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Probiotic Bacteria in Fish Sauce Fermentation: Current Uses, Health Benefits, Challenges and Future Perspectives

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Abstract

Background and Aim: Fish sauce is a traditional functional product produced by the fermentation of fish with salt. There are disadvantages in the sauce manufacturing such as the long fermentation time, inconsistency of quality, production of histamine and contamination risks. Starter culture of probiotic bacteria have become a feasible means to overcome these disadvantages to make a safer, higher-quality and healthier sauce through changing the micro flora. Micro flora modify the microenvironment and metabolism of sauce and improve the fermentation process by synthesizing desired metabolites and bioactive compounds. This study reviewed various effects of probiotic microorganisms during the fermentation of fish sauce concerning its product quality, food safety, flavor development and bioactive substances for functional characteristics.

Material and Methods: Web of Science, Scopus, PubMed and Google Scholar were searched to collect data. The following terms were combined in the searching for references linked to the lactic acid bacteria, fermented fish products, probiotics, *Pediococcus pentosaceus*, *Lactiplantibacillus plantarum*, *Bacillus licheniformis*, *Tetragenococcus halophilus* and fish sauce fermentation.

Results and Conclusion: Probiotic microorganisms, particularly *Lactiplantibacillus plantarum* and *Bacillus licheniformis*, were beneficial in accelerating protein degradation, improving the sensory characteristics of sauce, preventing proliferation of spoilage and microbes and decreasing generation of biogenic amines and histamine. These could boost bioactive peptides and group-B vitamin contents of sauces for better health and improved characteristics in functional products. Non-conventional fermentations and mixed starter cultures might promise in improving process efficiency and constancy in sauce production. Fermentation with probiotic has emerged as an excellent technology that can provide better and safer products and includes great potential to commercialization; however, *in vivo* research and scaling-up for industrial uses of the probiotic fish sauce are necessary.

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What is "already known":

- The traditional fermentation processes require native microorganisms, leading to extended fermentation periods, product diversity, and generation of biogenic amines including histamine.
- To overcome these challenges, the use of probiotic starter cultures containing strains like *Lactiplantibacillus plantarum*, and *Bacillus licheniformis* to enhance fermentation performance and ensure safety and product quality.
- Most research efforts so far have been of these probiotics separately and few studies have evaluated the impact of these microorganisms on the technological, functional and health properties.

What this article adds:	• This review critically evaluates mainstream probiotic alternatives used in fish sauce fermentation based on their technological effectiveness, safety, and biological function. • This review illustrates the benefits of probiotic starter culture addition to the fermentation process in terms of flavor generation, improvement of nutritional benefits, enhanced safety and, diminished histamine production. • Research needs were delineated with reference to multispecies starter cultures, omics, up-scaled application and clinical evaluation to guide future development and marketing of improved safer, high value probiotic fish sauces.

1. INTRODUCTION

Fish sauce is one of the most ancient fermented traditional fish products and a part of the cultural heritage of Southeast Asian countries such as Thailand, Vietnam, Philippines and Cambodia. The sauce produced via natural fermentation of fish in high salt is appreciated for unique flavor, unique aroma and savory taste as well as its biological qualities [1]. Production process involved the action of microorganisms and various chemical, biological reactions that converted high molecular weight proteins to low molecular products, which include smaller peptides and amino acids that can be used as savoring agents of these products. Because of its important cultural and consumption characteristics, fish sauce production has increasingly become interested by the scientists [2].

Regarding further investigation on the important roles of microorganism in fermented product, adoption of probiotic bacteria as starting cultures in fish sauce fermentation has been suggested as a novel alternative approach [3–4]. Fish sauce has widely been known as a popular traditional seasoning product and involved in research in recent years since food with health benefits has been popular. The last several years have included significant progress in Food Microbiology and the use of probiotic microorganisms to enhance the process and widen the product scope of fermented seafood. Therefore, probiotic-assisted fish sauce fermentation has shown significant promise in combining the conventional technique with biotechnology advancement [5].

Traditional fish sauce is mainly produced via natural fermentation and depends on native microbial flora. Although this natural process produces products with diverse flavors, due to the uncontrolled microbial composition, it is prone to producing biogenic amines such as histamine and other toxic compounds that can harm consumers. Histamine, in particular, can cause food poisoning called scombroids in high doses. Therefore, controlling the growth of microorganisms that produce these compounds and replacing them with safe and beneficial strains is greatly important [6].

Despite all these advantages, use of probiotics in fish sauce fermentation faces challenges. One of these challenges is the selection of strains that can survive in high salinity conditions and have effective fermentation activity. Furthermore, stability of these strains during the storage process and their transportation and quality assurance and sensory acceptance of the product by the consumers are other issues that need further research [7].

Regarding the growing global trend in consuming healthy fermented foods and the increasing demand for safe, nutritious and quality products, it seems that the use of probiotic bacteria in fish sauce production can not only solve the problems of traditional methods, but also provide new horizons for the development of this traditional food industry on a global scale. Recent scientific studies emphasize the high potential of this approach. In recent years, mass production of probiotic fish sauces with superior characteristics is expected [8–9]. The present study reviewed various effects of probiotic microorganisms during the fermentation process of fish sauce concerning product quality, food safety, flavor development and bioactive substances for functional characteristics of the fish sauce.

2. METHOD: LITERATURE SEARCH STRATEGY AND STUDY SELECTION

This study searched thoroughly all the literature associating to use of probiotics during fish sauce production. Web of Science, Scopus, PubMed and Google Scholar were searched electronically to collect published data. The following terms were combined in the searching for references linked to the lactic acid bacteria (LAB), fermented fish products, probiotics, *Pediococcus* (*P.*) *pentosaceus*, *Lactiplantibacillus* (*L.*) *plantarum*, *Bacillus* (*B.*) *licheniformis*, *Tetragenococcus* (*T.*) *halophilus* and fish sauce fermentation. Articles published in English from 2000 to 2026 and those published from 2020 to present

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time were highlighted to achieve a sufficient up-to-date data. All data collected in this review critically analyzed, compared and contrasted on the basis of technical characters, potential of each candidate in a probiotic capacity and safe character, as well as the strengths and weaknesses in using those candidates. Numerous articles have been investigated, including review articles, book chapters and scientific researches on probiotics, fish sauces and linked areas such as microbial community dynamics, probiotics action mechanisms, fish sauce fermentation, startup cultures of fish sauces, microbes' interactions in fish sauce starters, and functional and healthful characteristics. However, repeated studies and irrelevant areas as well as the abstracts of conferences or proceedings have not been included.

This comprehensive review article collected all information on probiotic bacterial species that could be used as start culture in fish sauce fermentation based on their functions, technical characteristics, safety assessment, with emphasis on their ecological characters, production of functional ingredients, effects on fermentation and health benefits. Additionally, the study suggested designs of the novel startup cultures, comparisons with commercially accessible probiotics and further prospects of probiotic fish sauces.

3. TRADITIONAL FISH SAUCE FERMENTATION

3.1. An Overview of Traditional Fish Sauce

Traditional fish sauce production consists of salting of fish (20–30%) and setting the fish at room temperature (RT) for a prolonged time. Traditional fish sauce is rich in salt and high degree of protein hydrolysis and therefore amino nitrogen, peptides and flavor active compounds are produced in the fish sauce [10]. Chemical composition of the fish sauce and quality attributes are affected by the fish species, concentration of salt, time of fermentation and microbial activity. Major components of compositional and quality characteristics of traditional fish sauce compared with that produced by nontraditional and probiotic-assisted fermentation methods are summarized in Table 1.

The importance of the total nitrogen, amino nitrogen, salt, moisture and pH was that these parameters indicated the overall degradation of protein and quality of the product, reflected the protein hydrolysis, provided the umami, provided for the preservation and selection of microbes, regulated the total protein, controlled the physical characteristics of the product and fixed the extent; to which, microbial activity and stability affected the product [11]. During these conditions, endogenous microbes contribute to the degradation of fish proteins and the generation of flavor associated compounds. Nonetheless, the final product microbial characteristics and quality depend on factors such as the type of fish, environment and initially inherent microorganisms [10].

3.2. Microbiology of Fish Sauce Fermentation

As reported, *Halobacterium* and *Bacillus* species, belonging to the genera of halophilic bacteria, play roles in fish sauce fermentation. In addition, lactobacilli and staphylococci can be isolated. However, several of these microbes can accumulate biogenic amines such as histamine to a quantity that causes health problems in humans when consumed too much (Figure 1) [11–12].

Table 1. Typical compositional and quality characteristics of traditional and other fish sauce types (%)

Sample Parameter	Traditional sauce	Nontraditional sauce	Probiotic sauce
Total nitrogen (g/l)	1.50 – 3.00	2-3.50	2.50-4
Amino nitrogen (g/l)	0.60-1.20	0.80 – 1.50	1-1.80
Salt (%)	20 - 30	10-20	10-20
Moisture (%)	60-70	60-75	60-75
pH	5.30 – 6.50	5-6.20	4.80-5.80

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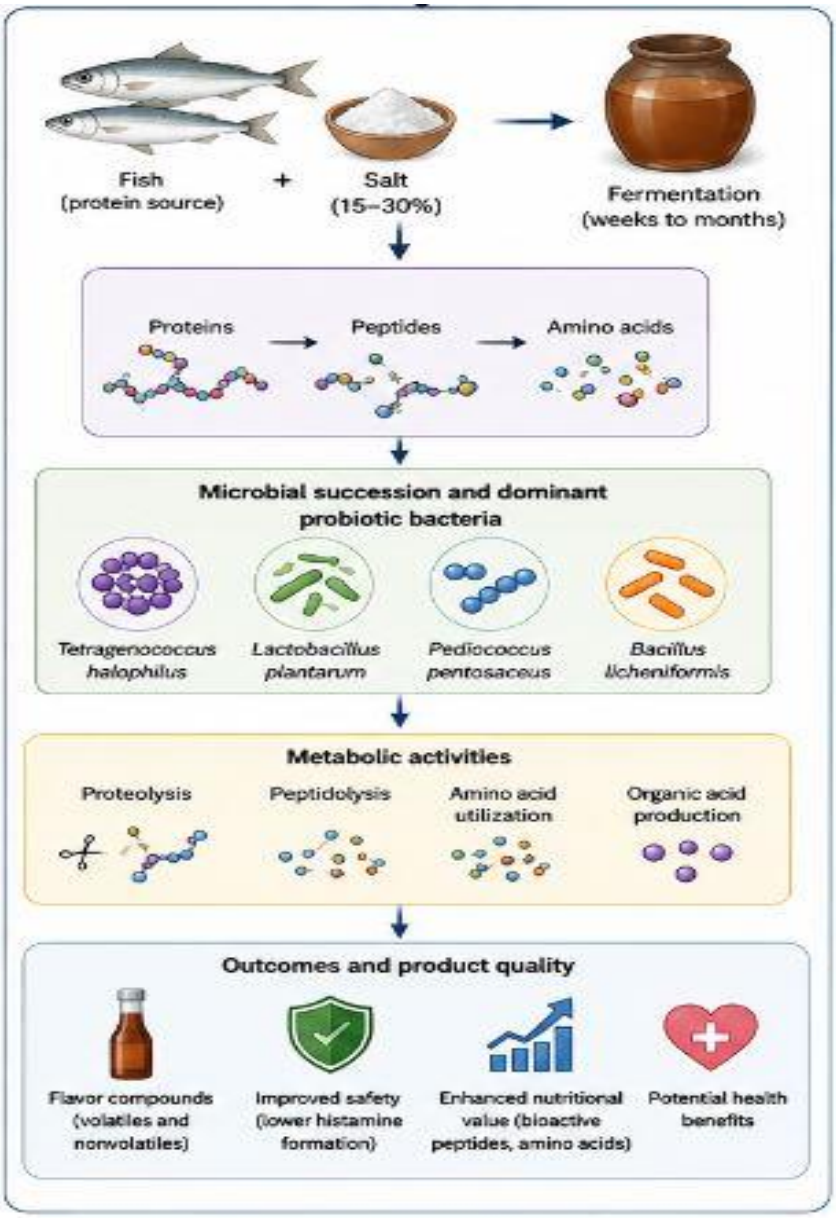

Figure 1. Schematic overview of fish sauce fermentation and associated probiotic microorganisms

4. DEFINITION AND CHARACTERISTICS OF PROBIOTIC BACTERIA IN FISH SAUCE

4.1. Probiotic Bacteria: Definition and Characteristics

Probiotic is a live microorganism. If consumed in sufficient quantities, it provide benefits on the health of the host. In the fish sauce fermentation process, probiotic microorganisms include genera of *Lactiplantibacillus* and *Bacillus*, especially *L. plantarum* and *B. licheniformis*, because of their characteristics and functional values (Table 2).

The *L. plantarum* can be used as a potential starter culture for the fermentation [13]. Mostly, this bacterial strain grow best at a medium salt concentration, except for certain *L. plantarum* isolated from fermented foods that able to survive in the high-salt environments. The *L. plantarum* is usually treated as a supplementary starter rather than the essential microorganisms in the traditional fish sauce fermentation with high-salt contents [14].

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Table 2. Technological and functional characteristics of *L. plantarum* and *B. licheniformis* probiotic strains used in fish sauce fermentation

Index Species	Salinity resistance	Antihistamine activity	Production of flavor compounds
L. plantarum	3 -6%	-	+
B. licheniformis	15%	-	+

4.2. Why Using Probiotics in Fish Sauce?

As a result of higher demand for high quality fermented products with the disadvantages of spontaneous fermentation, researchers have shown further attentions to incorporation of probiotic microorganisms in fish sauce fermentation processes. In traditional spontaneous fermentation, most of fermenting microbial community are based on autochthonous micro flora, leading to unstable fermentation efficiency and different product qualities. To get a better control on microbial process and fermentation procedure, starter culture inoculation of selected probiotic strains have been shown as a promising method [13–15]. In addition, consumer's awareness of health products with natural fermentation process has forced use of probiotics. Probiotic bacteria offer routes for standardizing fermentation environments, developing novel fish sauce products with desirable attributes and probiotic assisted fermentation, are becoming alternatives to the process of traditional spontaneous fermentation (Table 3) [16–17].

4.3. Technological and Nutritional Benefits of Probiotics in Fish Sauce

Probiotics act as proteolytic agents during the manufacturing of fish sauce. Probiotic secreted proteolytic enzymes are the most important by decomposing fish proteins and amino acids into other molecules. This helps improve flavors, product nutritional values, increase digestibility and contribute to increased yields of healthy beneficial compounds; as shown by Liu et al (2024) [18]. Further beneficial compounds such as bacteriocins and organic acids prevent contaminations [19]. Hence, probiotic technology can significantly extend shelf life, prevent spoilage and avoid health problems. Some probiotic strains enhance fish sauce organoleptic quality and provide desired flavor by producing umami taste via metabolic processing of amino acid metabolism and generating volatized compounds [20].

Moreover, probiotic microorganisms may improve accessibility of essential micronutrients and help producing bioactive peptides with antioxidant, antihypertensive and antibacterial characteristics. These actions can improve health benefits of fermented fish and facilitate their development as functional foods [21–22]. Beneficial effects of probiotic bacteria are species and strain dependents. Any particular probiotic strain may produce specific qualities such as desirable taste, protein degradation, ability of inhibition and production of bioactive products; hence, no specific probiotic has been verified to perform better than any others for all quality characteristics, including sensory, nutritional and health characteristics. Rather, the selection process should be aimed at targeting one particular product of desired technological and functional characteristics [23].

The enhancement in technology and nutritional characteristics resulted from the use of probiotics is mainly contributed from the metabolic functioning of microorganisms during the fermentation process. Therefore, probiotics do not need to survive to improve taste characteristics, enhance nutrition, decrease biogenic amines and improve fermentation yield or speed up fermentation. To use probiotics to benefit the consumers, it is necessary that a sufficient number of viable cells is achieved in the end products [24].

Table 3. Comparison of conventional and probiotic fish sauces

Sample Parameter	Conventional	Probiotic fish sauce
Microbial control	Limited	Improved
Functional properties	Low	High
Bioactive compounds	Moderate	Higher
Health benefits	Indirect	Potentially greater
Market value	Traditional	Value added

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4.4. Potential Health Benefits

In addition to the benefits for fermentation efficiency and quality of end products, probiotic microorganisms may include health benefits as well as those associated to fermentation. Emerging evidence have indicated that certain probiotic strains can positively alter the structure of gut microbes, improve gut integrity and function, promote immune homeostasis and participate in metabolic balance. However, extent of these health effects is often strain dependent and warrants by validation in human intervention trials [25–26].

4.5. Selection Criteria of Probiotic Strains for Fish Sauce Fermentation

In the production process, successful implementation of probiotic bacteria in the fermentation of fish sauce is primarily attributed to appropriate selection of microbial strains. To use probiotic bacteria to industrial fish sauce production, it is necessary to systematically assess the technological, safety and functional characteristics of probiotic microorganisms before selection as starter cultures; as not all probiotics are adapted to the harsh conditions of fish sauce fermentation [21].

4.5.1. Salt tolerance

Tolerance to high salt concentrations is one of the important selection parameters. The process of fish sauce production involves a naturally occurring habitat of 15–30% of NaCl, resulting in osmotic pressure, cellular damage and inhibition of metabolic activity. Probiotic starter culture must provide sustainable proliferation and metabolic activity to prevent detrimental effects on its own and host fish [3].

4.5.2. Acid and bile resistances

To cause a probiotic effect upon ingestion of microorganisms, probiotics should endure travelling the guts, consequently chosen strains should be tolerant to the acidity of the abdomen and bile. Assessment for bile salt as well as gastric acid susceptibility is widely used [27].

4.5.3. Antimicrobial activity

Good probiotic strains are expected with antimicrobial activities to pathogenic microorganisms that affect food quality and safety. Strains could increase the stability and safety of fermented food products by producing various antibacterial compounds such as hydrogen peroxide. Thus, strains with a wide range of antagonistic activities are favorite for use in fermented seafood [28].

4.5.4. Adhesion ability and colonization potential

Interaction with the intestinal epithelium adhesion ability of the probiotic is another vital factor, due to the temporary colonization in the gastrointestinal tract (GIT) and strengthening communication with the host's immune response between the probiotics. Moreover, probiotics with high adhesion rate usually perform better for efficacy [27].

4.5.5. Safety assessment

Probiotic choice is first affected by safety requirements. Strains suggested should not include virulence genes or toxin producing factors and have not to show pathogenic abilities or carry genes coding for antibiotic resistance that may be transmitted to pathogens. Therefore, genome sequencing has emerged as a valuable tool to check probiotic safety [21].

4.5.6. Technological performance

In addition to possessing probiotic traits, strains need to contain favorable technical characteristics, including rapid growth and consistent fermentation behavior, ability to produce flavor and compatibility with the processing parameters that occur at an industrial scale. Multifunctional strains possessing probiotic and technological characteristics are particularly interested for their use in the production of fish sauce. The identification of the correct strain involves an integrated scientific discipline encompassing microbiology, food science and technology, food genomics and risk assessment to produce beneficial fermentation and safe food [3].

5. MAJOR PROBIOTIC CANDIDATES USED IN FISH SAUCE FERMENTATION

5.1. *L. plantarum*

5.1.1. Biological characteristics of *L. plantarum*

Researchers have been interested in *L. plantarum*, an excellent probiotic strain. Although this bacterium is a great probiotic strain, its versatility is particularly attractive in food science. The *L. plantarum* is detected in several foods that have been fermented using multiple methods. The bacterium can tolerate acidic and high-salt conditions and survive when food is limited. This resilience makes *L.*

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plantarum one of the most common fermentation microorganisms in food products [13, 29].

5.1.2. Adaptation to fish-sauce fermentation conditions

The bacteria used in the fermentation of fish sauce must not only have the cells capable of surviving the challenging conditions in the environment but also possess characteristics that allow them to perform well within the complex ecological environment that describes fish sauce fermentation. The process; by which, fish sauce is fermented involves multiple stages of succession of various microorganisms undergoing biochemical reactions that alter the behavior and metabolism of the microbial population for a long time. The *L. plantarum* has demonstrated ability to adapt to the fermentation environment of fish sauce by demonstrating metabolic flexibility and a wide variety of substrate uses [15], while playing a critically biological role that contributes to these biochemical reactions in the end product. In addition, interactions between *L. plantarum* and other microorganisms during the fermentation of fish sauce likely alter and affect the ecological microorganisms and subsequently affect flavor generation, nutritional metabolism and stability of the final value added fermented products. These characteristics support the commercial use of *L. plantarum* in fish sauce fermentation. The *L. plantarum* is capable of using a full range of carbohydrate and nitrogen sources, which provide it with a competitive advantage during the fermentation. When used as a starter culture, *L. plantarum* provides consistency to the fermentation process and improve the quality of the final commercial products [30].

5.1.3. Mechanisms of action of *L. plantarum*

Multiple linked pathways are involved in producing technological and probiotic effects of *L. plantarum*. One of the most significant pathways is through the production of organic acids and particularly lactic acids. The accumulation of these acids leads to decreased pH of the surrounding area; thereby, making it increasingly difficult for other types of less desirable microorganisms to propagate. Additionally, several of the strains produce bacteriocins and other antimicrobial substances and thus provide added microbial safety (Figure 2) [3, 31].

While there are numerous studies demonstrating the antimicrobial characteristics of *L. plantarum*, there is a significant variability in the strength of the effects from strain to strain, depending on how the studies were completed; therefore, making it difficult to come to conclusions from direct comparisons of the results. There is limited clinical evidence showing that good probiotic fish sauce can help regulate the immune system through the actions of the probiotic microorganisms and cause unintended but beneficial probiotic effects through the combination of fermentation and probiotic microorganisms [8, 27].

5.1.4. Current uses and functional significance of *L. plantarum*

The *L. plantarum* has become a popular target species for research on fish sauce fermentation since it is able to adapt to various environments and show a wide range of genetic capabilities. Several authors demonstrated that *L. plantarum* was effective when used as the single strain or in combination with other cultures for starter production, resulting in higher product consistency and lower batch-to-batch variation [21].

The *L. plantarum* wide metabolic capability allows it help interact with other fermenting microorganisms. Latest omics-based studies have uncovered that the bacterium includes various stress response systems and regulatory pathways, which help it adapt to changing environmental conditions. That is why *L. plantarum* is considered as a good model organism for investigating microbial succession and metabolic interactions during fish sauce fermentation (Figure 3) [32].

In addition to the sheer possibilities of technical improvement, the scientific studies of *L. plantarum* strongly support its implementation for setting up standardized fermentation processes. However, several differences from one strain to another one still are reported and further research is needed to select strains that perform best and assess their effectiveness in conditions of industrial production [33, 35].

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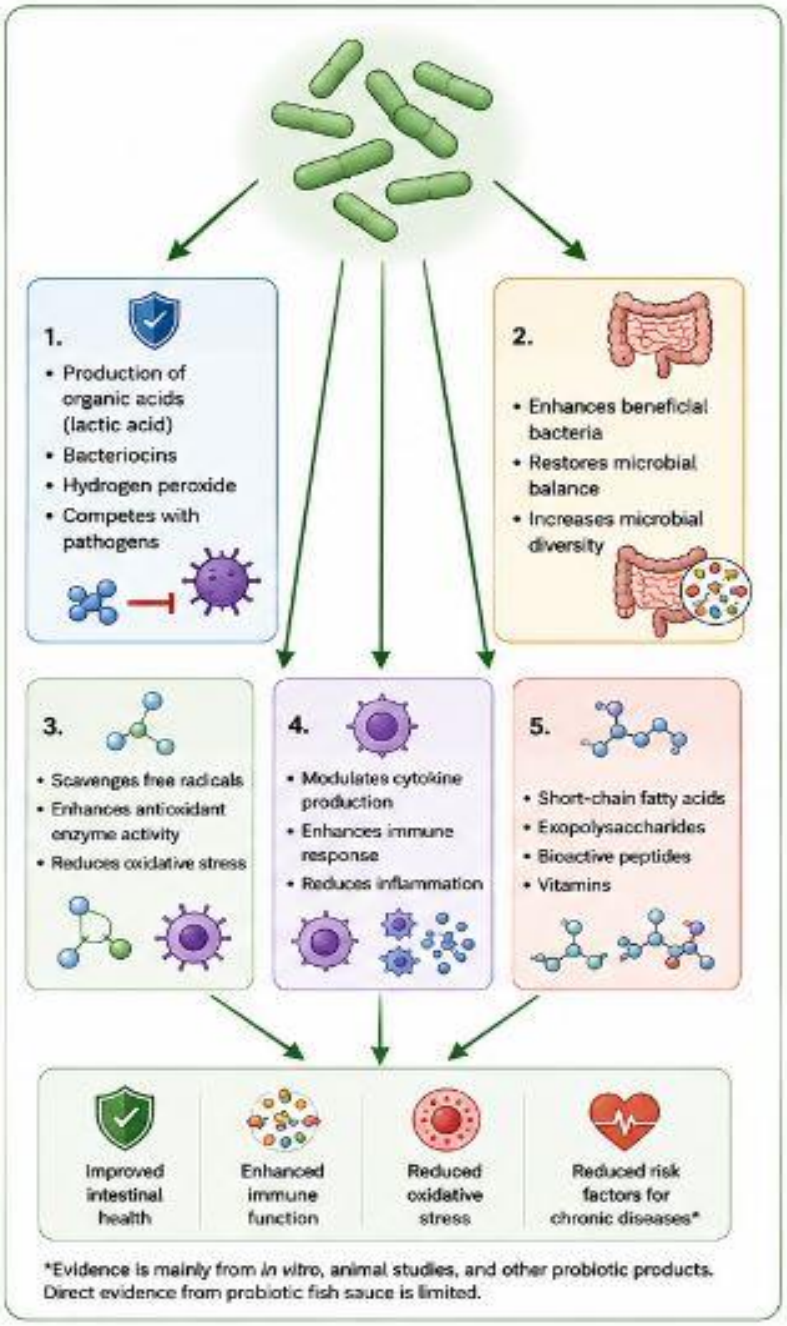

Figure 2. Suggested mechanisms underlying the probiotic effects

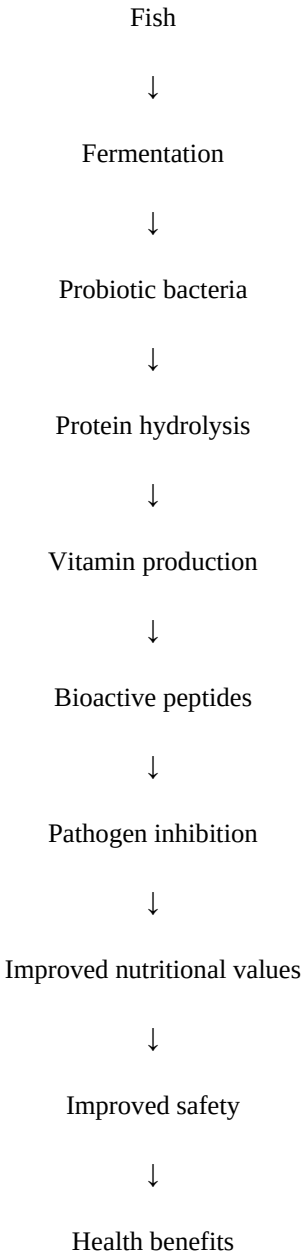

Figure 3. Overview of probiotic bacteria functions during fish sauce fermentation

5.2. *B. licheniformis*

5.2.1. Biological characteristics of *B. licheniformis*

The *B. licheniformis* is a Gram-positive bacterium that is capable of forming spores and this characteristic is the biggest reason that further attention is associated to it in food fermentation; giving it ability to adapt to various environmental conditions [36]. Similar to several LAB, *B. licheniformis* can resist even to the harsh processing conditions using its endospore formation that gives it a protective layer against heat, salinity and other environmental stresses. This characteristic, in fact, makes it appropriate for fermented food systems; where, it is usually difficult for microbes to survive [10]. This species is so common in nature and can be detected in many fermented foods. It has been isolated from seafood products and traditional fish fermented. A number of

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strains have been identified with positive effects on fermentation performance and food quality [37].

5.2.2. Technological and functional characteristics

Another significant capability of *B. licheniformis* on its interaction with the environment is the production of various extracellular enzymes and bioactive metabolites on a large scale. Due to these functionalities, the species has attracted attention in a number of food biotechnological processes as a potential element of multifunctional starter cultures. Moreover, isolates have shown antimicrobial activity and probiotic traits, but these characteristics are strictly strain specific [38].

Compared to LAB, *B. licheniformis* is typically seen as a further technologically resilient microorganism with great potential for industrial use. Then, thorough safety assessment and detailed characterization of individual strains are still necessary steps that need to be addressed before this bacterium is used extensively in the production of commercial fish sauce [36, 39, 40].

5.2.3. Comparison of *L. plantarum* with *B. licheniformis*

The *B. licheniformis* has been shown as further more resilient and stable microorganism under adverse environments, compared to *L. plantarum*, due to its natural ability to form spores. The *B. licheniformis* and *L. plantarum* are commonly used for fermented fish sauce; however, these microorganisms have distinct physiologic characteristics that result in differing functions for the fermentation. The *L. plantarum* is well known for its health promoting effects, ability to produce organic acids and antibacterial mechanisms, whereas *B. licheniformis* includes several further enzymes to facilitate the breakdown of proteins into smaller peptides that can be absorbed into the host's body. The two microorganisms, when used separately or in combination, can include synergistic effects and add values to the fermentation (Table 4) [37].

5.2.4. Other probiotic bacteria

Within probiotic microorganisms, *L. plantarum* has been the subject of most research in fermented seafood. Other bacterial species have also been documented to include important functions in fish fermentations with high-salt contents. Collecting additional knowledge on these probiotic candidates is critical for creating efficient starter cultures used in fish sauce production [41].

5.2.4.1. *Tetragenococcus halophilus*

Compared to most LAB, *T. halophilus* may be considered an extreme halophilic bacterium that is able to produce stable fermentation products under conditions of high-salt concentrations. For example, *T. halophilus* is able to grow on fish sauce at high salinity and this species produces a majority of aroma compounds associated with fish sauce. This makes *T. halophilus* a significant microorganism and an excellent candidate for use in high salinity fermentation products (Table 5) [35, 40].

Table 4. Comparison of *L. plantarum* with *B. licheniformis* in fish sauce fermentation

Characteristic	*L. plantarum*	*B. licheniformis*
Taxonomic group	Lactic acid bacterium	Spore forming bacterium
Salt tolerance	High	Moderate–High
Acid production	Strong	Limited
Proteolytic activity	Moderate	Strong
Bacteriocin production	High	Moderate
Probiotic evidence	Extensive	Moderate
Flavor enhancement	Moderate	Strong
Industrial application	Widely studied	Emerging

Table 5. Other probiotic bacteria associated with fish sauce fermentation

Index Microorganism	Fermented products	Fermentation conditions	Major findings	References
T.halophilus	Fish sauce	High salt (20 – 25%)	Improved flavor and microbial stability	Han et al., 2024
L. plantarum	Fermented fish products	Moderate salinity	Enhanced antimicrobial activity and probiotic potential	Stergiou et al., 2021; Alonso Garcia et al., 2021
P.pentosaceus	Fermented seafood	Moderate salinity	Bacteriocin production and pathogen inhibition	Shi et al., 2022
B. licheniformis	Fermented fish paste	Variable	Increased proteolysis and flavor development	Liu et al., 2024
L. pentosus	Fermented seafood	Moderate salinity	Antioxidant and probiotic activities	Alonso Garcia et al., 2021

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5.2.4.2. *Weissella* species

Members of the genus *Weissella* are greatly interested lately because they can make not only exopolysaccharides but also other metabolites that change the physicochemical conditions of fermented foods. When fish sauce is fermenting, the *Weissella* spp. can be involved in changing the texture and producing special flavors (Table 5) [40].

5.2.4.3. *P. pentosaceus*

As a result of its high production of bacteriocins, *P. pentosaceus* demonstrations a strong antagonistic activity against harmful microorganisms. Evidence suggests that it plays an important role in maintaining microbial balance during fermentation, indicating it as an appropriate starter culture for improving the microbiological quality of fish sauce (Table 5) [18, 40].

5.2.4.4. *Lactiplantibacillus pentosus*

The *L. pentosus* is significant for its capability of metabolizing a wide range of substrates and tolerance to high salinity conditions. Additionally, this includes the ability to enzymatically degrade proteins and, from a phylogenetic perspective, it is closely linked to *L. plantarum*. Therefore, it represents an excellent candidate for controlled fish sauce fermentation. However, there is limited research describing its use and further study is necessary to fully understand its technological and functional characteristics (Table 5) [41–42].

5.2.4.5. Comparative significance of alternative probiotic species

Despite that these bacteria include great characteristics, documents that link them to fish sauce fermentation are still quite insufficient if compared with the two well-known strains of *L. plantarum* and *B. licheniformis*. Further research is necessary to clarify their metabolic functions, how they interact with the native microbial community and their potential for producing on an industrial scale (Table 6) [41, 43–44].

5.2.5. Comparative assessment of probiotic bacteria used in fish sauce fermentation

Various species of bacteria have been studied for use in fish sauce fermentation. However, the research findings show that their efficiency as technology and their beneficial effects as probiotics can vary widely based on the strains and fermentation environment. Thus, it is important to compare these microorganisms to find out the best ones to make functional fish sauce products [28].

5.2.5.1. Overall comparison and critical perspectives

Overall, various types of bacteria can be used to make high quality fish sauce. The various kinds of bacteria involved include various technological and functional characteristics; hence, their ability to ferment depends on the characteristics of the bacteria as well as how they were grown during fermentation, resulting in various metabolic capabilities, enzyme production, probiotic characteristics and ability to adapt to their environment. Therefore, selecting the correct types of bacteria should be carried out based on the specific fermentation process and product quality requirements (Table 7) [31, 45].

5.2.5.2. Comparison of available studies

Because of differences in several factors, including design of the experiment (experimental setup), sources of fermentation substrate, environmental conditions used during fermentation, analytical methods to assess results produced by the fermentation process and criteria to assess the resulting fermented product, it is not easy to compare the research on fish sauce fermentation. The differences within these studies make the findings of each unique and; thus, finding similar results may not necessarily be possible when comparing results from various studies. Researchers must implement uniform experimental designs and consistently assess the results produced by the fermentation process results in greater reproducibility and better comparability of fish sauce fermentation research [20, 47].

Table 6. Comparison of major probiotic species used in fish fermentation

Index Species	Salt tolerance	Probiotic properties	Antimicrobial activity	Application in fish fermentation
L. plantarum	Moderate	Excellent	Strong	Widely used
B. licheniformis	Moderate – High	Good	Moderate	Potential starter culture
T. halophilus	Very high	Moderate	Moderate	High salt fermentation
P. pentosaceus	Moderate	Good	Strong	Fermented seafood
Weisella spp.	Moderate	Emerging	Moderate	Experimental

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Table 7. Comparative assessment of probiotic candidates for fish sauce fermentation

Bacteria Criterion	*L. plantarum*	*P. pentosaceus*	*B. licheniformis*	*T.halophilus*	Weisella spp.
Level of evidence in fish sauce fermentation	Extensive	Moderate	Moderate to extensive	Extensive	Limited
Overall suitability for high salt fermentation	Highly suitable and currently the most extensively investigated probiotic candidate	Promising complementary probiotic candidate requiring further validation	Highly promising, particularly for improving protein hydrolysis and flavor development	Highly suitable for saline fermentation environments	Promising but insufficiently validated for large scale fish sauce fermentation
Major limitation	Limited tolerance to very high salinity	Limited information on flavor development	Safety concerns and regulatory issues	Limited evidence regarding probiotic functionality	Limited evidence in fish sauce fermentation, strain dependent performance and variable salt tolerance
Main strengths	Strong probiotic potential, antimicrobial activity, biogenic amine reduction, production of bioactive peptides, contribution of nutritional and sensory quality	Antimicrobial activity, inhibition of spoilage microorganisms, potential probiotic properties, contribution to product safety	High proteolytic activity, flavor enhancement, production of bioactive compounds, tolerance to fish fermentation candidates	Antimicrobial activity, inhibition of spoilage microorganisms, potential probiotic properties, contribution to product safety	Production of exopolysaccharides, contribution to flavor development, antimicrobial activity and potential probiotic effects

6. CHALLENGES AND LIMITATIONS OF PROBIOTIC BACTERIAL USE IN FISH SAUCE PRODUCTION

The use of cultured probiotic bacteria in the production of fish sauce includes numerous benefits that range from nutritional and technological to health advantages; however, there are still several scientific, technological, economical and regulatory issues that have not been resolved. All of the highlighted issues can make it difficult to implement, market and receive consumer acceptance. Understanding the existent challenges is necessary to develop enhancing procedures for the functions of probiotics, while preserving quality and safety of the product (Table 8) [11].

Table 8. Advantages and limitations of probiotic fish sauce

Advantages	Limitations
Rich in dietary fibers and proteins, Enhanced bioavailability of nutrients, Production of amino acids and the bioactive peptides and other metabolites	The survival and viability of probiotics may be impaired by processing and storage
More interesting and sophisticated sensory characteristics, better complexity of taste and aroma production	Excessive salt levels could prevent certain probiotic strains from growing effectively and acting properly.
Decreasing content of biogenic amines such as histamine and providing increased security	Significant strain related variation may result in incompatible fermentation outcomes
Possible benefits to human health	Little clinical proof of health benefit in human
Enhanced safety by controlling proliferation of pathogenic and spoilage microorganisms	The difficulty in regulatory and standard challenges prevent commercial implementation
Enhanced fermentation activity and Faster protein hydrolysis.	Problems in retaining stable quality during industrial production
Potential development of value added functional fermented seafood products	Higher production and quality control costs
More consumer demand for health focused and functional food	Limited consumer awareness and acceptance in some markets

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6.1. High Salt Stress and Environmental Adaptation

Using probiotic bacteria to produce fish sauce can be challenging because of the excessive salt levels needed for producing fish sauce. Increased salinity causes osmotic distress to the bacterial cells and leads to decreased growth, metabolism and enzyme production. There are probiotic bacteria that include halotolerant characteristics; however, the strain's salinity tolerance varies greatly from one strain to another resulting in variation of survival rates and fermentation rates.

This variability of strains includes an effect on the activity of the probiotic and results in decreased stability of the overall fermentation process. Due to the numerous chemistry and physical factors affecting every fermentation batch, it is almost impossible to set the microbial population stable throughout the fermentation process and achieve a consistent level of product quality [28, 35].

6.2. Preserving Probiotic Viability During Processing and Storage

Preserving probiotic microorganisms alive through processing and storage is possibly the biggest challenge of producing probiotic fish sauce. The population of cells may slowly decrease if they are exposed to harsh conditions such as high salt, temperature fluctuation, oxidative stress and extended storage time, which can bring the instability and loss of functionality of the end product. Moreover, changes in the physicochemical parameters during fermentation and storage may affect microbial survival; thereby, decreasing the persistence of probiotic populations with time. Therefore, ensuring the supply of viable cells up to the point of consumption is a problem that needs solutions for a successful production of probiotic fish sauce [12, 34, 48].

6.3. Complexity of Microbial Ecosystems During Fermentation

Fish sauce is traditionally produced through complex interactions of various microorganisms that are present in the fermentation processes. Microorganisms that coexist in a competitive or cooperative manner affect microbial succession, metabolic activities and fermentation dynamics, which are responsible for variations in the quality and composition of the final products. Due to significant differences in the growth characteristics and metabolic capabilities of the various strains, the fermentation outcomes can be inconsistent. The environmental conditions and the native microbiota composition are usually the major factors affecting behavior of microbial communities; hence, fermentation processes can hardly be predicted and controlled. Thus, differences in microbial interactions may cause differences between observations in the laboratory and those at the industrial scale [13, 34, 49].

6.4. Histamine Formation and Microbiological Safety Concerns

The continuous production of histamine and other biogenic amines in fermented fish products is a major concern due to the negatively effect on the health of consumers as well as the effect on the quality of the products when high levels are present. Safety, including safety of probiotics, is the other major concern linked to the microorganisms, since some strains include the potential for undesirable metabolism or resistance to antimicrobial agents. The potential for the presence of virulent factors and harmful metabolites is greatly concerned due to their potential to decrease microbiological safety of the product. Overall, these difficulties demonstrate challenges in the production of safe and high-quality probiotic fish sauces (Figure 4) [17, 21, 35, 50].

6.5. Regulatory and Standardization Challenges

Regulatory and standardization issues are main challenges for the development and commercialization of probiotic fish sauce. First, regulatory requirements and definitions of probiotic products are different from one country to another one, which lead to discrepancies in product classification and approval system. Moreover, the lack of globally recognized standards for strain selection, fermentation conditions and quality control causes large differences between studies and production systems. These types of disparities make it difficult to compare scientific results and slow down the setting of common manufacturing and product specification standards [5, 39].

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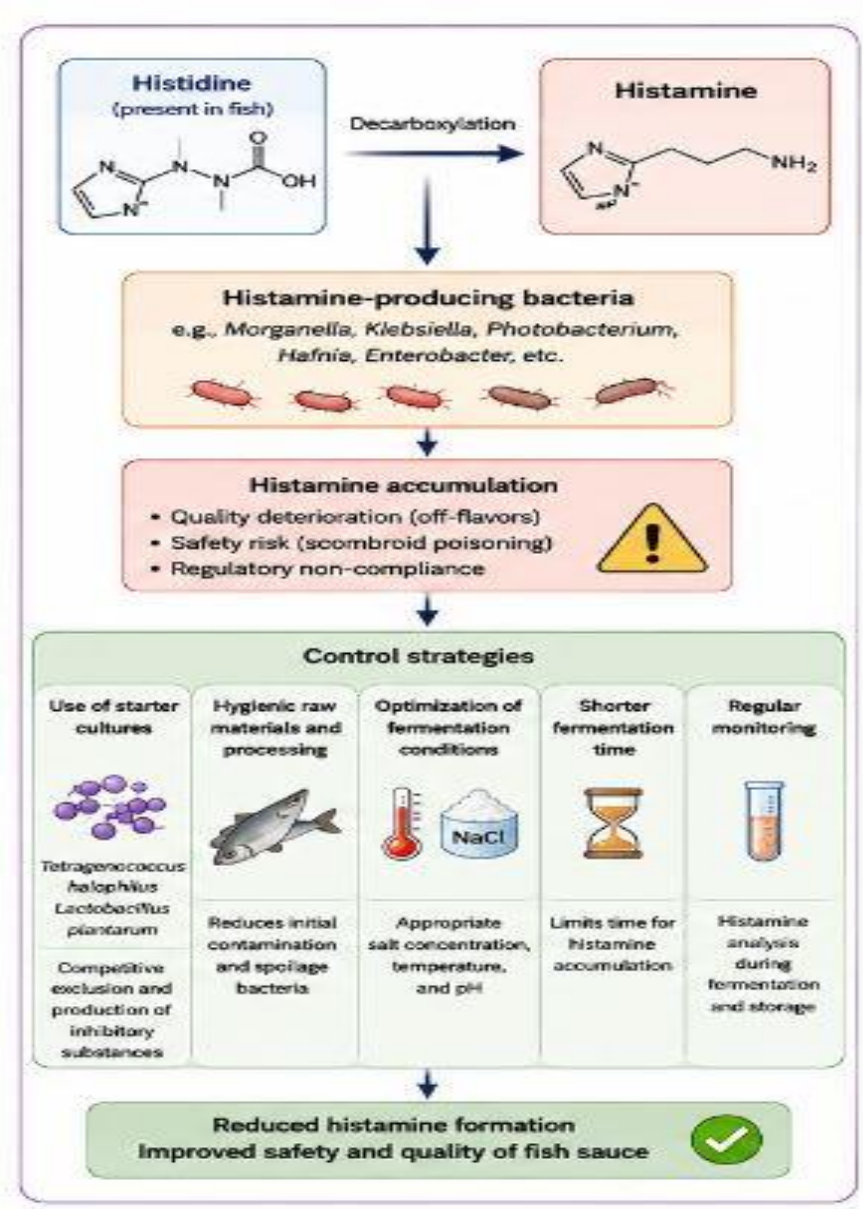

Figure 4. Histamine formation and control strategies in fish sauce fermentation

6.6. Economic and Industrial Scale Limitations

Although results in the laboratory are interesting, production of probiotic fish sauce on a large scale involves a set of economic and technological restrictions. Differences between laboratory studies and industrial productions include troubles in carrying out reproducibility, microbial stability and process similarities and each separate batch of product can differ its quality and dependability. The expenses that may involve the culture, process control and quality checks can represent significant issues for the commercialization of probiotic fish sauce [34, 49].

6.7. Insufficient Clinical Evidence

Evidence support the technical and functional capabilities of probiotics in fermented fish products, but most research include limitations regarding health effects of probiotic consumption as there are a few studies carried out directly on humans and even a fewer ones providing sufficient clinical data to support making health claims linked to the use of probiotic fish sauces. Most data collected have been through *in vitro* or animal studies, resulting in an incomplete understanding of how long-term

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consumption of probiotic fish sauces may affect human physiology and therefore limiting the use of probiotic based fish sauce as a functional food product [26–28, 35].

7. FUTURE PROSPECTS, RESEARCH PRIORITIES AND KNOWLEDGE GAPS

The potential of probiotic fish sauce is really brilliant as functional foods, which not only take care of the individuals' health but also are rich in nutrients. Since people worldwide are becoming further health conscious and demand further natural and fermented products, probiotic fish sauce can be the globally functional food. However, this is a big challenge which can be resolved only if there are simultaneous developments in microbiology, food biotechnology, industrial processing, regulatory structures and consumer education [10].

Segments of knowledge still inhibit the solution for the probiotic fish sauce. Most of the publications have been dealing with mainly microbial isolation and characterization. Relatively, a few references to microbial interactions and fermentation ecology are available. Furthermore, probiotic strains and indigenous microbiota are little known; hence, rendering it impossible to compute fermentation dynamics and optimize microbial communities (Table 9) [24].

7.1. Development of Mixed Starter Cultures

Each strain of probiotic includes various and unique functional and technological characteristics; therefore, research should be carried out on development and design of multiple strain starter cultures that contain functional traits that are complementary. The combination of highly salt tolerant microorganisms with probiotic and enzyme producing strains enhances the yield of the product, its microbiological stability, sensory characteristics and functional characteristics. A better understanding of microbial interactions within this type of consortium enhances the manufacturing process regarding performance and reliability of the manufactured food product [51].

7.2. Omics Based Approaches for Understanding Fermentation Ecology

Nowadays, recent developments in metagenomics, transcriptomics, proteomics and metabolomics represent a comprehensive technique for clarifying the microbial succession, metabolic functions of microorganisms and process of flavor and bioactive compound formations. However, studies use omics in the field of probiotic fish sauce fermentation are still little. However, combining multiple omics technologies with analyses helps achieve insights into the microbial community and guide the construction of starter culture design logically. Though the omics have been suggested as potentially valuable technologies, it is not readily used to enhance industrial fish sauce production due to technological sophistication and costs [24].

7.3. Genome Guided Strain Selection and Adaptive Assessment

An optimal approach to finding strong microorganisms resilient to harsh fermentation conditions may include using genome guided approach or adaptive laboratory assessment of strains. Through whole genome sequencing (WGS), strain safety can be assessed for better acceptance of potential candidate strains for fish sauce production [45].

Table 9. Knowledge gaps and further research priorities

Research area	Current limitations	Future directions
Clinical evidence	Lack of human studies	Clinical trials on probiotic fish sauce
Salt tolerance	Limited survival of some strains	Isolation of halotolerant probiotics
Starter cultures	Mostly single strains	Development of mixed starter cultures
Functional compounds	Limited characterization	Omics and metabolomics studies
Commercial application	Lack of standardization	Optimization of industrial processes

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7.4. Improvement of Probiotic Stability and Preservation Technologies

Further studies should highlight the innovations of state of the art strategies in enhancing stability and efficacy of probiotic microorganisms during storage and transport. Potential strategies such as microencapsulation, freeze drying, coating agents and packaging systems may contribute to preserve cell survivability and their probiotic function. Moreover, novel strategies of formulation and delivery techniques need to investigate values in functionality and use in commercial probiotic fish sauce production [34, 52].

7.5. Human Clinical Validation and Commercial Use

Further human interventional study design is needed to assess best dose, long-term security and evidence based claims. In parallel, product standardization, regulatory alignment and consumer admission are needed to support market rollout and industrial uses of the probiotic fish sauce products [15, 26].

7.6. Economic Viability

Many studies were developed at laboratory scale while there is lack of information concerning the production and commercialization in large scale. Further research must be carried out to develop novel technologies that can assess profitability and cost effectiveness production methods and standardization. Regulation harmonization and industrial scale optimization need to be developed to boost industrial scale production of probiotic fish sauce [53, 54].

7.7. Consumer Acceptance and Market Development

Consumer outlook describes further viability of probiotic fish sauces. In spite of growing knowledge on probiotics worldwide, a very little is understood regarding perception, sensory acceptance and market possibilities of probiotic seafood products. Further research must concentrate on consumer's perspective, sensory qualities and good communication methods to reinforce self-assurance and boost global market opportunities [55, 56].

The successful integration of results from the laboratory into industry is undoubtedly the most essential part for the creation of scientifically accepted commercial probiotic fish sauce in a wide scale; as must be implemented in a global market. The further studies continuously focus on inter disciplinarily science that includes microbiology, biotechnological, omics disciplines, clinical sciences and food engineering as the keys to revitalize traditional fish sauce fermentation and accelerate next generation functional seafood products (Figure 5) [24, 57].

8. CONCLUSION

Therefore, use of probiotics in fish sauce fermentation seems as a good strategy in renovation and modernization of traditional seafood fermentation system. Combination of microbiology science and fermentation helps create new generations for seafood with innovative and valuable food ingredients. This research includes the potential to lead to a better controlled, sustainable scientifically driven fermentation process.

9. DECLARATION

9.1. Acknowledgements

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9.2. Declaration of Competing Interest

The authors declare no conflict of interest.

9.3. Authors' Contributions

Conceptualization, ST and AM; methodology, ST; software, ST; validation, ST and AM; formal analysis, CM; investigation, ST and AM; resources, ST and CH; data curation, KH; writing-original draft preparation, ST, AM, CM and KH; writing-review and editing, ST, AM, CM and KH; visualization, ST and KH; supervision, KH; project administration, ST.

9.4. Using Artificial Intelligent Chatbots

No AI chatbots or tools were used in this study.

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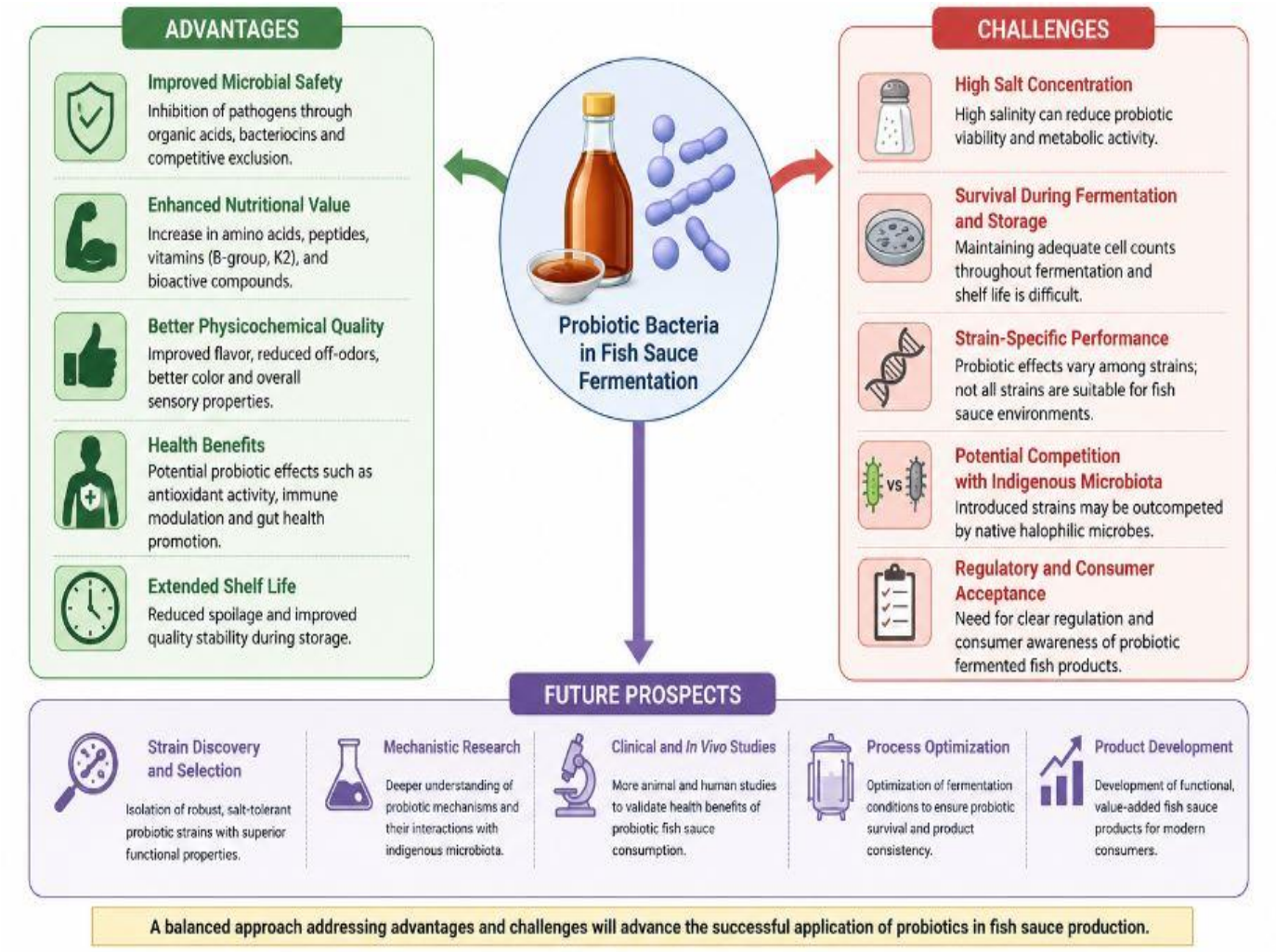

Figure 5. Advantages, challenges and future prospects of probiotic uses in fish sauce production

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باکتری‌های پروبیوتیک در تخمیر سس ماهی: کاربردهای فعلی، مزایای سلامتی، چالش‌ها و چشم‌اندازهای آینده

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چکیده

سابقه و هدف: سس ماهی یکی از فرآورده‌های سنتی و فراسودمند است که از تخمیر ماهی در حضور نمک تولید می‌شود و در بسیاری از کشورهای آسیایی از جایگاه تغذیه‌ای و اقتصادی ویژه‌ای برخوردار است. با وجود مزایای فراوان، فرآیند تولید این محصول با محدودیت‌هایی از جمله طولانی بودن دوره تخمیر، نوسان در کیفیت محصول، تشکیل هیستامین و سایر آمین‌های زیستی و احتمال آلودگی‌های میکروبی همراه است. در سال‌های اخیر، استفاده از باکتری‌های پروبیوتیکی به‌عنوان کشت‌های آغازگر، راهکاری مؤثر برای بهبود فرآیند تخمیر و ارتقای کیفیت و ایمنی سس ماهی مورد توجه قرار گرفته است. این میکروارگانیسم‌ها با تغییر فلور میکروبی، تنظیم ریزمحیط تخمیر و تولید متابولیت‌ها و ترکیبات زیست‌فعال مطلوب، موجب بهبود ویژگی‌های کیفی، تغذیه‌ای و فرآسودمندی محصول می‌شوند. مطالعه حاضر با هدف مرور کاربردهای باکتری‌های پروبیوتیکی در تخمیر سس ماهی و بررسی نقش آن‌ها در بهبود کیفیت، ایمنی، ویژگی‌های حسی و ارزش فرآسودمندی این محصول انجام شد.

مواد و روش ها: برای گردآوری اطلاعات، مقالات منتشرشده در پایگاه‌های اطلاعاتیWeb of Science ، Scopus، PubMed و Google Scholar مورد بررسی قرار گرفتند. جست‌وجوی منابع با استفاده از کلیدواژه‌های مرتبط با باکتری‌های اسیدلاکتیک، فرآورده‌های تخمیری ماهی، پروبیوتیک‌ها، *Pediococcus pentosaceus*، *Lactiplantibacillus plantarum*، *Bacillus licheniformis*، *Tetragenococcus halophilus* و تخمیر سس ماهی انجام شد.

یافته‌ها و نتیجه‌گیری: بررسی مطالعات نشان داد که باکتری‌های پروبیوتیکی، به‌ویژه *L. plantarum* و *B. licheniformis*، با تسریع هیدرولیز پروتئین‌ها، بهبود ویژگی‌های حسی، مهار رشد میکروارگانیسم‌های بیماری‌زا و فسادزا و کاهش تولید هیستامین و سایر آمین‌های زیستی، نقش مهمی در ارتقای کیفیت و ایمنی سس ماهی ایفا می‌کنند. علاوه بر این، این میکروارگانیسم‌ها از طریق افزایش تولید پپتیدهای زیست‌فعال و ویتامین‌های گروهB ، ارزش تغذیه‌ای و خواص فرآسودمند محصول را بهبود می‌بخشند. استفاده از روش‌های نوین تخمیر و کشت‌های آغازگر ترکیبی نیز می‌تواند موجب افزایش کارایی فرآیند، کاهش زمان تخمیر و یکنواختی بیشتر کیفیت محصول شود. به‌طور کلی، به‌کارگیری پروبیوتیک‌ها در تخمیر سس ماهی، فناوری امیدبخشی برای تولید محصولاتی ایمن‌تر، باکیفیت‌تر و دارای ارزش افزوده بیشتر به شمار می‌رود و ظرفیت بالایی برای توسعه در مقیاس صنعتی دارد. با این حال، انجام مطالعات بیشتر، به‌ویژه پژوهش‌های *in vivo* و ارزیابی‌های صنعتی در مقیاس بزرگ، برای تأیید اثرات سلامت‌بخش و تسهیل تجاری‌سازی این محصولات ضروری است.

واژگان کلیدی: پروبیوتیک، سس ماهی، کشت آغازگر، فرآورده‌های فرآسودمند، محصولات کم‌نمک، ایمنی مواد غذایی، ویژگی‌های حسی، غذاهای نوظهور

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