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## **Application of Lactic Acid Bacteria in Fish Fermentation**

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### **Abstract:**

Ensuring food quality and safety remains a major challenge in developing countries. Fish and its products, as rich nutritional sources, are prone to contamination and spoilage, which can lead to foodborne illnesses. Fermentation, one of the oldest and most effective preservation methods, offers cost-efficiency and improved organoleptic and nutritional properties. LAB (Lactic acid bacteria), a diverse group of microorganisms, play essential roles in the fermentation of traditional fish products by acidifying the matrix, improving texture, and enhancing microbial safety through competition with spoilage organisms and biogenic amine reduction. Fish fermentation results in products such as fish paste and sauces, which may promote consumer health. Moreover, LAB-fermented feeds enhance growth rates and gut probiotic levels. This study reviews the nutritional, microbial, and sensory aspects of fermented fish products, focusing on the metabolic control of LAB to improve flavor and safety, thereby supporting the production of high-quality and safe fish-based foods.

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### Introduction

Foodborne illnesses are recognized as a significant public health concern, causing high morbidity and mortality worldwide. It is estimated that 600 million people fall ill due to contaminated foods annually (Cortés-Sánchez et al., 2024; Faour-Klingbeil et al., 2020). Symptoms may range from mild gastrointestinal distress to severe conditions such as septicemia and meningitis. Fish and fishery products, due to their high protein content and nearly neutral pH, are highly perishable and susceptible to microbial spoilage (Cai et al., 2024; Friesema et al., 2022), despite being vital sources of omega-3 fatty acids, proteins, vitamin D, and selenium (Zang et al., 2020).

Fermentation has been widely adopted to prolong the shelf-life of fresh fish. This microbial process enhances food quality and is especially advantageous due to its simplicity, low cost, and the added nutritional and sensory value it imparts (Marti-Quijal et al., 2020). Fermented fish products appear in various forms such as sauces, pastes, and dried-fermented items (Takenaka et al., 2020), offering a more viable preservation alternative compared to freezing or drying (Zang et al., 2020). Historically, fermentation has ancient roots, and with the advancement of microbiology in the 19th century, better control of fermentation processes became possible (Manna et al., 2024).

LAB are Gram-positive, non-motile, non-spore-forming, catalase-negative, oxidase-negative, microaerophilic bacteria that ferment carbohydrates into lactic acid (Wang et al., 2021b). Morphologically rodshaped (bacilli) or spherical (cocci), they share similar physiological traits. LAB are nutritionally fastidious. requiring carbohydrates, amino acids, peptides, nucleic acid derivatives, and vitamins for optimal growth. Based on fermentation metabolism, they are classified as homo- or heterofermentative, and produce antimicrobial compounds that enhance food safety (Manna et al., 2024).

In addition to controlling spoilage, LAB also inhibit biogenic amine formation during fermentation (Cai et al., 2024) and contribute to flavor development via proteolysis, yielding aldehydes, alcohols, and esters (Kieliszek et al., 2021; Cai et al., 2024).

Furthermore, LAB widely are recognized probiotics. Intestinal as microbiota, influenced by diet, antibiotics, and environmental conditions, may become compromising immunity imbalanced, (Luan et al., 2023). LAB, as probiotic agents, improve immune responses and are considered alternatives to antibiotics in aquaculture. Studies have demonstrated LAB's role in modulating gut microbiota and enhancing host immunity (Lee et al., 2024). This review places special emphasis on current research into fermented fish products, particularly the role of LAB in ensuring product safety and flavor optimization.

### Conclusion Microbiology and Biological Preservation of Fish

The composition and proportion of bacterial microbiota in freshly caught fish are influenced by both endogenous and exogenous factors. Endogenous factors include species-specific traits such as host lineage, genotype, diet, parasite load, immune status, and life history. Exogenous factors encompass the bacterial composition of surrounding the environment, environmental features, and physicochemical parameters such temperature and water pollution levels. Microorganisms are typically present on the external surfaces of fish, including skin, gills, and intestines.

In cold-water fish species, microbial levels on the skin and gills typically range from 10<sup>2</sup> to 10<sup>4</sup> CFU/cm<sup>2</sup>, while in warmwater fish, these values range from 10<sup>3</sup> to 10<sup>6</sup> CFU/cm<sup>2</sup>. In the intestinal tract, microbial loads may range from 10<sup>3</sup> to 10<sup>9</sup> CFU/g, heavily influenced by the type and amount of food consumed. Warm-water

fish generally host mesophilic bacteria, whereas temperate water species harbor both Gram-negative bacteria, such as Pseudomonas and Vibrio, and Grampositive genera including Bacillus and Lactobacillus in varying proportions (Lee et al., 2024; Sylvain et al., 2020). In polluted waters, high levels of Enterobacteriaceae, including Escherichia coli and Salmonella, have been reported.

Microbial activity is a major factor contributing to fish spoilage. Although freshly caught healthy fish are usually sterile internally, post-mortem invasion by bacteria occurs through the gills, skin, and bloodstream (Rathod et al., Psychrotrophic and mesophilic bacteria can cause spoilage within 1 to 2 days at elevated temperatures (above 15°C), particularly between 20-35°C (Cortés-Sánchez et al., 2024). Spoilage-related bacteria include species of Aeromonas, Vibrio. Pseudomonas, capable of growing under both aerobic and anaerobic conditions (de Matos et al. 2020). Bacterial activity in fish tissues results in undesirable changes such as discoloration and off-odors due to the production of organic compounds (Rathod et al., 2022; Speranza et al., 2021).

The presence of pathogenic microorganisms in fish is also significant, as they may be transmitted to humans via direct contact or consumption. These pathogens are classified into indigenous and non-indigenous types (de Matos et al., 2020; Speranza et al., 2021). Overall, the microbiological profile of fish is critical for assessing spoilage, pathogenic contamination, and shelf life. Thus, careful monitoring is required throughout the food supply chain to prevent foodborne illnesses (Cortés-Sánchez et al., 2024).

Several methods exist to inhibit microbial growth and enhance fish preservation. Common techniques include icing, refrigeration, and freezing, which effectively slow microbial proliferation but are often limited by short shelf-life and high costs. Other methods include pulsed electric fields, pulsed light, electrolyzed water,

thermal processing, dehydration, and pH reduction (Speranza et al., 2021; Talledo Solórzano et al., 2020; Cortés Sánchez et al., 2024).

Biological preservation, such fermentation, represents promising method to extend shelf life and ensure food safety by utilizing natural microbiota and antimicrobial compounds (Ramos-Vivas et al., 2021). This approach involves the use of lactic acid bacteria, bacteriophages, and endolysins. These microorganisms contribute to food safety through resource antimicrobial compound competition, production, and interactions at the intestinal level. However, biological preservation should not be viewed as a substitute for quality assurance systems such as HACCP (hazard analysis systems and critical control which remain points), essential improving the safety and quality of fish products. Due to the high susceptibility of fish to microbial growth, strict hygiene protocols must be enforced to prevent spoilage and foodborne diseases (Gutiérrez Fernández et al., 2020; Alegre Vilas et al., 2020).

# **Definition and Types of Fermented Fish Products**

Fermented fish is a widely consumed product in Asian, African, and Northern European countries. It is distinct from other fish products like dried or frozen fish due to its unique appearance, complex flavors, and specific packaging methods. Fermented fish products are typically classified into three main types: whole fermented fish, fish paste, and fish sauce (Zang et al., 2020; Chan et al., 2023) (Table 1).

Fermentation processes may be natural or controlled. Key parameters such as salt concentration, temperature, starter cultures, and fermentation duration significantly influence the final product's quality and appearance (Zang et al., 2020). Packaging also varies by product type: fish sauce and paste are usually stored in bottles or jars, while solid fermented fish is commonly canned, and dried fish is sold openly in

traditional markets (Rasul et al., 2020). Compared to other preservation methods, fermented fish products offer superior taste and quality due to the biochemical changes imparted by fermentation (Chan et al., 2023). Fermented fish products can be categorized based on several key criteria (Figure 1): (1) Product Form: Fermented fish can appear in various physical forms, including whole fish, fish chunks, pastes, or even liquid forms. (2) Fermentation Duration: In general, fermentation periods can be classified into three stages: Freshly fermented fish: This typically maintains the original form of the fish and lasts from a few days to several weeks (Bamidele et al., 2023). Semi-fermented fish: This stage spans from several weeks to a few months. The fish often transforms into a paste or liquid, resulting in a more intense and complex flavor profile (Loyda et al., 2023).

Fully fermented fish: Representing the longest stage of fermentation, the process continues until the product reaches full readiness for consumption (Bellaggia et al., 2020). (3) Type of Fermentation or Process: Fermented fish production can be broadly divided into two main approaches: **Spontaneous** fermentation, where initiation of the process relies on the naturally occurring microorganisms present in the fish and its environment (Ma et al., 2022). Starter culture fermentation, which involves the intentional introduction of selected microbial strains typically isolated fermented traditionally from foods (Vinicius De Melo Pereira et al., 2020). Starter cultures may include yeasts, which produce aromatic compounds via carbohydrate fermentation (Cai et al., 2024),

Table 1 Summary of various fermented fish products and their fermentation parameters (Chan et al., 2023).

Products	Origin	Types of Products	Types of Fish	Pre- Treatment/Additive	Fermentation Time	Temperature
Nampla	Thailand	Fish sauce	Stolephrous spp. Ristrelliger spp. Cirrhinus spp.	Salt or brine, throughout, the process	6–18 months, depending on the size of fish	Ambient temperature
Budu	Malaysia	Fish sauce/paste	Stolephorus spp.Sardinella spp.Decaterus macrosoma	Fish added with salt before fermentation, and added with palm sugar, tamarind, and monosodium glutamate as flavourings	6–12 months	30–40 <sup>oc</sup>
Bakasang	Indonesia	Fish sauce/paste	Katsuwonus pelamis L.	Preconditioned in a warm place	7 days	$30-50^{\ 0C}$
Yu-Lu	China	Fish sauce	Engraulis japonicas, Channa asiatica	Preconditioned with salt at a 1:3 salt-to-fish ratio	2–6 months	20–25 °C
Shidal	India	Whole fish	Puntius spp. Setipinna phasa	Air-tight earthen container	3–5 months	Room temperature
Jeotgal (e.g.,myeolchijeot)	Korean	Whole fish	Anchovies	Only salt, or with the addition of Korean red peppers, soy sauce, and/or malted rice	Two months for jeotgal with low-salt levels (6–18%), and a few years for jeotgal with a high-salt content (over 20%)	10-30 <sup>0c</sup>
Katsuobushi	Japan	Whole fish	Euthynnus pelamis, Katsuwonus pelamis, Euphonia affinis, Auxis rochei, Auxis thazard, Sarda orientalis	Fish are dried (Arabushi)	6 months	Ambient temperature
Feseekh	Egypt	Whole fish	Mugil cephlus, Alestes baremose, Hydrocynus sp.	Low salt level after maturing phase; second has a high salt content and can be eaten after storing	Maturing phase:15–20 days, Storage phase:2–3 months	Room temperature

and bacteria, among which lactic acid bacteria (LAB) are the most prominent group (Liu et al., 2021). (4) Salt Content: Fermented fish products can also be classified based on their salt concentration High-salt (>20% of total weight), Medium-salt (typically 10–15%), Low-salt (3-8%) and Salt-free products. Salt acts as a natural preservative by lowering water activity and inhibiting the growth of spoilage and pathogenic microorganisms (Cai et al., 2024). Moreover, it enhances the flavor, promotes firmness, and improves the texture of the fish during fermentation (Wang et al., 2020). (5) Added Ingredients: Besides the main ingredients used in fermented fish production, some products include carbohydrate sources such as starch (e.g., rice, millet, rice bran, flour) and sugars (e.g., sucrose, glucose, fructose, maltose). These serve as energy sources for microbial growth, supporting rapid and proliferation of LAB yeasts. Simultaneously, the microbial metabolism of these carbohydrates generates flavor compounds like organic acids and esters. This metabolic activity plays a crucial role unique shaping the characteristics of fermented fish products (Feng et al., 2021).

## Lactic Acid Bacteria (LAB) Definition of LAB

Lactic acid bacteria (LAB) are Grampositive, non-spore-forming microorganisms that are commonly found in natural environments and fermented foods such as dairy products, meat, and alcoholic beverages. LAB encompass a wide range of genera including Streptococcus,

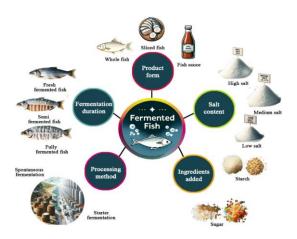


Fig 1 Classification of fermented fish.

Enterococcus, Lactobacillus, Aerococcus, Carnobacterium, Leuconostoc, Lactococcus, and Pediococcus. In addition, genera such as Enoeococcus, Sporolactobacillus, Tetragenococcus, Vagococcus, and Weissella are also classified as LAB, all belonging to the order Lactobacillales (Aguirre-Garcia et al., 2024; Bautist and Barrado 2023).

From a metabolic perspective, LAB ferment carbohydrates and primarily produce lactic acid (homofermentative). However, some species also generate other metabolites such as succinate, ethanol, acetate, and carbon dioxide (heterofermentative) (Table 2).

LAB are considered safe bacteria with recognized health benefits and probiotic properties. They play a significant role in improving the quality and safety of foods, making them a healthy dietary option for humans (Aguirre-Garcia et al., 2024; Freire et al., 2021).

**Table 2** Lactic acid bacteria, antimicrobial metabolites, and glucose fermentation metabolism (Cortés-Sánchez et al., 2024)

Microorganism	Produced Bacteriocin	Fermentation Metabolism	
Lactococcus lactis	Nisin	Homofermentative	
Pediococcus acidilactici	Pediocin	Homofermentative	
Lactobacillus sakei	Sakacin	Heterofermentative	
Enterococcus faecium	Enterocin	Homofermentative	
Leuconostoc mesenteroides	Mesenterocin	Heterofermentative	
Lactobacillus casei	Caseicin	Heterofermentative	
Lactobacillus helveticus	Helveticin	Heterofermentative	

In the food industry, LAB are widely used as biological preservatives, capable of inhibiting the growth of spoilage and pathogenic microorganisms. Their metabolic by-products influence the nutritional, sensory, and safety characteristics of fermented products, thereby enhancing their shelf life and overall quality (Rathod et al., 2022; Freire et al., 2021; Ramírez Ramírez et al., 2021).

### **Bacteriocins**

Bacteriocins are antimicrobial peptides synthesized by lactic acid bacteria (LAB) and secreted into the extracellular environment (Alegre Vilas et al., 2020). The production of these peptides is typically triggered under stress conditions such as pH fluctuations, redox potential changes, and nutrient limitations (Catagna Rodriguez., 2022).

Bacteriocins exert their antimicrobial through various mechanisms. effects including inhibition of peptidoglycan synthesis, interference with DNA, RNA, and protein metabolism, disruption of membrane integrity in target cells, and formation of ion channels leading to essential intracellular leakage of components (Negash and Tsehai., 2020; Alegre Vilas et al., 2020; Bautista and Barrado, 2023). Bacteriocins are influenced by factors such as proteolytic enzymes and food composition (fat and salt content), but they generally exhibit stability under heat and across a wide pH range. These peptides are colorless, odorless, and tasteless, enhancing their potential for application in food, pharmaceutical, and agricultural industries (Negash and Tsehai, 2020; Rodríguez et al., 2022). They are classified based on factors such as producing microorganisms, weight, molecular physicochemical properties, chemical structure, and mode of action (Alegre Vilas et al., 2020; Bautista and Barrado, 2023).

Classification of Bacteriocins (Catagna Rodriguez, 2022; Cortés-Sánchez et al., 2024):

- 1. Class I Lantibiotics: Small peptides (<5 kDa) containing unusual amino acids.
  - Subclasses include:
- Class Ia: Linear, cationic peptides (e.g., nisin)
- Class Ib: Globular, hydrophobic peptides
- Class Iia: Heat-stable peptides with specific sequences
- Class lib: Two-peptide pore-forming complexes
  - Class Iic: Small circular peptides
- Class Iid: Single post-translationally modified peptides
  - Class Iie: Multi-peptide systems
- 2. Class II Linear bacteriocins: Small (<10 kDa), heat-stable peptides
- 3. Class III High molecular weight bacteriocins: Large, structurally complex peptides
- 4. Class IV Complex circular peptides: Composed of proteins along with lipid or carbohydrate moieties
- 5. Class V Circular bacteriocins: Not post-translationally modified

In the food industry, bacteriocins are applied through several strategies: Direct addition of live bacteriocin-producing LAB, Incorporation of crude extracts or concentrates, Use of purified bacteriocins as food additives, and Immobilization or integration of bacteriocins into packaging materials (Bautista and Barrado, 2023; Catagna Rodriguez., 2022). These methods contribute to enhancing food safety and shelf life and can be combined with physicochemical treatments or natural preservatives (Bautista and Barrado, 2023). Numerous global studies have explored the application of LAB and their metabolites in food preservation (Table 3) (Cortés-Sánchez et al., 2024).

Several studies have evaluated the effects of lactic acid bacteria (LAB) and bacteriocins on the preservation and quality of fish fillets. In the study by Castillo-Jiménez et al., the effect of LAB immersion (Lactobacillus plantarum and Lactobacillus acidophilus) on Oreochromis niloticus fish fillets at 5°C for 30 days was examined.

Fillets containing LAB had 5.94 log CFU/g LAB and <2.7 log CFU/g coliforms and psychrophiles, while the control group showed higher levels of coliforms and psychrophiles. This study demonstrated that LAB, due to their production of organic acids and bacteriocins, can inhibit spoilage microbiota (Castillo-Jiménez et al., 2017).

In the study by Talledo et al., the physico-chemical properties and inhibition of microbial spoilage of Oreochromis sp. fish fillets at 3°C using LAB were investigated. LAB were able to maintain the quality and shelf life of the fillets for 30 days (Talledo Solórzano et al., 2020). In the study by Salazar et al., the effect of LAB antimicrobial extracts on tilapia fillets at 8°C for 10 days was examined. These extracts reduced the concentration of mesophiles and coliforms, helping to preserve the product (Salazar et al., 2011).

In the study by Kaktcham et al., the effect of semi-purified nisin produced by *Lactococcus lactis* subsp. on fish paste was compared with sodium benzoate. Nisin was able to inhibit microbial load more effectively and was introduced as an effective preservative to improve the safety and quality of fish paste without affecting its sensory properties (Kaktcham et al., 2019). These studies indicate that the use of LAB and bacteriocins can serve as effective methods for preserving the quality and shelf

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# Application of Lactic Acid Bacteria (LAB) in Fish Silage Production

Industrial processing of fish generates major waste (about 60% includes fins, scales, heads, entrails, and skeletons), which results in nearly 29 million tons of waste globally (Ramírez et al., 2021). These waste products can make up to 70% of the fish's initial weight and are used in fish silage production (Cortés-Sánchez et al., 2024). Silage is a semi-liquid paste product derived from whole fish or waste, and it is categorized into two types: chemical (using organic acids) and biological (fermented by LAB).

**Table 3** Studies on the application of lactic acid bacteria (LAB) or their metabolites for the preservation of fish and fish products

Fish/Byproduct	Application Conditions	Analyzed Microorganisms	Biocontrol	Limitations
Lubin fillets (Centropomus undecimalis)	Immersion in 2% sodium alginate coating containing L. reuteri at 24 and 48 h of fermentation and storage at 4 °C.	Aerobic, psychotropic, and enterobacterial microorganisms.	Reduction in the growth of aerobic, psychotropic, and enterobacteria compared to control fillets. Improves the color and texture of foods due to fermentation, maintaining a compact structure and reducing oxidation reactions.	NR
Cold smoked salmon	Use of L. sakei CTC494 inoculated (1% v/w) to a final concentration of4.6 log CFU/g. Vacuum packed and stored at 8 °C/21 days.	L. monocytogenes.	Growth inhibition of L. monocytogenes for 21 days.	NR

Silage is also recognized as a valuable protein source for animal feed possesses antibacterial and antioxidant properties (Ozyurt et al., 2020; Ramírez Ramírez et al., 2021). LAB fermentation can improve the nutritional quality of silage and inhibit the growth of fungi and harmful bacteria. Biological silage typically remains stable at a pH of 4 to 4.5 and acidity of 3.2% for around 30 days (Guapacha et al., 2021). However, fish silage has drawbacks such as content, which high water makes transportation economically inefficient, and its susceptibility to oxidation, which can lead to the formation of toxic products and reduced quality. Additionally, exposure to light and air can accelerate oxidation. Therefore. removing fat or adding antioxidants can help improve product stability, although synthetic antioxidants may be expensive or prohibited in some cases (Raeesi et al., 2023).

### **Role of LAB in Fermented Fish Products**

Fermented fish is a product made from fermentation spontaneous the microorganisms under natural conditions, exhibiting wide variety microorganisms (Feng et al., 2021). The microbial composition of these products varies depending on geographic location, raw materials, and production methods (Table 4). Initially, molds (such Aspergillus and Penicillium) proliferate rapidly (Cai et al., 2024), but fermentation progresses and salt penetration occurs, their populations decrease, and lactic acid bacteria (LAB) and yeasts become dominant. Over time, the pH decreases, and conditions become favorable for LAB growth, which contributes to an increase in umami flavor (a savory taste found in fermented products in Japan) (Wang et al., 2022c). Halophilic bacteria play an essential role in preserving safety and enhancing the flavor of fermented fish (Wang et al., 2022c). LAB such as Leuconostoc, Lacticaseibacillus,

Enterococcus. Lactococcus. and Pediococcus contribute to the fermentation process and final flavor, with their diversity varying depending on the region and production methods (Hu et al., 2022; Han et al., 2023). For instance, in two Filipino products, Tinabal and Patis, the LAB composition differs. These changes are linked to region-specific characteristics. Additionally, in traditional Korean fermented fish sauce, Tetragenococcus is identified as the dominant microorganism, which is dependent on the size of anchovies used (Cai et al., 2024). For example, fish is a key component of the human diet in West Bengal and Odisha, where over 90% of the population consumes fish. Due to the perishable nature of fish, it is dried by adding salt (3 to 4 kg per 100 kg of raw fish), which helps remove water and initiate partial fermentation. This drying process takes 7 to 10 days and contributes to the enhancement of the nutritional value and sensory properties of the products. One popular food product in these regions is Sukoti or Shutki, which is dried fish. bacteria (Enterococcus Various and Lactobacillus) and yeasts (Candida kyropetroorum and Candida bombicola) are involved in the fermentation process of Sukoti. Overall, the presence and activity of LAB in various fermented fish products are closely linked to the development of taste and product quality (Manna et al., 2024).

# **Impact of LAB on the Fermentation Process of Fish**

### **Microbial Population Structure**

A high microbial diversity in the gut flora is beneficial for maintaining gut health (Feng et al., 2021). However, in fermented products, simplicity in the microbial structure is crucial to ensure optimal quality (Bai et al., 2021). The addition of lactic acid bacteria (LAB) in the production of fermented fish reduces microbial diversity and prevents the growth of harmful microorganisms (Mayata-Apaza et al., 2021).

**Table 4** LAB in Spontaneously Fermented Fish (Cai et al., 2024)

LAB	Substrate	Product	Country
Levilactobacillus	Carp (Cyprinus carpio)	Suan zuo yu, Suan yu	China
Levilactobacillus brevis	Ngasang (Esomus danricus)	Hentak	India
Lactiplantibacillus plantarum	Sardines (Sardina pilchardus), Red tilapia (Oreochromis niloticus and Oreochromis mossambicus)	Mahyaveh Plaa-som	Iran, Thailand
Limosa reuteri	Red tilapia (Oreochromis niloticus and Oreochromis mossambicus)	Plaa-som	Thailand
Tetragenococcus	Anchovy (Engraulis japonicus), Carp (Cyprinus carpio)	Myeolchiaekjeot Yu-lu Suanyu	Korean, China, China
Tetragenococcus halophilus	Herring (Clupea harengus)	Surstr omming	Swedish
Enterococcus	Carp (Cyprinus carpio)	Suan yu	China
Enterococcus faecalis	Carp (Cyprinus carpio)	Suanyu	China
Enterococcus faecium	Sardines (Sardina pilchardus)	Mahyaveh	Iran
Pediococcus	Carp (Cyprinus carpio), Trout, arctic, char	Suan yu, Rakfisk	China, Norwegian
Pediococcus pentosaceus	shrimp or fish	Pha ak trey,Pha ak, kampus	Cambodia
Weissella	Carp (Cyprinus carpio)	Suan zuo yu, Suan yu	China
Leuconostoc	Carp (Cyprinus carpio), Trout, arctic, char	Suan yu, Rakfisk	China, Norwegian
Carnobacterium	Herring (Clupea harengus), Trout, arctic, char	surstr"omming, Rakfisk	Swedish, Norwegian
Lactococcus	Carp (Cyprinus carpio)	Suan yu	China
Marinilactibacillus psychrotolerans, Streptococcus infantis	Herring (Clupea harengus)	Surstr <sup>*</sup> omming	Swedish

### **Acidification**

Lactic acid bacteria (LAB) produce lactic acid by fermenting sugars and carbohydrates, leading to a decrease in pH and improved food safety. Fish products with a pH lower than 4.4 are safe for raw consumption (Cai et al., 2024). In the traditional Iranian fermented fish product "Mahiyeh," the use of a combined starter culture significantly increased the total acidity (Nazari et al., 2021). accumulation of organic acids in fermented foods not only prevents the growth of harmful microorganisms but also plays a vital role in regulating the flavor and texture of the food (Cai et al., 2024).

## Role of LAB in the Flavor, Aroma, and Texture of Fermented Fish Flavor

The flavor of fermented fish encompasses a wide range of tastes such as sweet, salty, sour, bitter, and spicy, with sourness and umami being key characteristics. Sourness is primarily due to organic acids like lactic acid and other acids such as formic acid, acetic acid, propionic

acid, n-butyric acid, isobutyric acid, n-valeric acid, and iso-valeric acid (Cai et al., 2024) (Table 5). LAB play a significant role in the fermentation process, contributing to appetite stimulation and saliva secretion (Bangar et al., 2022). Umami flavor in fermented fish is primarily derived from peptides, amino acids, and nucleotides, resulting from the breakdown of proteins and nucleic acids. Studies have shown that amino acids such as glutamic acid and aspartic acid contribute to the umami flavor associated with fermented fish (Wang et al., 2022c).

### **Aroma**

The aroma in fermented fish is created by compounds produced metabolic processes, including alcohols, aldehydes, ketones, and sulfur-containing compounds (Han et al., 2023). The impact of these compounds on aroma is evaluated using the odor activity value (OAV). identified 56 volatile Studies have compounds in fermented fish sauce, 12 of exceed OAV threshold. the Compounds like 3-methylthiopentanal and

**Table 5** Effect of LAB on flavoring substances in fermented fish (cai et al., 2024)

LAB	Substrate	Product	FAA	Non-volatile compounds	Volatile compounds
Pediococcus pentosaceus, Pediococcus lactis, Lactiplantibacillus plantarum, Latilactobacillus sakei	Carp (Hypophthalmichthys molitrix)	Fermented fish-chili paste	Glu↑, Asp↑, Lys↑, Ala↑, Gly↑	Lactic acid↑, Citric acid↓, Malic acid↓	Branched-chain, alcohols↑, Phenolic↑, Ethyl esters↑, Ethanol↓, Aldehydes↓
Lactiplantibacillus plantarum, Pediococcus pentosaceus, Pediococcus acidilactici	Carp (Ctenopharyngodon idellus)	Zhayu	Glu↑, Asp↑, Thr↑, Lys↑, Tyr↑, His↑	Acetic acid↑, Butanoic acid↑, 4- methylpentanoic acid↑	Saturated alcohols↑, Terpenoids↑, Phenols↑, Dimethyl sulfide↑, Ketones↓
Enterococcus rivorum, Enterococcus lactis	Crucian (Carassius auratus)	Suanyu	Glu↑, Ala↑, Leu↑	Tartaric acids↑, Malic acids↑, Lactic acids↑, 5'-AMP↑, 5'- IMP↑, Citric acid↓	Hexanal↑, Nonanal↑ (Z)- 2-, heptenal↑, (E)-2- heptenal↑, (E)-2- hexenal↑, 1-hexanol↑, 1- octen-3-ol↑, Ethyl hexanoate↑
Latilactobacillus fermentum, Lactiplantibacillus plantarum, Lactococcus lactis subsp. Cermoris	Nile tilapia (Oreochromis niloticus)	Fish sauce	Glu↑, Ser↑, Gly↑, Thr↑, Ala↑, Lys↑	Lactic acid↑, Acetic acid↑, Citric acid↓, Malic acid↓, Pyruvic acid↓, Succinic acid↓	1-hexanol↑, Acids↑, Esters↑, Aliphatic aldehydes↓
Lactiplantibacillus plantarum, Staphylococcus xylosus, Saccharomyces cerevisiae	Carps (Cyprinus carpio)	Suanyu	His↑, Arg↑, Tyr↑, glu↑	-	-
Tetragenococcus halophilus	Anchovy (Stolephorus sp.)	Nam pla	Glu↑, Val↑, Phe↑, Ser↓, Arg↓	-	2-Methylpropanal↑, 2- Methylbutanal↑, 3- methylbutanal↑, 2- methylpropanal↑, Ethyl acetate↑, Dimethyl disulfide↓
Lactiplantibacillus plantarum	Grass carps	Suanzhayu	Asp↑, Glu↑	-	1-hexanol\(\cap2\), 3-methyl-1-butanol\(\cap2\), 1-octen-3-ol\(\cap2\), Heptanal\(\cap2\); Hexanal\(\cap2\), Benzaldehyde\(\cap2\), Nonanal\(\cap2\); 3-octanone\(\cap2\), Ethyl hexanoate\(\cap2\), Ethyl octanoate\(\cap2\)

3-methylbutanal are recognized as the most influential flavor contributors (Wang et al., 2020a). Aldehydes and ketones have pleasant aromatic properties with nutty, fruity, and chocolate-like flavors and low odor thresholds, significantly influencing the flavor of fermented fish (Yang et al., 2020).

Alcohols, with mushroom, spice, and citrus aromas, contribute significantly, with 1-Octen-3-ol being identified as a key flavor compound in these products (Zhang et al., 2022). The interaction between alcohols and acids leads to the production of esters, enhancing the sweetness and richness of the aroma of fermented fish

(Gao et al., 2020). Additionally, volatile fatty acids with short- and medium-chain lengths (4-12 carbon atoms) have lower odor thresholds, while long-chain fatty acids (over 12 carbon atoms) have a lesser impact on flavor (Cai et al., 2024). The aroma of these fish is mainly due to the degradation of amino acids and lipid oxidation (Feng et al., 2021), with LAB playing a key role in protein hydrolysis into free amino acids and the production of free fatty acids (FFAs). These FFAs then undergo oxidation, producing volatile compounds like aldehydes and ketones. Moreover, compounds like esters are formed from interactions between alcohols

and acids, which contribute to the richness of the aroma (Cai et al., 2024).

### **Texture**

Texture significantly contributes to the sensory appeal of fermented fish products, including characteristics such as hardness, springiness, adhesiveness, and chewiness. These textural attributes are primarily influenced by microorganisms and active enzymes. The gelling of fish proteins notably impacts the overall texture of fermented fish. Changes in firmness arise from the denaturation and coagulation of muscle proteins, while springiness and adhesiveness represent the development of internal bonds in the gel-like network of muscle proteins (Xu et al., 2021). LAB produce organic acids that lead to a decrease in pH, which in turn causes the coagulation of muscle proteins. This coagulation enhances the gel network structure, increasing the hardness of the fish meat (Cai et al., 2024). Additionally, high salt content fermented fish contributes to increased hardness of the meat influencing water retention and loss (Wang et al., 2020). Conversely, the reduction in springiness in fermented fish can be attributed to the proteolytic activity of endogenous proteases (Cai et al., 2024).

# Impact of LAB in Enhancing Food Safety of Fermented Fish

# Reduction of Pathogens and Spoilage Microorganisms

In many cultures, fermented fish is preferred to be consumed raw. For example, "Bagoong," a Filipino fermented fish product made from a mixture of raw fish, salt, sugar, and dill, is consumed without a heating step, which could potentially lead to contamination pathogenic by microorganisms and health issues such as diarrhea and malnutrition (Cai et al., 2024). Both fungi, including Candida albicans, Debaryomyces hansenii, Aspergillus flavus, and Penicillium digitatum (Xu et al., 2021), and bacteria, including Pseudomonas, Clostridium perfringens, Shewanella putrefaciens, Morganella morganii, and Hafnia alvei, are responsible for spoilage (Wang et al., 2023d; Zhang et al., 2022). Fungi typically produce toxins, while bacteria destroy the tissue and produce odors in fermented fish products. Unfortunately, fish meat has high water activity, is rich in protein and fat, and provides an ideal environment for the microorganisms. growth of various Therefore, controlling the growth and reproduction of these potentially harmful microorganisms in fermented fish is crucial to ensure food safety (Cai et al., 2024).

Traditional fermented fish production often involves adding substantial amounts of salt to control the growth of pathogenic and spoilage bacteria. However, excessive salt consumption can lead to various health including hypertension, issues. heart disease, vascular problems, stroke, and stomach cancer. Moreover, high salt levels may promote the formation of N-nitroso compounds and biogenic amines during fermentation (Han et al., 2023). LAB play a key role here, as they effectively inhibit the growth of Vibrio bacteria through carbohydrate metabolism into lactic acid (Cai et al., 2024). LAB also prevent the development of pathogenic and spoilage bacteria through the secretion of bacteriocins. For instance, plantarum Lactiplantibacillus 1-24-LJ inhibits effectively the growth proteobacteria, including harmful strains like Escherichia coli and Salmonella, while promoting the growth of beneficial LAB strains (Zhang et al., 2023).

### **Biogenic Amines**

During fermentation, proteins are broken down into free amino acids, which can lead to the production of biogenic amines such as histamine. This transformation occurs when amino acids are decarboxylated by amino acid decarboxylases, producing various biogenic amines. Additionally, biogenic amines can also arise from aldehydes and ketones, influenced by aminotransferases (Xu et al., 2021).

Excessive consumption of these amines, particularly histamine, can lead to side effects such as headaches and respiratory problems. To optimize the management of biogenic amines in fermentation, reducing temperature and moisture is effective, but may increase costs and prolong the process. Efforts to control the production of biogenic amines focus on inhibiting microbial amino acid decarboxylase activity and promoting microorganisms that degrade amines (Cai et al., 2024). Some LAB strains have the suppress biogenic ability to production. For example, specific strains like Lactobacillus plantarum can inhibit amine-producing biogenic bacteria, reducing histamine content in fermented fish (Zhang et al. 2022). Furthermore, LAB's ability to reduce the accumulation of biogenic amines is strain-dependent, requiring precise validation for each strain (Zhang et al., 2021).

### **Discussion**

Fish are a nutritious and essential food but due to their specific source. characteristics, such as pH and water activity, they are prone to spoilage and contamination. Biological preservation using LAB as a chemical-free alternative can enhance the shelf life and safety of fish products. The use of LAB in the production of fermented fish aids in improving flavor aroma while inhibiting harmful bacteria. Factors such as temperature (optimal temperature around 30°C), water activity, and salt concentration influence LAB performance. Maintaining appropriate pH is also essential for LAB efficiency, as pH levels above 8 or below 2 can reduce their activity. The production of fermented fish faces challenges such as carbohydrate deficiency, but additional carbohydrates can be introduced during fermentation. Indeed, the use of LAB offers numerous benefits, such as enhanced safety, consistent product quality, and the ability to fine-tune flavor profiles. However, this process is not without challenges. Fermented fish, worldwide, involves a wide

range of preparation methods, varying in fish species, initial microbial content, and variables such as temperature, pH, and salinity. These variations can introduce fluctuations in the fermentation process. Consequently, screening, identification, and functional evaluation of specific LAB strains suited to different fermented fish production conditions become a significant challenge. Moreover, fermented fish, as a regional delicacy, possesses a unique set of flavors and characteristics that make it distinctive. The introduction of LAB as a starter culture inevitably alters microbial composition during fermentation, consequently influencing the flavor, aroma, and texture of the final product. The challenge lies in whether consumers accustomed to traditional fermented fish flavors will embrace these new profiles. Furthermore, navigating the landscape of food safety regulations and labeling in different regions poses another challenge. Ensuring the consistency and safety of fermented fish products as they transition from small-scale production to large-scale commercial efforts complex is a undertaking. Addressing these limitations and challenges requires a combination of scientific technological research. innovation, and a steadfast commitment to maintaining the highest standards of food safety and quality control. This review highlights the pivotal role of fermentation, particularly through LAB action, preserving and enhancing fish products. LAB contribute to the safety of fish products by inhibiting the growth of spoilage microorganisms, lead to the development of unique flavors, alter sensory properties, and make them appealing to a wide range of tastes across various cultures. In today's advanced food preservation technology landscape, the role fermentation has shifted preservation to flavor enhancement in fermented foods. Research clearly shows LAB's capacity to impact the flavor of fermented fish. However, there are currently few studies that shed light on

specific metabolites produced by LAB to modulate flavor and the optimal accumulation levels of these compounds to maximize the flavor profile of fermented fish. Therefore, deliberate control and precise modulation of characteristic metabolites should be the focal point for improving quality. Fermented fish products are mainly consumed in specific regions yet and have not been widely commercialized globally. With a better understanding of the fermentation process, the global reach of these products is expected to materialize soon. The health benefits associated with consuming fermented fish can aid in expanding its market. Improving the fermentation process by optimizing temperature, pH, and fermentation time according to the type of important to maximize fish is fermentation potential. Furthermore, deeper study of fermented fish compounds is necessary to improve their sensory properties. Hygiene practices and quality control systems such as HACCP can increase the safety of these products. The future of fermented fish products lies in improving fermentation technology and evaluating quality, and the use of Omics studies (aiming to collectively describe and quantify biological molecules that form the structure, function, and dynamics of an organism or organisms) in evaluating the microbiome of these products is expected to increase.

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