

A smear of pure isolates was prepared on a clean slide and allowed to air-dry and heat-fix. The heat-fixed smear was flooded with crystal violet dye for 1 minute and rinsed under tap water for 3 seconds. Then, the slide was flooded with iodine solution for 1 minute and rinsed under tap water for 3 seconds. After rinsing, the smear was decolorized with 96% of ethanol for 20 seconds and washed slide gently under tap water for 3 seconds. Thereafter, the smear was counterstained with safranin and dried using absorbent paper. Finally, the air-dried smear was observed under the oil immersion objective. After the Gram staining, gram-negative bacteria are stained pink/red and gram-positive bacteria are stained blue/purple (Gram, 1884).

Motility Test

Pure broth culture of LAB was taken by a sterile needle and stabbed straight vertically into a test tube containing motility medium [Tryptose (10g/ L), NaCl (5g/ L), and Agar (5g/ L), final pH adjusted to 7.2 ± 0.2] to the bottom of the tube and incubated anaerobically, using anaerobic jar (BBL, Gaspak System, Hitech e-601, China) at 32°C for 24 h. A positive motility test was indicated by a turbid area diffusing away from the line of inoculation and a negative test was indicated by growth along the inoculation line only, or no further growth (Shields and Cathcart, 2011).

Endospore Test

A smear of isolates was prepared on a clean glass slide and allowed to air-dry followed by heat fixing and placed on wire gauze at the top steaming (Boiling water in a beaker), then flooded with 0.5% (w/v) malachite green solution and steamed for 5 minutes. After cooling, the slide was washed with tap water and counterstained with safranin, and stayed for 30 seconds. The slide was washed with tap water, air-dried, and then observed under the oil immersion objective (×1000) for pink color indicating vegetative cell and green color in the confirmation for the presence of endospore (Schaeffer and Fulton, 1933). LAB are non-spore formers and are negative for spore test, hence no green colored expected.

3.5.2. Biochemical test

KOH-test (Test for Lipopolysaccharide)

Two drops of 3 % KOH solution were placed on a clean microscopic slide. A colony was aseptically picked from MRS agar using an inculcating loop and stirred in the KOH solution for 10 seconds to 2 minutes. The inoculating loop was raised slowly from the mass when the KOH solution becomes viscous, the thread of slime followed the loop for 0.5 to 2 cm or more (for Gram-negative bacteria) and no slime as the watery suspension did not follow the loop (for Gram-positive bacteria) (Gregerson, 1978).

Catalase Test

A catalase test was carried out by flooding young colonies with a 3% solution of H_2O_2. The formation of bubbles indicated the presence of catalase and no bubbles for the absence of catalase enzyme (MacFaddin, 1980).

Cytochrome oxidase test

The pure isolates of LAB from the plate were rubbed on filter paper then three drops of the oxidase reagent were added onto the rubbed filter paper. Then, isolates were considered oxidase-positive when the color changed to dark blue and negative when the color was not changed within 30 seconds (Kovacs, 1956).

Carbohydrate fermentation test

For carbohydrate fermentation, 1 ml of overnight culture transferred to 10 ml of Phenol Red Broth containing 2% of glucose, inserted with Durham tube and incubated for 48 hrs at 32°C. Finally, the change of color from red to yellow indicated the production of acid (fermentation only), bubble formation (CO_2) in Durham tubes indicated the positive result for gas production. No color change showed no fermentation (Hassan, 2018).

3.5.3. Physiological test

3.5.3.1. Temperatures Tolerance

Loopful of the selected overnight LAB cultures were streaked on MRS agar and incubated anaerobically at 15, and 45°C for 48h. Thereafter, the growth of LAB was used to designate that the isolates are tolerant to that specific temperature (Tambekar and Bhutada, 2010).

3.5.3.2. Salt tolerance

Loopful overnight cultures of LAB cultures were separately inoculated into MRS agar supplemented with 2, 4.5 & 6.5% NaCl and incubated anaerobically at 32ºC for 48 h. Then, the growth of the culture was considered that the isolates were tolerant to the specific salt concentration (Ayo-Omogie & Okorie, 2016).

3.6. Evaluation of Probiotic Properties of LAB

3.6.1. Acid tolerance test

From overnight culture, 1ml of LAB was transferred into sterile MRS broth adjusted to pH 2, and 3 using HCl and NaOH and incubated anaerobically at 32ºC for 24 h. After incubation, a loopful culture was streaked on MRS agar and incubated anaerobically at 32ºC for 48 h. The growth of LAB on MRS agar showed as pH tolerant (Ayo-Omogie & Okorie, 2016).

3.6.2. Bile tolerance test

For bile tolerance, 1 ml of the overnight culture was added to MRS broth previously adjusted with bile salts at different concentrations of 0.3 & 0.5% and incubated anaerobically at 32°C

for 24h. Then, the survival of the isolates was assessed by inoculating a loopful of isolates on MRS agar and incubating anaerobically at 32°C for 24 h. Finally, the growth LAB on MRS showed bile tolerance (Kim *et al*., 2018).

3.6.3. Evaluation of Antimicrobial Activity of LAB

The antimicrobial activity of the selected LAB isolates cell-free supernatant (CFS) against *Bacillus cereus (*ATCC® 25923™), *Staphylococcus aureus subsp. aureus* (ATCC® 25923™), *Salmonella enterica subsp. enterica serovar* Typhimurium (ATCC®13311™), *Escherichia coli* (ATCC®25922™), and *Candida albicans* (ATCC®14053™) were tested by the Agar well diffusion assay. A volume of 2 mL overnight cultures of LAB was transferred to the Eppendorf tube and centrifuged at 10,000 rpm for 10 minutes adjusted at 4°C. The CFS was removed from cell pellets carefully and filtered by membrane filtration (0.45 µm). On the other hand, the cell density of each overnight activated reference pathogens was adjusted to turbidity standard of 0.5M McFarland and swabbed on pre-dried surfaces of Muller Hinton Agar (MHA) using a sterile cotton swab. A well with a 6 mm diameter was prepared using a sterile cork-borer on previously inoculated MHA and each well was filled with 100 µl of CFS of LAB isolates. The plates were incubated anaerobically at 32°C for 24 h and the zone of growth inhibition (mm) was measured using a transparent ruler. Accordingly, the antimicrobial activities of all the isolates were assessed (Appendix 2) (Muthukumar and Kandeepan, 2015).

3.6.4. Antibiotic Sensitivity Test

Antimicrobial susceptibility testing of the isolates was done using the disk diffusion method. Briefly, overnight LAB cultures were swabbed using cotton swab onto MRS Agar (Oxoid) and the following standard drug discs with their respective potency (µg/disc): chloramphenicol (30), ciprofloxacin (5), clindamycin (2), erythromycin (15), kanamycin (30), penicillin G (10), and streptomycin (10) were placed using forceps on the medium and incubated anaerobically at 32°C for 24 h. Then, the zones of inhibition were measured manually with a transparent ruler. resistant for purpose of analysis.

3.7. Selection of Probiotic

LAB isolates that had the best antimicrobial activity (≥15mm), tolerated pH 2, grown in a wider range of temperature, tolerated 0.5% bile salt, and were resistant to common antibiotics were selected for the aquarium experiment (Kosin and Rakshit, 2006).

3.8. Isolation of common fish Pathogens

3.8.1. Isolation of *Salmonella* and *Shigella* spp

For isolation of *Salmonella* and *Shigella* spp., 10 g of fish gut sample was mixed with 90 ml of Buffered peptone water (BPW). For fish body surface, 4cm^2 swab from a surface was mixed with 9ml BPW and incubated at 37°C for 24 hrs. Then 1 ml pre-enrichment broth culture was added to 10 ml of Rappaport-Vassiliadis enrichment broth and again incubated at 37°C for 24 hrs. Thereafter, a loopful of suspension from a tube was streaked onto Salmonella and Shigella agar (Ashrafudoulla *et al.,* 2021). Then biochemical tests like KOH, endospore, catalase, oxidase, triple sugar fermentation, urease, citrate utilization**,** motility, sulfide, and indole production were conducted for confirmation of the presumptive Salmonella/Shigella isolates.

Triple Sugar fermentation test

The butt was stabbed and the slant was streaked and incubated at 37°C for 24 hrs to detect fermentation of glucose, sucrose, and lactose as well as production of H_2S (Osama, 2021). The presence of alkaline (red) slant and acid (yellow) butt, with or without production of H2S was considered as presumptive for *Salmonella* spp.

Citrate utilization test

The slant was streaked and the tube was incubated at 37°C for 24 hrs to determine citrate utilization as a sole source of carbon (Osama, 2021). The presence of growth and color change from green to blue was considered as presumptive for *Salmonella spp.*

Motility, Sulphide and Indole production test

The SIM medium was stabbed to the bottom and incubated at 37°C for 24 hrs for the determination of H_2S production, indole production, and motility (Osama, 2021). The non-utilization of indole and absence of deep red color at the surface of agar was considered as presumptive for *Salmonella* spp.

Urease test

Urea Agar Base slant was streaked and the tube was incubated at 37°C for 24 hrs to assess the hydrolysis of urea (Anupam *et al.,* 2015). No color change was considered as negative and thus presumptive for *Salmonella spp.*

3.8.2. Isolation of *Staphylococcus aureus*

From the gut, S. *aureus* was isolated by taking 10 g of the gut and mixed with 90 ml of peptone water and swab fish body surface using a cotton swab with an area of $4cm^2$ and a tip end that swabbed the surface was cut using sterile tweezers. Then, 1ml of the homogenates were serially diluted up to 10^{-6}. Finally, 0.1 ml were spread plated on Mannitol Salt Agar (MSA) and incubated at 37°C for 48 hrs. Then, golden-yellow colonies surrounded by red color from MSA were subjected to tests like Gram staining, motility, oxidase, catalase test. Coagulase, and DNAase test

DNase test

Touch a colony of the pure culture with a loop and was inoculated onto a small area of the DNase test agar plate, in the middle of one of the marked sections, to form a thick plaque of growth 5-10 mm in diameter after incubation. Incubated the plate at 37°C for 18-24hr.

Coagulase test

Coagulase test was done using slide test. Briefly, a colony of the pure isolates was emulsified in a drop of distilled water on two ends of a clean glass slide to make thick suspensions. One was labeled as test and the other was as control. A loopful of human blood plasma was added to one of the suspensions and mixed gently. Clumping within 10 seconds was observed for coagulase-positive organisms

3.8.3. Isolation of *Listeria* species

For detection of *Listeria spp.*, 10 g of fish sample was mixed with 90 ml of Buffered peptone water (BPW) and homogenized using a shaker at 250 rpm (for the gut) and a swab vortexed with 9 ml BPW (for surface) were incubated at 37ºC for 24hrs. Then, 1 ml of homogenates was transferred to 10 ml of *Listeria* enrichment broth and incubated at 37◦C for 24hrs. After that, a loop full of culture was streaked onto Listeria Selective Agar and incubated for 24 hrs at 37ºC. Finally, Listeria colonies with dark halos and suspicious colonies were subjected to biochemical tests (Shivaramu, 2015). The biochemical tests like KOH, endospore, catalase, oxidase, triple sugar fermentation, urease, citrate utilization, motility, sulfide, indole production, carbohydrate fermentation, and β hemolysis Activity were conducted.

Carbohydrate fermentation of *Listeria spp*

The ability of microorganisms to ferment certain sugars was performed by inoculating 1 ml of overnight culture to 10 ml of Phenol Red Broth containing 2% of glucose, sucrose, mannitol,

and xylose separately inserted with Durham tube and incubated for 24 hours at 37°C. Finally, the change of color from red to yellow indicated the production of acid (fermentation only), bubble formation (CO_2) in Durham tubes indicated the positive result for gas production. No color change showed no fermentation (Rhaiem, 2016)

β hemolysis Activity

The *Listeria spp* β haemolysis activities were conducted by streaking of overnight cultures onto the blood agar supplementing with 5% (v/v) sheep blood and incubated at 37°C for 48 h. Then, lyses zones or clear zone around colonies were considered β-haemolytic activity (Hitchins, 1998).

3.8.4. Isolation of *Pseudomonas* species

For detection of *Pseudomonas* spp, from appropriate dilutions from gut and body surface, 0.1 ml of the aliquot was spread plated onto Pseudomonas Agar Base and incubated at 37°C for 48 hrs (Su *et al*., 2018). Then a biochemical test like KOH test, endospore, motility, catalase, oxidase, urease test, citrate utilization, Indole test, sulfide production test, and sugar fermentation tests (glucose, lactose, sucrose, maltose, and mannitol). The biochemical characters showed gram-negative, positive citrate utilization test, positive urease test, acid and abundant gas production from glucose, lactose, sucrose, maltose, and mannitol sugar fermentation test.

3.8.5. Isolation of *Escherichia coli*

For detection of *Escherichia coli*, from appropriate dilutions, 0.1 ml of the aliquot was spread plated onto Eosin methylene blue (EMB) and incubated at 37°C for 24-48 hrs (Su *et al*., 2018). Then a series of biochemical tests like, urease production, citrate utilization, Triple Sugar fermentation, and indole and sulfide production, motility, and MR-VP were conducted.

Methyl Red Voges –Proskauer (MR-VP) test

This medium was stabbed to the bottom and incubated at 37°C for 24 hrs for the determination of acid production for methyl red and acetoin production in Voges – Proskauer. Methyl red was added to the MR tube. A red color indicates a positive result (glucose can be converted into acidic end products such as lactate, acetate, and formate). A yellow color indicates a negative result; glucose is converted into neutral end products. For Voges -Proskauer first alpha-naphthol and then potassium hydroxide was added to the VP tube. The culture was allowed to sit for about 15 minutes for color development to occur. If acetoin was produced,

then the culture turns a red color (positive result); if acetoin was not produced then the culture appears yellowish to copper in color (a negative result). The non-production of red color as a result of the absence of deep red color in the medium was considered as presumptive for *Salmonella spp*

3.9. Pre and post-assessment of pathogens from an aquarium

The pre and post-assessment of pathogens from the aquarium was done by taking samples first from the aquarium before probiotics were applied (pre-assessment) and after probiotics were applied, 30 days later (post-assessment of pathogens). Five treatments (control, T1, T2, T3, and T4) and the detail of each treatment was written under section 3.10. Pathogens such as *Pseudomonas spp, S. aureus*, *E. coli, Listeria spp, Salmonella spp,* and *Shigella spp* were assessed in accordance with the previous procedure (Section 3.8).

3.10. Probiotic preparation and treatment

The best selected probiotic bacteria from African catfish (AFG10 and AFG8), and Nile tilapia (NTG8), both isolated from the fish, and JUT89 (isolated from Ethiopian traditional alcoholic beverage *Tej*, previously characterized and identified by Reda Nemo, PhD. Candidate) were used. The probiotic application was done following the procedure used earlier by Fakhri *et al*. (2019) and Masjudi *et al*. (2020). Briefly, a total of 10 glass aquaria having 5 treatments (control, T1, T2, T3, and T4) was used in this experiment (Table 1). Each aquarium was filled with 100 L of chlorine-free water in a 144 L capacity glass aquarium**.** Then**,** 30-day old larvae (Artificially produced) of African catfish were distributed to 10 glass aquaria with each having 23 fish per aquarium. Then, in the first aquarium, only feed (Phytoplanktons and Zooplanktons) without probiotic (control), Treatment 1 [feed+0.5ml of *Lactobacillus* spp 1 (isolated from African catfish)], Treatment 2 [feed+0.5ml of *Lactobacillus* spp 2 (isolated from African catfish)], Treatment 3 [feed+0.5ml of *Lactococcus* spp (isolated from Nile Tilapia)], Treatment 4 [feed+0.5ml of *Lactobacillus* spp (isolated from Tej)] each with a duplicate.

The probiotics at the concentration of 6 to 8 log CFU/ml (Checked by spread plating method on MRS) were activated in Research and Post Graduate Laboratory every 3 days (72 hrs) and moved to Aquaculture and Fisheries Laboratory, Jimma University, Department of Biology, using an anaerobic jar where the aquarium is present. The probiotics were inoculated into each aquarium water by using sterile syringes at three-day intervals with a dose of 0.5ml/100L and lasted one month. Regarding feed, initially, the larvae were fed on live feed (LF), and artificial dry feed (DF) at an inclusion level of 50% DF+50% LF**.** The diet was offered daily to the fish at a rate of 10% of their body weight throughout probiotic and feed treatments at a rate of three

times per day. Accumulated wastes and feed remnants were removed from each aquarium at a two-day interval by siphoning off 50% of the water volume per aquaria, followed by an equal replenishment of the volume of water. Moreover, all aquaria were supplied with an aerator (Sebo sb-648A, China) and aqua heater (Jebo 2010, power 300W, and frequency 50-60, China). Finally, mean weight, weight gain (Mean final weight – Mean initial weight), and mean total length, was analyzed (Fakhri *et al*., 2019; Masjudi *et al*., 2020).

Table 1. Summary of the experimental design for probiotic treatments

Aquarium	Treatment type	Components
1	Control	Only feed
2	T1	0.5ml of *Lb* spp$^{1(ACF)}$+feed
3	T2	0.5ml of *Lb* spp$^{2(ACF)}$+feed
4	T3	0.5ml of *Lc* spp$^{1(NT)}$+feed
5	T4	0.5ml of *Lb* spp$^{1(T*)}$+feed

Where, ACF= African catfish, NT= Nile tilapia, T*=Tej

3.11. Data Analysis

The one-way analysis of variance (ANOVA) and Tukey Multiple Range Test was conducted to figure out the differences among the groups' means at a significance level of $P<0.05$. All statistical analyses were carried out using SPSS version 20.

4. Results

4.1.Characterization of LAB

A total of 99 isolates were considered as presumptive LAB. Out of the 99 presumptive, 80(80.81%) were confirmed as LAB and grouped into *Lactobacillus*, *Lactococcus,* and *Leucnostoc* spp (Appendix 4). Among LAB isolates, *Lactobacillus* spp were the dominant (91.25%) followed by *Lactococcus* (7.5%). 92.5% of the isolates were homofermentative, 15% and 53.75% grew at 15°C, and 45°C respectively. Moreover, all isolates (100%) were tolerated 2 and 4 % NaCl (Table 2).

Table 2. Morphological and physiological characteristics of LAB isolated from fish

Characteristics	Category		
	I	II	III
Shape	Rod	Cocci	Cocci
Arrangement		Pair or short chain	Single
Gram reaction	+	+	+
Catalase Test	-	-	-
Motility test	-	-	-
Oxidase	-	-	-
Endospore	-	-	-
Fermentation	Homo/Hetro	Hetro	Homo
Growth at temperature			
15	+/-	+	+
37^{o}C	+	+	+
45 ^{o}C	+/-	-	-
Tolerance to NaCl (%)			
2%	+	+	+
4.5%	+	+	+
6.50%	+/-	+	+/-
Possible Identity	*Lactobacillus*	*Leucnostoc*	*Lactococcus*
No of isolates (%)	73 (91.25%)	1(1.25%)	6 (7.5%)

Where, **"+"** = Positive/Presence/ Growth; **"-"** = Negative/Absence/ No growth, **"** +/-**"=** Some grow/ others didn't

4.2.Evaluation of probiotic properties

4.2.1. Acid and Bile tolerance

From a total of 80 isolates of LAB, 64 (80%) tolerated pH3 and 48(60%) tolerated pH2, respectively (Table 3, Appendix 1). However, 32 (40%) isolates of LAB couldn't survive pH 2 (Appendix 1). On the other hand, 80 (100%) of the isolates survived 0.3% bile salt. Moreover,

75 (93.75%) survived 0.5% bile salts whereas 5 (6.25%) isolates including NTG18, NTS6, AFG26, AFS4, and AFS17 didn't survive 0.5% bile salt (Table 3).

Table 3. Acid and Bile tolerance of LAB isolates

S.N	Isolates Code	Expected Genus	Acid tolerance		Bile tolerance	
			pH2	pH3	0.3%	0.5%
1	NTG1	*Lactobacillus* spp	+	+	+	+
2	NTG4	*Lactobacillus* spp	+	+	+	+
3	NTG6	*Lactobacillus* spp	+	+	+	+
4	NTG7	*Lactobacillus* spp	+	+	+	+
5	NTG8	*Lactobacillus* spp	+	+	+	+
6	NTG9	*Lactobacillus* spp	+	+	+	+
7	NTG10	*Lactobacillus* spp	+	+	+	+
8	NTG14	*Lactobacillus* spp	+	+	+	+
9	NTG15	*Lactobacillus* spp	+	+	+	+
10	NTG16	*Lactobacillus* spp	+	+	+	+
11	NTG17	*Lactobacillus* spp	+	+	+	+
12	NTG18	*Lactobacillus* spp	+	+	+	-
13	NTG19	*Lactobacillus* spp	+	+	+	+
14	NTG20	*Lactobacillus* spp	+	+	+	+
15	NTS1	*Lactobacillus* spp	+	+	+	+
16	NTS2	*Lactobacillus* spp	+	+	+	+
17	NTS3	*Lactobacillus* spp	+	+	+	+
18	NTS4	*Lactobacillus* spp	+	+	+	-
19	NTS5	*Lactobacillus* spp	+	+	+	+
20	NTS6	*Lactobacillus* spp	+	+	+	-
21	NTS7	*Lactobacillus* spp	+	+	+	+
22	NTS8	*Lactobacillus* spp	+	+	+	+
23	NTS9	*Lactobacillus* spp	+	+	+	+
24	NTS10	*Lactobacillus* spp	+	+	+	+
25	AFG8	*Lactobacillus* spp	+	+	+	+
26	AFG5	*Lactobacillus* spp	+	+	+	+
27	AFG11	*Lactobacillus* spp	+	+	+	+
28	AFG30	*Lactobacillus* spp	+	+	+	+
29	AFG10	*Lactobacillus* spp	+	+	+	+
30	AFG26	*Lactobacillus* spp	+	+	+	-
31	AFG6	*Lactobacillus* spp	+	+	+	+
32	AFG1	*Lactobacillus* spp	+	+	+	+
33	AFG20	*Lactobacillus* spp	+	+	+	+
34	AFG14	*Lactobacillus* spp	+	+	+	+
35	AFG15	*Lactobacillus* spp	+	+	+	+
36	AFG3	*Lactobacillus* spp	+	+	+	+
37	AFG38	*Lactobacillus* spp	+	+	+	+
38	AFS7	*Lactobacillus* spp	+	+	+	+
39	AFS17	*Lactobacillus* spp	+	+	+	-
40	AFS3	*Lactobacillus* spp	+	+	+	+
41	AFS18	*Lactobacillus* spp	+	+	+	+
42	AFS4	*Lactobacillus* spp	+	+	+	-
43	AFS12	*Lactobacillus* spp	+	+	+	+
44	AFS11	*Lactobacillus* spp	+	+	+	+
45	AFS13	*Lactobacillus* spp	+	+	+	+
46	AFS15	*Lactobacillus* spp	+	+	+	+
47	AFS6	*Lactobacillus* spp	+	+	+	+
48	AFS14	*Lactobacillus* spp	+	+	+	+

Where, AFG= African Catfish Gut isolate, AFS= African Catfish Surface isolate, NTG= Nile Tilapia Gut isolate, NTS= Nile Tilapia Surface isolate, "+ ", tolerant to, "– "Non-tolerant to"

4.2.2. Evaluation of Antimicrobial activity of LAB isolates

A total of 43 isolates of LAB, which tolerated the pH2 and 0.5% bile salt were subjected to antimicrobial activity. Accordingly, the highest antimicrobial activity (≥ 15mm zone of inhibition) against all pathogens was exhibited by isolates coded NTG8, AFG8, and AFG10 (*Lactobacillus* species isolated from guts of African catfish and Nile tilapia). Out of 43, 17 (39.53%) isolates inhibited four and above pathogens. However, 24 (60.47%) showed antimicrobial activity on less than four pathogens (Appendix 2) (Appendix 3).

Table 4. Antimicrobial activity of LAB isolates against reference human pathogens

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

Where, AFG= African Catfish Gut isolate, AFS= African Catfish Surface isolate, NTG= Nile Tilapia Gut isolate, NTS= Nile Tilapia Surface isolate. The similar letter along column indicates the absence of significant difference ($P > 0.05$) whereas different letters indicate a significant difference ($P<0.05$)

4.2.3. Antibiotic Susceptibility of LAB Isolates

From 43 isolates, 17 (39.53%) showed antimicrobial activity against 4 to 5 pathogens. On the other hand, the majority of LAB isolates were susceptible to chloramphenicol and

erythromycin (64.71% each) followed by clindamycin (17.65%). However, all isolates were highly resistant (100%) to kanamycin, ciprofloxacin, streptomycin, and penicillin G (Table 5).

Table 5. Antibiotic sensitivity patterns of LAB isolates against different commercial drugs.

S.N	Species	Site	Code	Kanamycin (mm)	Clindamycin (mm)	Ciprofloxacin (mm)	Chloramphenicol (mm)	Streptomycin (mm)	Erythromycin (mm)	Penicillin G (mm)
1	Nile tilapia	Gut	NTG4	R	26S	R	29S	R	26S	R
2			NTG8	R	24S	R	22S	R	23S	R
3			NTG7	R	23S	R	24S	R	23S	R
4			NTG19	R	R	R	19S	R	23S	R
5			NTG15	R	R	R	22S	R	21S	R
6		Surface	NTS6	R	11R	R	25S	R	24S	R
7			NTS7	R	R	R	R	R	R	R
8	African catfish	Gut	AFG10	R	9R	R	23S	R	21S	R
9			AFG24	R	10R	R	22S	R	25S	R
10			AFG8	R	10R	R	27S	R	26S	R
11			AFG20	R	R	R	R	R	R	R
12			AFG30	R	R	R	R	R	R	R
13			AFG6	R	R	R	R	R	R	R
14		Surface	AFS4	R	R	R	R	R	R	R
15			AFS15	R	9R	R	25S	R	25S	R
16			AFS18	R	R	R	R	R	R	R
17			AFS14	R	9R	R	22S	R	23S	R

Where, AFG= African Catfish Gut isolate, AFS= African Catfish Surface isolate, NTG= Nile Tilapia Gut isolate, NTS= Nile Tilapia Surface isolate, R= Resistant, S= Susceptible

4.3. Prevalence of common fish pathogens

Overall, 60.71% of fish samples were positive for *S. aureus***.** The frequency of isolations of pathogens varied among the fish samples. Accordingly, *S. aureus* was detected in all surfaces of African catfish and Nile tilapia. However, a lower number (14.29% and 28.57%) of *S. aureus* was detected in the gut of African catfish and Nile tilapia, respectively (Table 6). Furthermore, 32.14% of fish samples were positive for *Salmonella* spp. with a higher (57.14%) prevalence of *Salmonella spp*. on the surface of Nile tilapia but lower in the gut of Nile tilapia and African catfish (14.29% each). On/in both surface and gut of African catfish and Nile tilapia, *Listeria* spp, *Shigella* spp, *Pseudomonas* spp, and *E. coli* were not detected (Table 6).

Table 6. Prevalence of common fish pathogens in fish samples

Sample source	Frequency (%) of Isolation of pathogens					
	S.aureus	*Listeria* spp.	*Salmonella* spp.	*Shigella* spp.	*Pseudomonas* spp.	*E.coli*
African catfish surface	7(100)	ND	3 (42.85)	ND	ND	ND
African catfish gut	1(14.29)	ND	1(14.29)	ND	ND	ND
Nile tilapia surface	7(100)	ND	4(57.14)	ND	ND	ND
Nile tilapia gut	2(28.57)	ND	1(14.29)	ND	ND	ND
Overall prevalence	17 (60.71)	ND	9 (32.14)	ND	ND	ND

Where, ND= Not detected

4.4. Antimicrobial Susceptibility patterns of *S. aureus* and *Salmonella spp*.

All (17 isolates) of *S. aureus* isolated from the guts of African catfish and Nile tilapia were susceptible to ciprofloxacin, chloramphenicol, and erythromycin. However, all *S. aureus* isolated from the surface and gut of both fish were highly resistant to Penicillin G (100%). Moreover, 100% of *S. aureus* isolated from guts of Africa catfish and Nile tilapia were also resistant to streptomycin (Table 7). On the other hand, 100% of *Salmonella* spp isolated from the surface of Africa catfish and gut of Nile tilapia were susceptible to chloramphenicol. Furthermore, all *Salmonella* spp isolated from the gut of African catfish were also susceptible to kanamycin, ciprofloxacin, and streptomycin (Table 8).

Table 7. Antimicrobial susceptibility patterns of *S. aureus* isolated from fish samples

antimicrobial agents	Disc potency (µg/ml)	African catfish				Nile tilapia			
		Surface		Gut		Surface		Gut	
		Resistance Frequency (%)	Sensitive Frequency (%)	Resistance Frequency (%)	Sensitive Frequency (%)	Resistance Frequency (%)	Sensitive Frequency (%)	Resistance Frequency (%)	Sensitive Frequency (%)
Erythromycin (E)	15	4(57.14)	3(42.86)	-	1(100)	5(71.43)	2(28.57)	-	1(100)
Clindamycin (CD)	2	5(71.43)	2(28.57)	1(100)	-	6(85.71)	1(14.29)	1(100)	-
Kanamycin (K)	30	1(14.29)	6(85.71)	-	1(100)	3(42.86)	4(57.14)	1(100)	-
Ciprofloxacin (CIP)	5	2(28.57)	5(71.43)	-	1(100)	1(14.29)	6(85.71)	-	1(100)
Penicillin (P)	10	7(100)	-	1(100)	-	7(100)	-	1(100)	-
Streptomycin (S)	10	-	7(100)	1(100)	-	4(57.14)	3(42.86)	1(100)	-
Chloramphenicol (C)	30	1(14.29)	6(85.71)	-	1(100)	6(85.71)	1(14.29)	-	1(100)

Table 8. Antimicrobial susceptibility patterns of *Salmonella* spp isolated from fish samples

Antimicrobial agents	Disc potency (µg/ml)	African catfish				Nile tilapia			
		Surface		Gut		Surface		Gut	
		Resistance Frequency (%)	Sensitive Frequency (%)	Resistance Frequency (%)	Sensitive Frequency (%)	Resistance Frequency (%)	Sensitive Frequency (%)	Resistance Frequency (%)	Sensitive Frequency (%)
Clindamycin (CD)	2	3(100)	-	1(100)	-	3(75)	1(25)	1(100)	-
Kanamycin (K)	30	2(75)	1(25)	-	1(100)	2(50)	2(50)	1(100)	-
Ciprofloxacin (CIP)	5	1(25)	2(75)	-	1(100)	1(25)	3(75)	-	1(100)
Streptomycin (S)	10	2(75)	1(25)	-	1(100)	1(25)	3(75)	1(100)	-
Chloramphenicol(C)	30	-	3(100)	1(100)	-	-	4(100)	-	1(100)

The multidrug resistance (MDR) patterns of *S. aureus* isolated from Nile tilapia revealed that 33.3% of the isolates were resistant to four antibiotics: E/CD/CIP/P, resistance to Erythromycin, Clindamycin, Ciprofloxacin, and Penicillin followed by 25% to five antibiotics (Table 9). The highest MDR observed in *S. aureus* (16.7%) was resistance to four antibiotics (mainly E/CD/CIP/P). The maximum number of antibiotics resisted by *S. aureus* was six antibiotics (E/CD/K/S/P/C) and the pattern was observed in one isolate only. Generally, MDR to four and five antibiotics dominated the resistance pattern for Nile tilapia (Table 9). Moreover, *S. aureus* isolated from African catfish were resistant to one to five antibiotics (20% each) (Table 9).

Table 9. MDR patterns of *S. aureus* isolated from fish samples

Types of fish	No. of antimicrobial resistance	Antimicrobial resistance pattern	No. of isolates (%)	Total (%)
African catfish	One	P	1 (20)	1(20)
	Two	C/P	1 (20)	1(20)
	Three	CD/S/P	1 (20)	1(20)
	Four	E/CD/CIP/P	1 (20)	1(20)
	Five	E/CD/CIP/P/K	1 (20)	1(20)
Nile tilapia	Two	K/P	1(8.3)	2(16.7)
		CD/P	1(8.3)	
	Three	S/C/P	1(8.3)	2(16.7)
		CD/P/CIP	1(8.3)	
	Four	K/P/S/C	1(8.3)	4(33.3)
		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)
		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)

Where: CD; Clindamycin, P; Penicillin, E; Erythromycin, C; Chloramphenicol, K; kanamycin, S; Streptomycin, CIP; Ciprofloxacin.

The MDR profile of *Salmonella* spp. isolated from Africa catfish showed the highest resistance (50%, 2/4) of the isolates towards two antibiotics (CD/E and CD/C) followed by four (E/CD/K/S) and five (E/CD/K/S/CIP) antibiotics (one isolate each). Moreover, the maximum number of antibiotics resisted by *Salmonella* spp, isolated from African catfish was five antibiotics (Table 10). On the other hand, the *Salmonella* spp (n=5) isolated from Nile tilapia resisted the maximum of five antibiotics (E/CD/CIP/S/K) (Table 10). A total of four isolates, out of five, of the *Salmonella* spp, showed similar resistance to two of the antibiotics (E/CD)

Table 10. MDR of *Salmonella* spp. isolated from fish samples in Jimma city

Types of fish	No. of antimicrobial resistance	Antimicrobial resistance pattern	No. of isolates %	Total %
African catfish	Two	CD/E	1 (25)	2(50)
		CD/C	1 (25)	
	Four	E/CD/K/S	1 (25)	1(25)
	Five	E/CD/K/S/CIP	1 (25)	1(25)
Nile tilapia	One	E	1 (20)	1(20)
	Two	E/CD	1 (20)	1(20)
	Three	E/CD/K	1 (20)	1(20)
	Four	E/CD/K/S	1 (20)	1(20)
	Five	E/CD/CIP/S/K	1 (20)	1(20)

Where: CD; Clindamycin, C; Chloramphenicol, K; kanamycin, CIP; Ciprofloxacin, S; Streptomycin and E; Erythromycin.

4.5. Pre and Post-assessment of pathogens from an aquarium

The pre and post-assessment of pathogens in an aquarium was done to observe the antimicrobial activity of the selected probiotic bacteria. Accordingly, in the initial analysis of the water, pathogens such as *E. coli*, *Pseudomonas* spp, *Salmonella* spp, and *Listeria* spp were detected. However, after the probiotic LAB strains (*Lactobacillus spp* coded AFG10 and AFG8) were

introduced into an aquarium, all pathogens previously detected in the aquarium were inhibited. Moreover, the aquarium into which *Lactococcus spp* NTG8 was introduced inhibited all pathogens, except *E. coli*, while the aquarium which had *Lactobacillus* spp of JUT89 inhibited only *Salmonella spp*. However, all pathogens persisted throughout 30 days in the control group (Table 11).

Table 11. The Pre and Post-assessment of the status of pathogens in an aquarium (N=2)

Treatment	Administered Probiotics	*S. aureus*		*Pseudomonas spp*		*Listeria spp*		*E. coli*		*Salmonella spp*	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Control	Code	–	–	+	+	+	+	+	+	+	+
1	AFG10[1]	–	–	+	–	+	–	+	–	+	–
2	AFG8[1]	–	–	+	–	+	–	+	–	+	–
3	NTG8	–	–	+	–	+	–	+	+	+	–
4	JUT89[1]	–	–	+	+	+	+	+	+	+	–

Where, "+" = Present, "–" = Absent, NT = Nile tilapia, AF = African catfish, G = Gut, Pre= Pre-treatment, Post= post-treatment

4.6. Growth performance of fish

The mean initial and final weight and length, and weight gain (WG), of fry under different probiotic treatments are presented in Table 12. The mean initial weight and length of the fish were the same for all treatments at the beginning of probiotic treatments. During the 30 days of probiotic treatments, the highest fish growth performance, both in weight and length, was recorded in T1 (AFG10) and T2 (AFG8), with slightly higher values for T1. In contrast, the least growth values were observed in the control and T4 (JUT89), while performance in T3 (NTG8) was intermediate between T1 & T2 (with the highest values) and T4 and control (with the lowest values) (Table 12). The variation in the final mean weight and length between the control and the treatment groups is statistically significant ($P = 0.02$). However, the final mean weight and length were statistically significant only between T1 and the control ($P = 0.04$ for weight; $P = 0.04$ for length). The weight gain (WG), followed a similar pattern, as the variation in the final mean weight and length. The variations in mean WG was statistically significant between T1 and control ($P = 0.04$ for WG; P

= 0.03 for AGR) whereas there is no statistically significant difference between the rest of the treatment groups (T2, T3, and T4) and the control (P > 0.05).

Table 12. Growth parameters of African catfish fry treated with different probiotic strains

Week	Treatment	MW (g)	MTL (cm)	WG (g)
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}
	T1	0.09 ± 0.01^{a}	1.92 ± 0.35^{a}	0.05 ± 0.01^{a}
	T2	0.08 ± 0.01^{ab}	1.72 ± 0.35^{a}	0.04 ± 0.01^{bc}
	T3	0.08 ± 0.01^{ab}	1.92 ± 0.26^{a}	0.05 ± 0.01^{ab}
	T4	0.07 ± 0.01^{c}	1.70 ± 0.46^{a}	0.03 ± 0.01^{c}
2	Control	0.10 ± 0.05^{c}	2.42 ± 0.35^{a}	0.07 ± 0.05^{a}
	T1	0.2 ± 0.09^{a}	2.78 ± 0.56^{a}	0.16 ± 0.09^{a}
	T2	0.18 ± 0.08^{ab}	2.68 ± 0.39^{a}	0.13 ± 0.08^{a}
	T3	0.17 ± 0.08^{ab}	2.52 ± 0.52^{a}	0.06 ± 0.00^{a}
	T4	0.10 ± 0.00^{c}	2.25 ± 0.19^{a}	0.03 ± 0.01^{a}
3	Control	0.15 ± 0.08^{b}	2.82 ± 0.26^{c}	0.11 ± 0.08^{a}
	T1	0.40 ± 0.15^{a}	3.72 ± 0.29^{a}	0.36 ± 0.15^{a}
	T2	0.35 ± 0.10^{a}	3.45 ± 0.39^{ab}	0.31 ± 0.10^{a}
	T3	0.33 ± 0.19^{a}	3.38 ± 0.35^{ab}	0.29 ± 0.19^{a}
	T4	0.17 ± 0.08^{b}	2.98 ± 0.37^{bc}	0.10 ± 0.05^{a}
4	Control	0.35 ± 0.10^{b}	3.38 ± 0.29^{b}	0.31 ± 0.11^{b}
	T1	1.28 ± 0.78^{a}	4.70 ± 0.92^{a}	1.24 ± 0.78^{a}
	T2	1.20 ± 0.38^{a}	4.65 ± 0.68^{a}	1.16 ± 0.38^{ab}
	T3	0.90 ± 0.83^{ab}	4.30 ± 1.08^{a}	0.86 ± 0.83^{ab}
	T4	0.51 ± 0.25^{b}	3.80 ± 0.67^{b}	0.47 ± 0.25^{ab}

Where: T = Treatment; MW = Mean weight; MTL = Mean total length; WG = Weight gain; T1 = *Lactobacillus* species one isolated from African catfish**,** T2 = *Lactobacillus* species two isolated from African catfish; T3 = *Lactococcus* species one isolated from Nile tilapia**,** T4= *Lactobacillus* species one isolated from Tej. The similar letter along column indicated no significant difference (P> 0.05) whereas different letters indicated a significant difference (P<0.05).

5. Discussion

Aquaculture is a fast-growing food sector in many developing countries (Akter *et al.,* 2019). African catfish and Nile tilapia are nowadays most common in fish farming activities (Gashaw and Wolff, 2014; Tesfahun, 2018). In the present study, isolates of LAB from Nile tilapia and African Catfish were dominated by *Lactobacillus spp* (91.25%) followed by *Lactococcus* spp (7.5%). Similarly, Muthukumar and Kandeepan (2015**)** reported a maximum population of *Lactobacillus* from freshwater fishes. The dominance of *Lactobacillus* spp could be due to the resistance of the isolates to harsh conditions, mutualistic relationship with other microbes, and the ubiquitous nature of the genus. The dominance of *Lactobacillus* spp in fish is due to their ability to adhere to cells, persistence, and multiplication under harsh conditions, production of bacteriocins, resistance to low pH, and tolerance to high temperature (Belicova *et al.*, 2013). *Lactobacillus spp* is a ubiquitous microorganism and lives widely in the intestinal tracts of several fish species in mutualistic relationships with the host (George *et al*., 2018).

In this study, 64 (80%) of LAB isolates tolerated pH3 while 48 (60%) isolates tolerated pH2. Moreover, 100% and 89.58% of the isolates survived 0.3% and 0.5% bile salt, respectively. Similarly, Muthukumar and Kandeepan (2015) also reported that 100% of the LAB isolates resisted 0.3% bile salts. Furthermore, Maragkoudaki *et al.* (2005) also reported 100% tolerance to 0.3% bile salt of lactobacilli of dairy origin isolated and characterized for their probiotic potential. In higher to the present study**,** Peristiwati *et al.* (2019) reported that all the LAB isolates resisted 0.5% bile salt. The pH 2 condition was a very extreme condition for the growth of microorganisms, including lactic acid bacteria which generally could be adapted to habitats with a fairly low pH atmosphere. Chemlal *et al* (2012) stated that almost all strains of their isolates were resistant to pH 3 but none tolerated pH2. According to Chemlal *et al* (2012), acidification of cell walls was one of the causes of bacterial cell death at low pH, in conditions with very acidic exposure., High acidity can also cause membrane damage and the release of intracellular components that can cause cell death.

The tolerance of bacteria to such a harsh environment produced by acid and bile is attributed to the production of ATPase, bile salt hydrolases, and other enzymes (Hussain *et al.*, 2021). Acid and bile resistance are prerequisites for probiotic function because ingested strains need to survive the harsh environment of the gastrointestinal tract (Archer & Halami, 2015). LAB isolates

could tolerate high acid through proton pumps, changes in cell membrane composition and cell density, DNA and protein damage repair (Guan & Liu, 2020).

In the current study, 17 (39.53%) isolates showed antimicrobial activity against 4 pathogens, namely: *S. aureus*, *E. coli*, *Salmonella* Typhimurium, and *Bacillus cereus***.** Moreover, 5 isolates of LAB, including *Lactobacillus* spp. also showed antimicrobial activity against all the 5 test pathogens. Similarly, Kato *et al*. (2016) reported *Lactobacillus spp* isolated from fish had the highest antimicrobial activity against selected gram-positive and gram-negative bacteria. The antimicrobial activity of LAB could be due to the production of primary metabolites like bacteriocin and secondary metabolites like organic acids and hydrogen peroxide. LAB also produce acetic acid, ethanol, aroma compounds, exopolysaccharide, and several important enzymes (Amarantini *et al.*, 2019). Most strains of *Lactobacillus spp* produce hydrogen peroxide as antimicrobial effects by attributing to a strong oxidizing effect on the lipid membrane and cellular proteins of the target organisms (Kurutas, 2016). Hence, antimicrobial activity is one of the most important criteria for the selection of probiotic bacteria as it can inhibit the growth of undesired microorganisms.

In this study, the majority of LAB isolates were susceptible to chloramphenicol and Erythromycin (64.7% each) followed by Clindamycin (52.9%). However, the isolates were highly resistant to penicillin G**,** Streptomycin, Ciprofloxacin, and Kanamycin (100%). The resistance of the LAB isolates to antibiotics could be due to the presence of resistance gene(s). The susceptibility of LAB could be the indication of LAB populations that neither possessed nor acquired the resistance gene so far (Beyan *et al.*, 2011). In Chloramphenicol susceptible LAB isolates, the cells will not grow because the drug suppresses growth by binding to the bacterial ribosome (blocking peptidyl transferase) and inhibiting protein synthesis and besides letting lipids diffuse through the bacterial cell membrane **(**Wongtavatchail *et al*, 2004) because of the drugs lipid solubility. According to Bulajic and Radulovic, (2012) report, the resistance of LAB isolates is accounted to the isolate's passion for potentially transmissible plasmid-encoded antibiotic resistance genes. The use of antibiotics in the rearing of fish is inappropriate because it gives rise to antibiotic resistance to pathogens (Dahiya, *et al*., 2020). On the contrary, antimicrobial resistance of probiotic bacteria is one of the most important criteria for the selection of good probiotic strains (Georgieva *et al.*, 2015; Acharya *et al*., 2019) mainly because their resistance to commonly used antibiotics ensures

their survival in GIT irrespective of the potential use of the drugs by consumers of the probiotics. The possibility of transmission of the resistance genes to potential pathogens that tolerate the inhibitory activity of LAB could not be ruled out although the likely chance is rare.

The predominant pathogen in the present3 study was generally *S. aureus* (60.71%) followed by *Salmonella spp***.** (32.14%) while *Listeria spp, Shigella spp, E. coli,* and *Pseudomonas spp* were not encountered. Moreover, majorities of the pathogens were detected from the surface of the fish. Similarly, Anwar *et al.* (2012) reported that fish from Lake Tana is contaminated with pathogens like *E. coli, Pseudomonas spp, Salmonella spp, Shigella spp,* and *Klebsiella spp*. The higher prevalence of *Salmonella spp*. and *S. aureus* in the present study could be due to cross-contamination during fishing, also the contamination of the river from which the samples were taken. Similarly, Isaac, **(**2019) stated that many factors cause the spread of disease-causing pathogens including, poor quality of water, lack of quality feeds, poor knowledge of disease prevention and treatment by the farmers, poor pond and tank management, high stocking densities, and lack of proper advice on fish farm management. The same could also be the reason for the dominance of pathogens on the surface of fish.

S. aureus showed high susceptibility to each of Ciprofloxacin, Erythromycin, and Chloramphenicol. Similarly**,** Bizuneh *et al* (2021) reported several *S. aureus* isolates susceptible to Ciprofloxacin (100 %) and gentamycin (89.09 %). The reason for susceptibility is Lack of resistance gene (naturally), failure to acquire the resistance genes (plasmid) because of the absence of other resistance microbes in the harsh environment (Azage & Kibret, 2017; Wamala *et al*, 2018). In the current study, 100% of *S. aureus* isolates were resistant to Penicillin G. This was higher to Beyene *et al*. (2017) who reported that 95.3% of the isolates were resistant to Penicillin G. The resistance of *S. aureus* to Penicillin G could be due to the production of penicillinase enzyme (a type of ß-lactamase) that hydrolyzed the beta-lactam ring of penicillin and they are the most commonly used antibiotics in Ethiopia (Beyene *et al***.** 2017).

In this study isolates of *Salmonella spp,* were highly susceptible to ciprofloxacin and chloramphenicol. However, the highest frequency of resistance of *Salmonella spp* to Clindamycin was observed in African Catfish and Nile Tilapia. This is similar to Yildirim *et al*, 2011, which stated *Salmonella spp* resisted 97% of clindamycin. The study in Sarab, Iran by Akbarmehr (2012), showed that *Salmonella spp*. were highly susceptible to chloramphenicol (100 %) followed by

ciprofloxacin and gentamycin (91.89 %each). However, isolates of *Salmonella spp*. exhibited resistance to streptomycin and tetracycline (29.72 % each) and ampicillin (13.51 %). In general, *Salmonella spp* and *S. aureus* were abundant in fish surface and gut. This may be due to contamination of the water body from where the samples were collected.

In the current study, despite some differences among each treatment (probiotic source and type), inoculation of LAB as a live probiotic in the culture unit of African catfish larvae revealed higher growth performance than the control. Among the probiotics, the highest growth performance was observed in treatment with *Lactobacillus* spp (AFG10) followed by *Lactobacillus* spp (AFG8) and *Lactococcus* spp (NTG 8). Similarly, Putra *et al*. (2017), reported a significant increment in growth performance through the application of probiotics to the African catfish fry culture unit. The highest growth performance observed with the use of probiotics (*Lactobacillus* spp) isolated from African catfish could be due to an easy adaption of the probiotics originally isolated from the same host (host-specific).

Host-derived (same origin) probiotics can offer significant advantages in terms of survival, growth performance, and decreasing infection rate because their physiological activities are at optimum level in the same natural habitats (Nguyen *et al*., 2017). Moreover, Masjudi *et al*. (2020) documented that the application of probiotics in water significantly increased the growth performances of Tapah (*Wallago leeri*) juveniles**.** *Lactobacillus* AFG 10 and AFG 8 inhibited all pathogens in water. Good probiotics affect fish health by improving several qualities of water since they modify the bacteria composition of the water and sediments (Venkateswera, 2007).

Among treatment groups, T4 (*Lactobacillus* spp, JUT89) isolated from *Tej*, was the least performed strain in terms of growth performance parameters. This could be due to differences in the source from where the probiotic was isolated (non-aqua, beverage-based probiotics), which takes time to adapt to the new environment. The results of pre-and post-assessment of pathogens in aquarium showed that JUT89 inhibits only *Salmonella* spp. This result indicated that the *Lactobacillus* spp isolated from *Tej* takes more time to adapt to the new environment and inhibit the pathogens. Generally, better growth enhancement in treatments than the control is possibly due to higher activity of probiotics in releasing digestive enzymes and promoting appetite, production of vitamins, breakdown of indigestible components (Hoseinifar *et al*., 2015). LAB is considered as a favorable microorganism for use as probiotics in aquaculture due to their abilities to stimulate

host gastrointestinal development, digestive function, mucosal tolerance, stimulating an immune response, and improved disease resistance sustaining the normal growth, health, and well-being of farmed fish because they serve as nutrients source, vitamins, and digestive enzymes, and they could also significantly contribute to feeding consumption, nutrients uptake and host's growth rate (Nath *et al.*, 2019; Ringo, 2020**)**.

6. Conclusion

A total of 80 LAB isolates were characterized and grouped into *Lactobacillus*, *Lactococcus*, and *Leucnostoc* spp. Among the LAB isolates *Lactobacillus spp* was the predominant (91.25%) in both African catfish and Nile tilapia. As probiotic potential evaluation, majorities of the isolates of LAB resisted pH2 and 0.5% bile salts while all LAB isolates resisted pH 3 and 0.3% bile salts. Moreover, 39.53% of cell free supernatant of LAB inhibited four and above pathogens with the highest antimicrobial activity (≥ 15mm zone of inhibition) exhibited by isolates coded by NTG8, AFG8, and AFG10. In addition, majority of LAB isolates were susceptible to chloramphenicol, erythromycin, and clindamycin. However, all isolates of LAB were highly resistant (100%) to penicillin G**,** streptomycin, ciprofloxacin, and kanamycin.

On the other hand, the overall 60.71% of *S. aureus* and 32.14% *Salmonella spp* were detected in surfaces and gut of African catfish and Nile tilapia. However, *Listeria*, *Shigella*, *Pseudomonas* spp, and *E. coli* were not detected in both fish. Thus, the presence of *S. aureus* and *Salmonella spp* could affect the production of fish besides of the risk for human beings.

Although probiotics are expected to inhibit pathogens, the pre and post-assessment of pathogens during probiotic administration showed some probiotic inhibit the growth of pathogens others didn't. Among these, the aquaria with *Lactobacillus* spp AFG10 and AFG8 isolated from African catfish inhabited all pathogens (*E. coli*, *Pseudomonas* spp, *Salmonella* spp, and *Listeria* spp) which were detected in the pre-assessment test. However, an aquarium that had *Lactococcus spp* (NTG8) isolated from Nile tilapia inhibited all pathogens, except *E. coli* and *Lactobacillus* spp of JUT89 inhibited only *Salmonella* spp. Moreover, probiotics from different source showed difference activity on fish growth performance. The highest fish growth performance, both in weight and length was recorded in *Lactobacillus* spp (AFG10) and *Lactobacillus* spp (AFG8). Among probiotics, the least growth values were observed in *Lactobacillus* spp (JUT89). In comparison to control, all aquaria with probiotics enhanced the growth performance of fish.

7. Recommendations

- Both African catfish and Nile tilapia had promising LAB isolates. So, better to isolate more probiotics from them.
- In most cases, the surfaces of Africa catfish and Nile tilapia were contaminated with pathogens than the gut. Hence, proper sanitation is mandatory for further use.
- *S. aureus* and *Salmonella* spp were the most prevalent. These pathogens are related to sanitation. Hence, proper sanitation in the aquarium and original source (river) is critical
- The probiotics AFG10 and AFG8 (*Lactobacillus* spp) which are originally isolated from African catfish are promising probiotics that inhibited all pathogens and had a good performance (increase both height and weight). So, better to use the potential probiotics that are isolated from the same fish.
- The Identification of the LAB isolates and pathogens was done to the genus level using conventional tests. Thus, better to identify the isolates and pathogens using the molecular approach.

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List of Appendix

Appendix 1. pH tolerance of probiotic strains

Site	Code	Acid	
Gut		pH2	pH3
	NTG1	+	+
	NTG2	-	-
	NTG3	-	-
	NTG4	+	+
	NTG5	-	-
	NTG6	+	+
	NTG7	+	+
	NTG8	+	+
	NTG9	+	+
	NTG10	+	+
	NTG11	-	-
	NTG12	-	-
	NTG13	-	-
	NTG14	+	+
	NTG15	+	+
	NTG16	+	+
	NTG17	+	+
	NTG18	+	+
	NTG19	+	+
	NTG20	+	+
Surface	NTS1	+	+
	NTS2	+	+
	NTS3	+	+
	NTS4	+	+
	NTS5	+	+
	NTS6	+	+
	NTS7	+	+
	NTS8	+	+
	NTS9	+	+
	NTS10	+	+
GUT	AFG8	+	+
	AFG40	-	+
	AFG16	-	+
	AFG39	-	-
	AFG5	+	+
	AFG13	-	-
	AFG11	+	+

	AFG18	-	+
	AFG30	+	+
	AFG9	-	-
	AFG2	-	-
	AFG25	-	+
	AFG17	-	+
	AFG4	-	-
	AFG10	+	+
	AFG26	+	+
	AFG6	+	+
	AFG1	+	+
	AFG20	+	+
	AFG14	+	+
	AFG13	-	-
	AFG15	+	+
	AFG37	-	-
	AFG3	+	+
	AFG28	-	+
	AFG38	+	+
	AFG24	-	+
	AFG33	-	+
	AFG34	-	-
Surface	AFS7	+	+
	AFS17	+	+
	AFS3	+	+
	AFS10	-	+
	AFS19	-	+
	AFS18	+	+
	AFS9	-	+
	AFS20	-	+
	AFS4	+	+
	AFS1	-	+
	AFS12	+	+
	AFS11	+	+
	AFS13	+	+
	AFS15	+	+
	AFS16	-	-
	AFS6	+	+
	AFS5	-	-
	AFS2	-	+
	AFS14	+	+
	AFS22	-	-
	AFS 26	-	-

Where, AFG= African Catfish Gut isolate, AFS= African Catfish Surface isolate, NTG= Nile Tilapia Gut isolate, NTS= Nile Tilapia Surface isolate, "+ ", tolerant to, "– "Non-tolerant to"

Appendix 2. Antimicrobial activity of LAB isolates

SN	Site	Species	Code	*B.cereus*	*E.coli*	*S.aureus*	*S.Typhi*	*C.albicans*
1	Gut	*Oreochromis niloticus*	NTG1	12.5 ± 0.71	0 ± 0	11 ± 0	12 ± 0	14 ± 1.4
2			NTG2	0 ± 0	0 ± 0	12 ± 0	11.5 ± 0.7	0 ± 0
3			NTG3	0 ± 0	0 ± 0	18.5 ± 0.7	13 ± 0	0 ± 0
4			NTG4	14 ± 0	0 ± 0	17.5 ± 0.7	16 ± 0	17.5 ± 0.7
5			NTG5	15.5 ± 0.71	0 ± 0	13 ± 1.4	15 ± 1.4	0 ± 0
6			NTG6	14 ± 0	14.5 ± 0.71	11 ± 0	0 ± 0	0 ± 0
7			NTG7	14.5 ± 0.71	15.5 ± 0.71	14 ± 0	14.5 ± 0.7	0 ± 0
8			NTG8	16.5 ± 0.71	15.5 ± 0.71	16.5 ± 2.1	15 ± 0	15.5± 0.71
9			NTG9	13.5 ± 0.71	12.5 ± 0.71	0 ± 0	12.5 ± 0.7	0 ± 0
10			NTG10	15.5 ± 0.71	14.5 ± 0.71	0 ± 0	13.5 ± 0.7	0 ± 0
11			NTG11	17.5 ± 0.71	14.5 ± 0.71	0 ± 0	13.5 ± 0.7	0 ± 0
12			NTG12	16 ± 0	0 ± 0	0 ± 0	15.5 ± 0.7	0 ± 0
13			NTG13	15.5 ± 0.71	18 ± 0	0 ± 0	12.5 ± 0.7	0 ± 0
14			NTG14	14.5 ± 0.71	14.5 ± 0.71	0 ± 0	11.5 ± 0.7	0 ± 0
15			NTG15	14.5 ± 0.71	14 ± 1.41	0 ± 0	0 ± 0	0 ± 0
16			NTG16	13.5 ± 0.71	13.5 ± 0.71	0 ± 0	6 ± 8.5	0 ± 0
17			NTG17	15.5 ± 0.71	15.5 ± 0.71	0 ± 0	0 ± 0	0 ± 0
18			NTG18	14 ± 0	12 ± 1.41	11.5 ± 0.7	13 ± 1.4	0 ± 0
19			NTG19	16 ± 1.41	13 ± 1.41	14 ± 0	11.5 ± 0.7	0 ± 0
20			NTG20	15.5 ± 0.71	14 ± 1.41	0 ± 0	14.5 ± 0.7	0 ± 0
21	Surface		NTS1	15 ± 1.41	13.5 ± 2.12	11.5 ± 0.7	13 ± 1.4	0 ± 0
22			NTS2	15 ± 0	0 ± 0	13 ± 1.4	13.5 ± 0.7	0 ± 0
23			NTS3	0 ± 0	0 ± 0	12 ± 0	0 ± 0	0 ± 0
24			NTS4	12.5 ± 0.71	0 ± 0	12.5 ± 0.7	0 ± 0	0 ± 0
25			NTS5	15 ± 1.41	12 ± 1.41	12 ± 1.4	13 ± 0	0 ± 0
26			NTS6	17 ± 0	15 ± 1.41	12 ± 0	13.5 ± 0.7	0 ± 0
27			NTS7	14.5 ± 0.71	13.5 ± 0.71	13 ± 1.4	14.5 ± 0.7	0 ± 0
28			NTS8	14 ± 0	13.5 ± 0.71	11 ± 0	14 ± 1.4	0 ± 0
29			NTS9	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
30			NTS10	0 ± 0	14 ± 1.41	12 ± 0	11.5 ± 0.7	0 ± 0
31	Gut	*Clarias gariepinus*	AFG8	16 ± 1.41	17 ± 0	15.5 ± 0.7	16 ± 0	15.5± 0.71
32			AFG40	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
33			AFG16	13 ± 1.41	0 ± 0	14.5 ± 0.7	12.5 ± 0.7	13.5 ± 0.7

34		AFG39	14 ± 1.41	16.5 ± 2.12	17 ± 0	20 ± 1.4	0 ± 0
35		AFG5	15 ± 0	0 ± 0	0 ± 0	0 ± 0	13 ± 0
36		AFG13	14.5 ± 0.71	16 ± 1.41	13.5 ± 0.7	12.5 ± 0.7	0 ± 0
37		AFG11	12.5 ± 0.71	14.5 ± 0.71	13.5 ± 2.1	14 ± 1.4	0 ± 0
38		AFG18	14.5 ± 2.12	14.5 ± 0.71	14.5 ± 0.7	15 ± 0	0 ± 0
39		AFG30	15 ± 1.41	15 ± 0	14.5 ± 0.7	13 ± 1.4	0 ± 0
40		AFG9	13.5 ± 0.71	14.5 ± 0.71	15.5 ± 0.7	15.5 ± 0.7	0 ± 0
41		AFG2	13.5 ± 0.71	0 ± 0	0 ± 0	0 ± 0	0 ± 0
42		AFG25	0 ± 0	17 ± 1.41	15.5 ± 0.7	16 ± 0	0 ± 0
43		AFG17	14.5 ± 0.71	18 ± 0	16 ± 0	15.5 ± 0.7	0 ± 0
44		AFG4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	14 ± 0
45		AFG10	17 ± 0.71	16.5 ± 0.71	16 ± 0	16.5 ± 0.7	16 ± 0
46		AFG26	0 ± 0	14.5 ± 0.71	15 ± 0	15.5 ± 2.1	0 ± 0
47		AFG6	13.5 ± 0.71	14 ± 0	13 ± 1.4	16 ± 0	0 ± 0
48		AFG1	13.5 ± 0.71	16 ± 0	15 ± 1.4	13.5 ± 0.7	0 ± 0
49		AFG20	14 ± 1.41	13 ± 0	13.5 ± 0.7	14 ± 1.4	0 ± 0
50		AFG14	0 ± 0	13 ± 0	13 ± 1.4	13.5 ± 0.7	0 ± 0
51		AFG13	14 ± 0	16 ± 1.41	13 ± 0	13.5 ± 0.7	0 ± 0
52		AFG15	0 ± 0	11.5 ± 0.71	0 ± 0	0 ± 0	0 ± 0
53		AFG37	14 ± 1.41	14.5 ± 0.71	15.5 ± 0.7	13.5 ± 0.7	13.5 ± 0.7
54		AFG3	12.5 ± 0.71	15 ± 1.41	15.5 ± 0.7	15 ± 1.4	0 ± 0
55		AFG28	12.5 ± 2.12	13.5 ± 0.71	14 ± 0	13.5 ± 0.7	0 ± 0
56		AFG38	0 ± 0	13 ± 1.41	15.5 ± 0.7	14.5 ± 0.7	0 ± 0
57		AFG24	14 ± 1.41	13.5 ± 0.71	15 ± 0	14 ± 0	14.5 ± 0.7
58		AFG33	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
59		AFG34	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
60		AFG19	12.5 ± 2.12	13 ± 1.41	13.5 ± 0.7	13 ± 1.4	0 ± 0
61	Surface	AFS16	16 ± 0	15.5 ± 0.71	11.5 ± 0.7	15 ± 1.4	0 ± 0
62		AFS17	5.5 ± 7.78	0 ± 0	0 ± 0	14 ± 1.4	0 ± 0
63		AFS9	14 ± 1.41	13.5 ± 2.12	15 ± 1.4	14.5 ± 0.7	0 ± 0
64		AFS4	15 ± 0	15.5 ± 0.71	17 ± 0	18.5 ± 0.7	0 ± 0
65		AFS14	14 ± 0	15.5 ± 0.71	16 ± 1.4	13 ± 0	15.5 ± 0.7
66		AFS12	14.5 ± 0.71	13 ± 1.41	0 ± 0	0 ± 0	0 ± 0
67		AFS20	16 ± 1.41	15 ± 0	14 ± 1.4	13 ± 1.4	13 ± 1.4
68		AFS2	13.5 ± 2.12	18 ± 2.83	15 ± 0	14.5 ± 0.7	14.5 ± 0.7
69		AFS15	14 ± 0	15.5 ± 0.71	13.5 ± 0.7	16 ± 0	0 ± 0
70		AFS3	12 ± 1.41	14 ± 0	14.5 ± 0.7	12.5 ± 0.7	0 ± 0
71		AFS5	12.5 ± 0.71	13.5 ± 2.12	16 ± 0	15.5 ± 0.7	0 ± 0
72		AFS10	12.5 ± 0.71	12.5 ± 2.12	14.5 ± 0.7	16 ± 0	0 ± 0
73		AFS19	11.5 ± 0.71	12.5 ± 2.12	15.5 ± 0.7	15 ± 0	0 ± 0
74		AFS1	12.5 ± 0.71	15 ± 0	15.5 ± 2.1	14.5 ± 0.7	0 ± 0
75		AFS7	12 ± 0	16 ± 0	14.5 ± 0.7	16 ± 0	0 ± 0
76		AFS6	11.5 ± 0.71	15 ± 1.41	17 ± 0	12.5 ± 2.1	0 ± 0
77		AFS13	12.5 ± 0.71	16 ± 0	0 ± 0	0 ± 0	0 ± 0
78		AFS8	12.5 ± 0.71	17.5 ± 2.12	15 ± 0	15.5 ± 0.7	0 ± 0
79		AFS18	14.5 ± 0.71	15 ± 0	14.5 ± 0.7	17 ± 1.4	0 ± 0
80		AFS11	14.5 ± 0.71	14 ± 0	14.5 ± 0.7	16 ± 0	0 ± 0

Where: AF=African catfish, NT=Nile tilapia S= Surface G= Gut

Appendix 3. Results of one-way ANOVA

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
B.cereus	Between Groups	32.529	16	2.033	2.560	.031
	Within Groups	13.500	17	.794		
	Total	46.029	33			
E.coli	Between Groups	435.059	16	27.191	46.225	.000
	Within Groups	10.000	17	.588		
	Total	445.059	33			
S.aureus	Between Groups	470.529	16	29.408	37.032	.000
	Within Groups	13.500	17	.794		
	Total	484.029	33			
S.typhi	Between Groups	529.941	16	33.121	62.562	.000
	Within Groups	9.000	17	.529		
	Total	538.941	33			
C.albican	Between Groups	1248.059	16	78.004	884.042	.000
	Within Groups	1.500	17	.088		
	Total	1249.559	33			

Appendix 4. Morphological, Biochemical, and physiological tests for LAB isolates

										Temperature			Salt			Expected Genus
Site	S.N	Code	Gram	Shape	Catalase	Oxidase	Fermentation Type	Endospore	Motility	15°C	37°C	45°C	2%	4.5%	6.5%	
Gut	1	NTG1	+	Rod	-		Hetro	-	-	+	+	+	+	+	-	*Lactobacillus*
	2	NTG2	+	Rod	-		Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	3	NTG3	+	Rod	-		Hetro	-	-	-	+	-	+	+	+	*Lactobacillus*
	4	NTG4	+	Rod	-		Hetro	-	-	+	+	+	+	+	+	*Lactobacillus*
	5	NTG5	+	Rod	-		Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	6	NTG6	+	Rod	-		Homo	-	-	+	+	-	-	-	-	*Lactobacillus*
	7	NTG7	+	Rod	-		Homo	-	-	-	+	+	+	+	-	*Lactobacillus*
	8	NTG8	+	Coccus	-		Homo	-	-	+	+	-	+	+	+	*Lactococcus*
	9	NTG9	+	Rod	-		Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	10	NTG10	+	Rod	-		Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	11	NTG11	+	Rod	-		Homo	-	-	+	+	+	-	-	-	*Lactobacillus*
	12	NTG12	+	Rod	-		Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	13	NTG13	+	Rod	-		Homo	-	-	+	+	+	-	-	-	*Lactobacillus*
	14	NTG14	+	Rod	-		Homo	-	-	+	+	+	-	-	-	*Lactobacillus*
	15	NTG15	+	Rod	-		Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	16	NTG16	+	Rod	-		Homo	-	-	+	+	+	+	+	-	*Lactobacillus*
	17	NTG17	+	Rod	-		Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	18	NTG18	+	Coccus	-		Homo	-	-	+	+	-	+	+	-	*Lactococcus*
	19	NTG19	+	Rod	-		Homo	-	-	-	+	+	+	+	+	*Lactobacillus*
	20	NTG20	+	Rod	-		Homo	-	-	+	+	+	-	-	-	*Lactobacillus*
Surface	21	NTS1	+	Coccus	-		Homo	-	-	+	+	-	+	+	+	*Lactococcus*
	22	NTS2	+	Rod	-		Hetro	-	-	+	+	+	+	+	+	*Lactobacillus*
	23	NTS3	+	Coccus	-		Hetro	-	-	+	+	-	+	+	+	*Leuconostoc*
	24	NTS4	+	Coccus	-		Homo	-	-	+	+	-	+	+	+	*Lactococcus*
	25	NTS5	+	Rod	-		Homo	-	-	+	+	+	-	-	-	*Lactobacillus*
	26	NTS6	+	Rod	-		Homo	-	-	-	+	+	-	-	-	*Lactobacillus*

	27	NTS7	+	Coccus	-	Homo	-	-	+	+	-	+	+	-	*Lactococcus*
	28	NTS8	+	Rod	-	Hetro	-	-	-	+	+	-	-	-	*Lactobacillus*
	29	NTS9	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	30	NTS10	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
Gut	31	AFG8	+	Rod	-	Homo	-	-	+	+	-	+	+	+	*Lactobacillus*
	32	AFG40	+	Rod	-	Hetro	-	-	+	+	-	+	+	+	*Lactobacillus*
	33	AFG16	+	Rod	-	Homo	-	-	+	+	-	-	-	-	*Lactobacillus*
	34	AFG39	+	Coccus	-	Homo	-	-	+	+	-	+	+	+	*Lactococcus*
	35	AFG5	+	Rod	-	Homo	-	-	+	+	-	+	-	-	*Lactobacillus*
	36	AFG13	+	Rod	-	Homo	-	-	+	+	+	-	-	-	*Lactobacillus*
	37	AFG11	+	Rod	-	Homo	-	-	+	+	-	+	+	+	*Lactobacillus*
	38	AFG18	+	Rod	-	Homo	-	-	+	+	-	-	-	-	*Lactobacillus*
	39	AFG30	+	Rod	-	Homo	-	-	-	+	-	+	+	+	*Lactobacillus*
	40	AFG9	+	Rod	-	Homo	-	-	+	+	+	-	-	-	*Lactobacillus*
	41	AFG2	+	Rod	-	Homo	-	-	-	+	-	+	+	+	*Lactobacillus*
	42	AFG25	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	43	AFG17	+	Rod	-	Homo	-	-	+	+	-	-	-	-	*Lactobacillus*
	44	AFG4	+	Rod	-	Homo	-	-	+	+	+	-	-	-	*Lactobacillus*
	45	AFG10	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	46	AFG26	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	47	AFG6	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	48	AFG1	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	49	AFG20	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	50	AFG14	+	Rod	-	Homo	-	-	-	+	-	+	+	-	*Lactobacillus*
	51	AFG13	+	Rod	-	Homo	-	-	+	+	-	-	-	-	*Lactobacillus*
	52	AFG15	+	Rod	-	Homo	-	-	+	+	-	+	-	-	*Lactobacillus*
	53	AFG37	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	54	AFG3	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	55	AFG28	+	Rod	-	Homo	-	-	+	+	+	+	-	-	*Lactobacillus*
	56	AFG38	+	Rod	-	Homo	-	-	+	+	-	+	+	-	*Lactobacillus*
	57	AFG24	+	Rod	-	Homo	-	-	+	+	-	+	+	+	*Lactobacillus*

	58	AFG33	+	Rod	-	Homo	-	-	+	+	-	-	-	-	*Lactobacillus*
	59	AFG34	+	Rod	-	Homo	-	-	+	+	+	-	-	-	*Lactobacillus*
	60	AFG19	+	Rod	-	Homo	-	-	+	+	+	+	+	-	*Lactobacillus*
Surface	61	AFS1	+	Rod	-	Homo	-	-	-	+	-	+	+	+	*Lactobacillus*
	62	AFS9	+	Rod	-	Homo	-	-	+	+	-	+	+	+	*Lactobacillus*
	63	AFS19	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	64	AFS12	+	Rod	-	Homo	-	-	+	+	+	-	-	-	*Lactobacillus*
	65	AFS16	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	66	AFS3	+	Rod	-	Homo	-	-	+	+	-	+	+	+	*Lactobacillus*
	67	AFS11	+	Rod	-	Homo	-	-	+	+	-	+	+	+	*Lactobacillus*
	68	AFS13	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	69	AFS10	+	Rod	-	Homo	-	-	+	+	+	+	+	-	*Lactobacillus*
	70	AFS4	+	Rod	-	Homo	-	-	+	+	-	+	+	+	*Lactobacillus*
	71	AFS18	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	72	AFS5	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	73	AFS6	+	Rod	-	Homo	-	-	+	+	+	+	+	+	*Lactobacillus*
	74	AFS14	+	Rod	-	Homo	-	-	-	+	-	+	+	+	*Lactobacillus*
	75	AFS2	+	Rod	-	Homo	-	-	+	+	-	+	+	+	*Lactobacillus*
	76	AFS15	+	Rod	-	Homo	-	-	-	+	-	+	+	-	*Lactobacillus*
	77	AFS20	+	Rod	-	Homo	-	-	+	+	+	+	+	-	*Lactobacillus*
	78	AFS7	+	Rod	-	Homo	-	-	-	+	+	+	+	+	*Lactobacillus*
	79	AFS22	+	Rod	-	Homo	-	-	-	+	-	+	-	-	*Lactobacillus*
	80	AFS26	+	Rod	-	Homo	-	-	-	+	-	+	-	-	*Lactobacillus*

Appendix 5. Some Pictures of the study

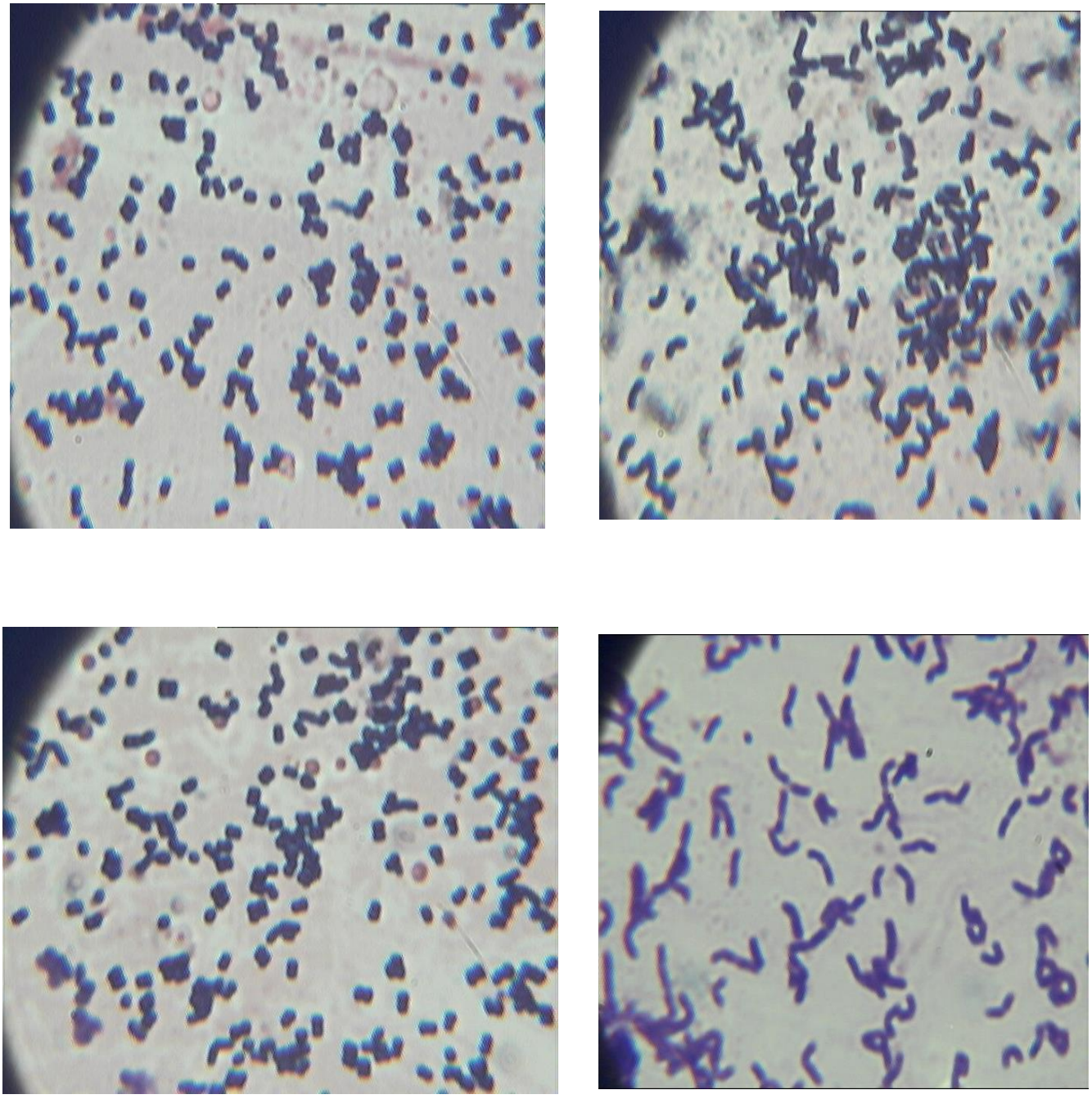

Figure: Microscopic View of Some LAB Isolates

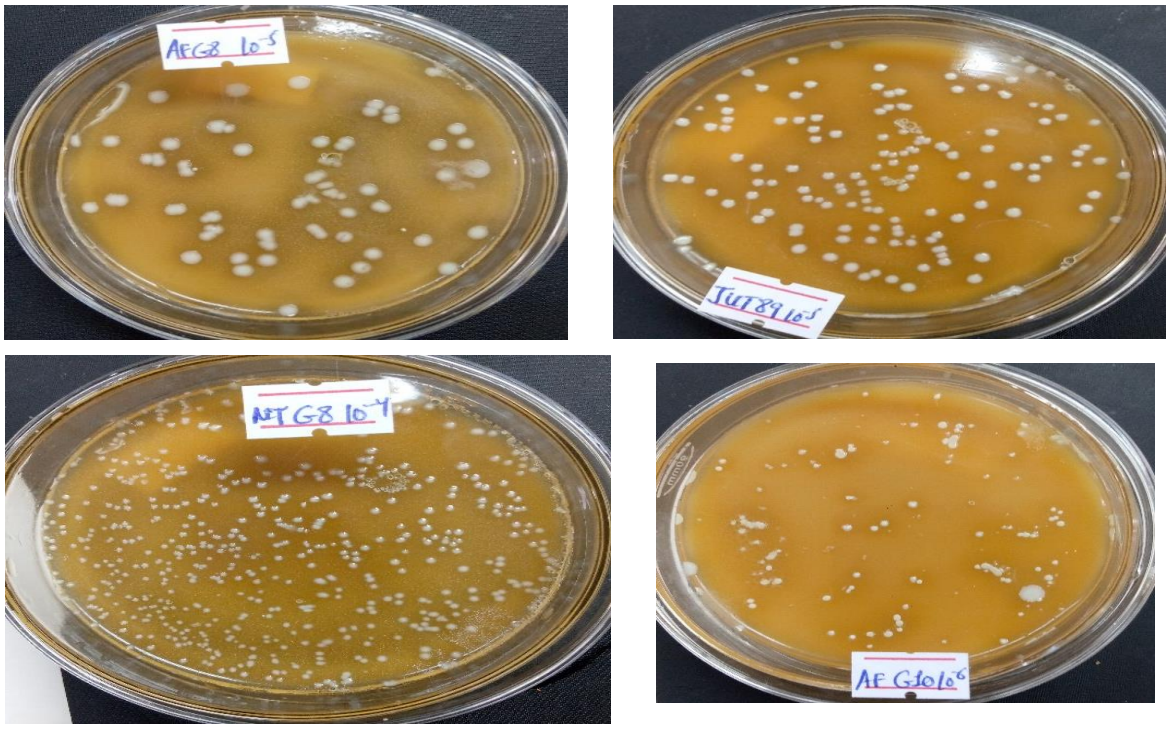

Figure: Some LAB isolates on MRS Agar

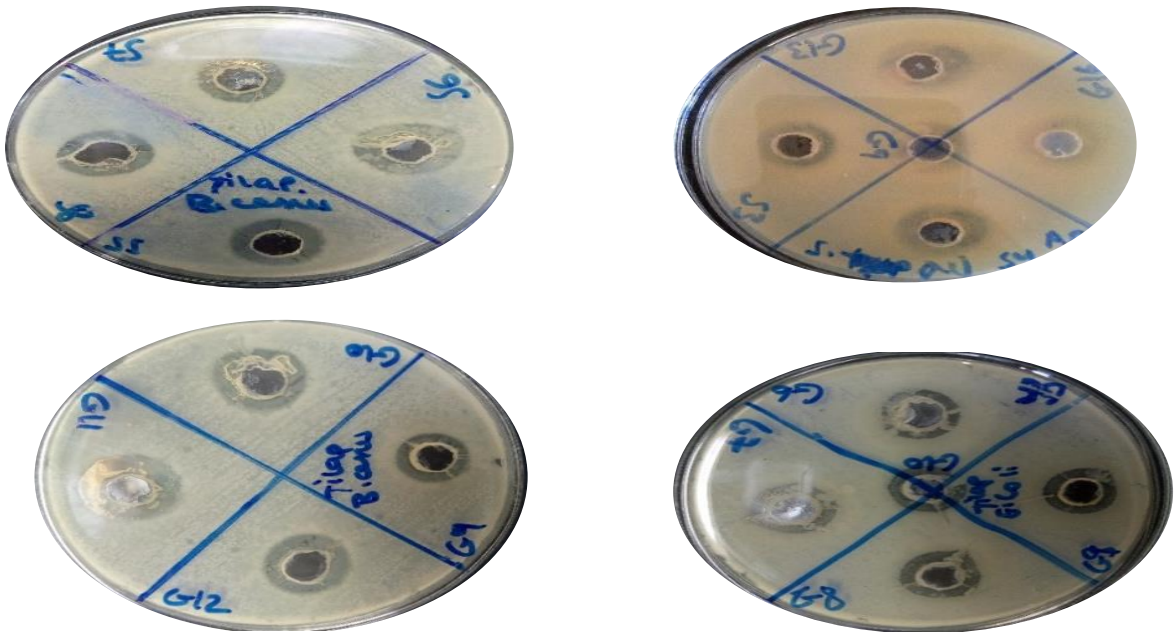

Figure: Antimicrobial activity of some LAB isolates

Figure: Sample preparation and dissection

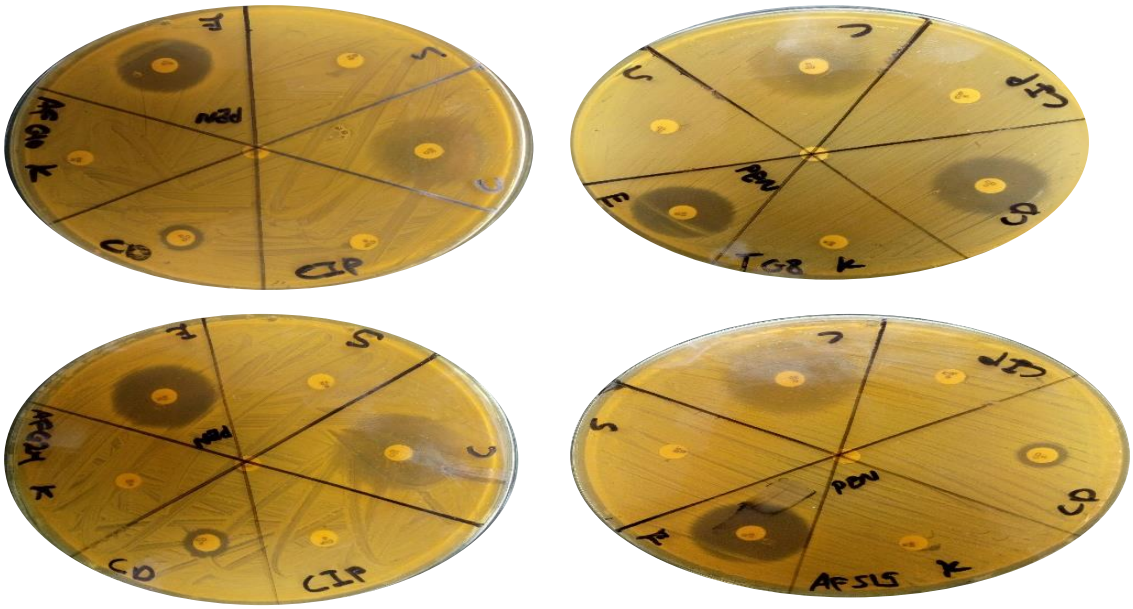

Figure: Antibiotic susceptibility of some LAB isolates

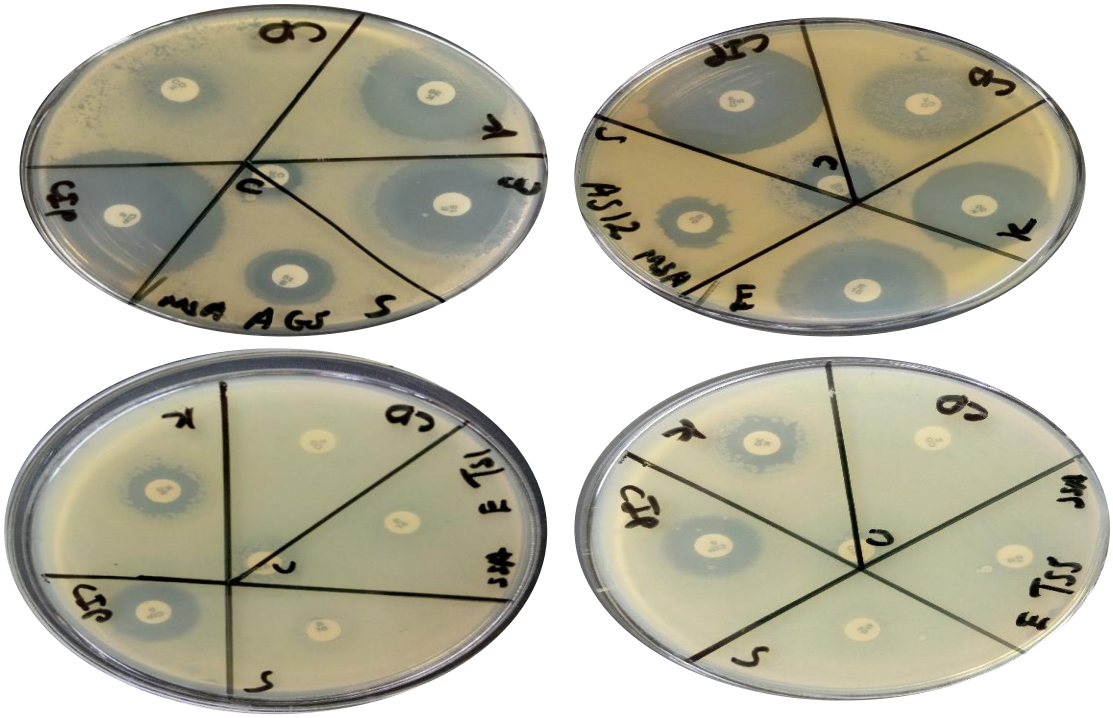

Figure: Antibiotic susceptibility of some pathogens

Figure: Some biochemical tests for pathogens

Figure: Aquarium setup

Figure: Admiration of Probiotics