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Probiotics from Fermented Fish

Nilofar Yasmin, Khusboo Kaur and Kaushal Sood

Abstract

The term 'Probiotics' is used to describe live microorganisms, which, when administered in adequate quantities, confer health benefits. The term probiotics was first introduced in 1965 by Lilly and Stillwell, who defined it to be microorganisms acting as growth promoters for other microorganisms. These microorganisms may include *Lactobacillus*, *Streptococcus*, *Bifidobacterium*, *Saccharomyces*, *Aspergillus*, *Enterococcus* etc., as well as a mixture of other microorganisms. The chapter focuses on providing a comprehensive and up-to-date review of probiotics that have been isolated from fermented fish-based products.

Keywords: probiotics, lactobacillus, fermented food, fermented fish

1. Introduction

The term 'Probiotics' conventionally refers to the substances produced by microorganisms that stimulated the growth of others. With the advancement of knowledge in the subject, the use of the term was later extended to describe the tissue extracts that stimulated microbial growth. This definition was further evolved to animal feed supplements which exerted a beneficial effect by contributing to intestinal flora [1]. With further advancement of knowledge in the field, the term *prebiotics* [2] was introduced to describe food supplements that were non-digestible by the host but were able to exert beneficial effects by selective stimulation of growth or activity of intestinal microorganisms. A combination of the two, probiotics and prebiotics, was referred to as *conbiotics* by certain authors while *synbiotics* by others [2, 3]. However, due to limited research in this field, the health benefits of prebiotics are yet to be verified. Over the recent years, functional foods have gained popularity due to their beneficial health effects, which have partly been attributed to their probiotic components [4]. Over the decades, the definition of probiotics has been refined by several workers. Vergin [5] suggested the action of the probiotic diet towards the intestinal microbiota in describing "the microbial balance of the body" [5]. Parker [6], defined probiotics as: "organisms and substances which contribute to intestinal microbial balance". This was the first time that probiotics were mentioned in the context of gut health. In 1989, Fuller [7] further refined the definition to "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". In the following years, the definition was extended to include mono- or mixed cultures of microorganisms that beneficially affected the host by improving the properties of the indigenous microbiota [8].

However, the widely accepted and currently in use definition is the one put forth by the World Health Organization:

"Probiotics are live microorganisms which, when administered in adequate amounts confer a health benefit on the host."

To summarize:

Prebiotic: A prebiotic is a non-viable food component that confers a health benefit by modulation of the gut microbiota.

Probiotics: These are live microorganisms, they confer health benefits to the host when administered in adequate doses.

Synbiotic: A product that contains both probiotics and prebiotics.

2. Probiotics: a brief history

Fermented dairy and other food products were produced and utilized for nutritional and therapeutic purposes long before the discovery of microorganisms. The discovery of fermentation was itself an incidence of serendipity. However, with the discovery of Lactic acid-producing bacteria by Pasteur in 1857, it was Pasteur and his successors who had a significant impact on the understanding of the microbiology involved in the process of fermentation [9]. The idea of using beneficial bacteria attracted interest along with the advances in microbiology and biotechnology in the following decades.

Research on the application of probiotic microorganisms in aquaculture started over two decades ago. Microorganisms, especially lactic acid bacteria (LAB), have long been associated with food fermentation. Dating back to 3200 BC, when the Egyptians produced fermented milk and dairy products during the Pharaonic period [10, 11]. Applications of probiotics in the field of animal husbandry gained popularity in the 1960s. In the 1980s, the most common probiotics for animal feeds belonged to three bacterial and one yeast genera: *Lactobacillus*, *Streptococcus*, *Bacillus*, and *Saccharomyces* spp. *Lactobacillus* sp. is recognized to produce potent antimicrobial compounds in order to establish their preservative and probiotic effects [12, 13] and have been consumed in the form of diverse food supplements through thousands of years and are “generally regarded as safe” (GRAS) [14, 15].

3. Probiotics: qualifying characteristics

Probiotics are an innate component of a healthy intestinal microbiota in humans and other animals. These colonize the gut through the diet or other non-dietary sources that are consumed by the organism. Novel species and strains of probiotic bacteria are being constantly identified with the exploration of previously unexplored sources. However, prior to incorporating such potential probiotic strains into products, their efficacy has to be carefully assessed based on a battery of criteria (Figure 1).

Foremost among such criteria is the safety of the host. Most of the probiotics in use today have been isolated from natural sources with a long history of safe use. Acid and bile salt stability of such strains are self-evident properties as these were able to colonize the intestinal tract. The development of probiotic products requires that the strains should also have antimicrobial activity and antibiotic resistance to the commonly administered drugs. Adhesion to intestinal cells and colonization of the gut are among the other primary requisites [3–5, 7, 16–19].

Acidic conditions (pH < 3.0) in the stomach act as a natural barrier to microorganisms and prevents most of them from passing into the intestine. Acid tolerance

Figure 1.
 Probiotics: Characteristic criteria.

is, hence, a preliminary character for any strain that is expected to have probiotic effects [16, 20]. Resistance to pH 3.0 for 2 h is one standard test to determine the low pH tolerance of potential probiotic isolates [21]. The exact mechanism of tolerance to low pH conditions is not yet known. The next barrier for a potential probiotic to survive is the bile salt in the intestine, the normal level of which is around 0.3%, but may range up to the extreme 2.0% during the first hour of digestion. In conjunction with acid tolerance, it has been used widely as a selection criterion of potential probiotics [22]. Bile resistance of potential probiotic strains is related to the activity of the enzyme- bile salt hydrolase (BSH) which catalyzes the hydrolysis of conjugated bile, hence reducing its toxic effects [23]. In addition, according to Ganzle et al. [24] bile resistance can be increased due to the protective effect of some food components.

The potential of lactic acid bacteria and probiotic yeast to inhibit the growth of other microorganisms in the intestine is a valuable feature for considering their application in the development of functional foods. The antagonistic property of the probiotic strains against pathogenic bacteria may be exerted by either competitive exclusion, a decrease of redox potential, inter-bacterial aggregation, or production of antimicrobial substances including organic acids, other inhibitory primary metabolites such as hydrogen peroxide, and special compounds like bacteriocins and antibiotics [25, 26]. This property enables the probiotics to alter the resident intestinal flora and modify it for the benefit of the host [27].

The ability of probiotic strains to endure and survive in the presence of antibiotics ensures the maintenance of healthy intestinal microbiota during the treatment of microbial infections. LAB has been shown to exhibit susceptibility to a broad spectrum of antibiotics. Although isolates of lactobacilli with strong resistance to penicillin, cephalosporins, and bacitracin have been recovered from the human gastro-intestinal tract and dairy products, in most of these cases, this resistance is not transmissible and represents an intrinsic characteristic of the organism [17, 28].

4. Probiotics: health benefits

The health benefits of probiotics were proposed over a century ago by Eli Metchnikoff when he postulated that manipulating the intestinal microbiome could enhance health and delay senescence [29]. There is now sufficient scientific evidence supporting the incorporation of probiotics in the diet for health benefits. The best documented benefits include- relief from bowel disorders such as lactose intolerance, antibiotic-associated diarrhea, and infectious diarrhea, and allergy. Emerging

evidence has indicated the potential role of probiotics in managing different kinds of cancers as well. Multiple *in vivo* studies have indicated that the administration of specific strains of lactic acid bacteria could prevent the establishment, growth, and metastasis of transplantable and chemically induced tumors [30]. In human subjects, probiotic therapy has been suggested to reduce the risk of colon cancer through the inhibition of transformation of procarcinogen to active carcinogens, binding/inactivating mutagenic compounds, producing antimutagenic compounds, suppressing the growth of pro-carcinogenic bacteria, reducing the absorption of mutagens from the intestine, and enhancing immune function [31, 32]. However, evidence is still lacking to establish a basis for probiotic therapy in cancer prevention.

Probiotics are known to exert their effects by influencing the intestinal microflora and protecting against infections, alleviating lactose intolerance, reducing blood cholesterol levels, improving weight gain and feed conversion ratio, and also stimulating the immune system [33]. Lactic acid bacteria (LAB) are a part of normal gut microflora in humans and some other animals and are known to produce lactic acid, hydrogen peroxide, diacetyl, acetaldehyde, and bacteriocins which are able to inhibit the growth of harmful microorganisms [34, 35].

Probiotics are mostly administered as live supplements in diet and exert diverse effects on the host. These influence the intestinal luminal environment and the innate and adaptive immune response systems [34, 36].

The use of probiotics for enhancing bio-growth parameters and in improving disease resistance ability has been well documented in aquaculture of fish for human consumption [37–41] but research on the effect of feeding probiotics in ornamental fishes is still an under-explored research territory.

Although most probiotics known so far are Gram-positive, with *Lactobacillus* and *Bifidobacterium* being the main species used for treatments of intestinal dysfunctions [42], some Gram-negative bacteria, such as *Escherichia coli* Nissle 1917 (EcN) [43], also known as “Mutaflor,” have also been reported to function as probiotics. Mutaflor has been used in Germany for many years in the treatment of chronic constipation [44] and colitis [45]. Probiotic bacteria have been shown to modulate intestinal microbiota through the modulation of luminal pH and the production of antimicrobial compounds [46, 47]. In addition to the foregoing, probiotics have also been reported to enhance the intestinal barrier function [48]. These effects collectively contribute to the management of inflammatory bowel disease [46].

There is strong evidence that the administration of probiotics is able to down-regulate over-expressed immune responses in subjects with autoimmune/immune-inflammatory disorders and enhance specific aspects of immune function in healthy subjects. Schifffrin and colleagues reported enhanced phagocytic capacity of peripheral blood leucocytes (polymorphonuclear and monocytes) in healthy human adults administered with specific strains of probiotics [49–52]. The effectiveness of probiotics in enhancing the immunogenicity of mucosal and systemic vaccines has also been reported. It has been reported that probiotic administration could induce antibody responses to completely unrelated antigens and to themselves [53, 54].

5. Probiotics from fermented fish

Probiotics have been obtained from a wide variety of traditionally fermented and preserved products that include dairy-based items like fermented milk, cheese, buttermilk, milk powder, and yogurt [55, 56]. Non-dairy food sources like soy-based products, cereals, and a variety of fermented juices have also proved to be promising [57, 58]. With more and more sources being explored, new strains and species of probiotics are being added to the list.

Fish and their products have emerged to be a potential source of novel probiotics that can be utilized to enhance the value of human nutrition [59]. Fish gut confers a congenial environment for colonization of bacteria abundant in the aquatic environment. Most of the probiotic bacteria isolated from the fish gut are either aerobes or facultative anaerobes. Worldwide, fishes have been consumed in diverse formats. Among some ethnic groups, there has been a tradition to preserve fish by drying and fermenting for enhanced shelf-life. In the North-eastern states of India, freshwater fish have been fermented by traditional practices into products such as Utonga-kupsu, Hentak, and Ngari. Workers have studied the bacterial communities in these products and isolated *Lactococcus lactis* subsp. *cremoris*, *L. plantarum*, *Enterococcus faecium*, *Lactobacillus fructosus*, *Lactobacillus amylophilus*, *Lactobacillus coryniformis*, *Bacillus subtilis* and *B. pumilus*, *B. cereus*, *Staphylococcus aureus* and Enterobacteriaceae population. Most of these have been characterized as probiotics [60, 61]. Similar explorations have reported several strains of probiotics from a variety of other fishes. The table in the following section (**Table 1**) summarizes various such sources and probiotic strains isolated from them.

Country/state/ region	Fish species	Bacteria isolated	Accession No.	References
Manipur (India)	<i>Puntius sophore</i>	<i>Lactococcus plantarum</i>	JX 847611	[60, 62]
		<i>Lactobacillus fructosus</i>		
		<i>Lactobacillus amylophilus</i>		
		<i>Enterococcus faecium</i>		
		<i>Lactobacillus coryniformis</i> subsp. <i>torquens</i>	KU945827	[63]
		<i>Lactobacillus lactis</i> subsp. <i>cremoris</i>		
		<i>L.brevis</i>		
		<i>Bacillus coagulans</i>	JX847608	[64]
Meghalaya (India)	<i>Danio</i> spp.	<i>Bacillus subtilis</i>	KX953135	[65]
		<i>Lactobacillus rossiae</i> isolate LS6	JN680708	[66]
		<i>L. plantarum</i> isolate LS5	JN680707	
		<i>L. rossiae</i> isolate LS4	JN680706	
		<i>Lactobacillus pentosus</i> isolate LS3	JN680705	
		<i>Lactobacillus pobuzihii</i> isolate TTp4	HQ141620	
		<i>L. pobuzihii</i> isolate TTp6	HQ141621	
		<i>L. pobuzihii</i> isolate TTp12	H Q141622	
		<i>L. pobuzihii</i> isolate TTp13	H Q141623	
Assam (India)	<i>Puntius</i> spp.	<i>L. pobuzihii</i> isolate TTp14	HQ141624	
		<i>Staphylococcus</i> sp.	KR706310	[67]
NE India	<i>Puntius</i> sp.	<i>Staphylococcus</i> <i>piscifermentans</i>		[68]
		<i>Staphylococcus arlettae</i>		
		<i>S. condiment</i>		
		<i>Staphylococcus sciuri</i>		
		<i>Staphylococcus warneri</i>		
		<i>S. nepalensis</i>		
		<i>Staphylococcus hominis</i>		

Country/state/ region	Fish species	Bacteria isolated	Accession No.	References
Malaysia	<i>Parastromateus niger</i> BLOCH	<i>Pediococcus pentosaceus</i>		[69]
		<i>Lactobacillus plantarum</i>		
		<i>L. pentosus</i>		
	<i>Stolephorus</i> spp.	<i>Lactobacillus casei</i>		[8]
		<i>Lactobacillus plantarum</i>		
Thailand	<i>Chitala ornata</i>	<i>Lactobacillus plantarum</i>		[7]
	<i>Channa micropeltes</i>	<i>L. pentosus</i>		
		<i>Staphylococcus simulans</i>	MG798679.1	[70]
Phillipines	<i>Chanos chanos</i>	<i>Leuconostoc mesenteroides</i>		[71]
		<i>P.cerevisiae</i>		
		<i>Enterococcus faecalis</i>		
		<i>Pacidilactici</i>		
		<i>Leu.paramesenteroides</i>		
		<i>L. plantarum</i>		
	<i>Loriculus philippensis</i>	<i>P. pentosaceus</i>		
		<i>Streptococcus equinus</i>		
		<i>Leuconostoc</i> sp.		
		<i>Lactobacillus</i> sp.		
	<i>Eleutheronema tetradactylum</i>	<i>P. halophilus.</i>		

Table 1.
Probiotics isolated from fish.

The processes like fermentation, salting, drying, and smoking are the popularly followed traditional methods of preservation of fish [72, 73]. As evident from the list (**Table 1**) lactic acid bacteria have been found to be predominant in most of the fermented fish products. However, the microbial diversity of these products also encompasses some species of *Micrococcus*, *Lactococcus*, *Enterococcus*, *Bacillus*, *Staphylococcus*, and *Enterobacteriaceae*. Conventionally, culture-based methods have been employed to identify LAB in food samples, and isolates are evaluated for probiotic properties under controlled conditions. With the advances in molecular techniques, the isolation and identification of microorganisms missed by culture-dependent methods have now been achieved. Consequently, as new microbial metabolites, such as bacteriocins, defensins, and other antimicrobial compounds are being reported, an extensive database for identification and comparison of potential novel products is now available [71]. Several strains of probiotic bacteria were isolated from various fish species (African catfish, European eel, Bream, Perch, Rudd) and most of these were reported to be *Lactobacillus* isolates which were able to inhibit pathogens by acid productions [75]. Various probiotic strains of *Bacillus subtilis* have been reported from the gastrointestinal tract of carps [75], coastal fishes [76], bivalves [77], shrimp culture ponds [78], and shrimp larvae-rearing medium [79]. Multiple studies supported that *B. subtilis* could reduce

pathogenic bacteria in aquaculture. The *Lactobacillus* species associated with the traditionally fermented fish product—Tungtap (a fermented product of ethnic tribes of the state of Meghalaya in India) were found to possess many health-promoting probiotic properties [66]. *Alcaligenes* sp. isolated from the gastrointestinal tract of *Tor tambroides*, function as an important probiotic that promote gut microbiota composition, improve gut health including bacterial nutritional enzyme activity, volatile short-chain fatty acids (VSCFA) production and gut morphology, and enhance production performance of Malaysian Mahseer (*T. tambroides*) [80].

The fish gut microbiota embodies diverse enzyme-producing microorganisms capable of producing multiple hydrolytic enzymes that aid in the digestion of carbohydrates, proteins, and lipids [81, 82]. *Bacillus* spp. has been reported from Utonga-kupsu, Hentak, and Ngari (traditional fermented fish of Manipur, North-East India) alongside *Staphylococcus*. These have also been reported from other fermented fish products such as Namplaa and Kapi (from Thailand) and have been shown to exhibit amylase, protease, and cellulase activities that can improve the quantity, availability, and digestibility of dietary nutrients in the body in addition to other probiotic effects [65, 83]. *S. simulans* PMRS35 isolated from *budu*, a traditional Thai salt-fermented fish-based product, possessed high lipase and protease activities and a vast array of desirable probiotic characteristics [70]. In any fermented food, the diverse microorganisms are capable of producing many useful enzymes like oxidase, β -galactosidase, amylase, *etc.* which are essential for aesculin hydrolysis, starch hydrolysis, nitrate to nitrite reduction, and other important biochemical conversions and can hence be useful in bioremediation as well [84].

Although the above list is not comprehensive, it represents the potential of fish and their products as a source of novel probiotics. The knowledge of the health benefits of fermented fish products has been utilized by many cultures worldwide and this information can be utilized for the development of probiotic products for human consumption.

6. Future prospects

The incorporation of probiotics from fish and fish products into the development of functional foods containing known probiotic strains can provide alternatives in therapeutics and ensure food security. Isolation and standardization of bacteriocins and other metabolites from probiotics can lead to the development of functional foods for individuals surviving on a vegan diet.

7. Conclusions

The host- probiotic relationship can be regarded as evolutionarily one of the most primitive associations. It represents a dynamic relationship that is influenced by dietary and other intrinsic and extrinsic factors. The kind of diet consumed by the host plays an important role in the maintenance of the probiotic microbiome in the body. On the other hand, a healthy probiotic microbiome in the host ascertains good growth and health of the host. The various health benefits and the potential role of probiotics in various human diseases have been highlighted in this chapter. As the kind of diet consumed influences the gut microbiome significantly, it, therefore, becomes essential to explore this intricate food-host-probiotic relationship in order to understand human health and diseases. The traditional food- preparation practices evolved through close observation of the effect of food on human and animal health. Hence, exploration of such traditionally prepared foods can reveal

some novel probiotics with potential therapeutic applications. In this chapter, some of such sources of probiotics have been listed. However, there is an urgent need to study these in detail as most of them have not been completely characterized to the extent of their utilization for human applications.

Conflict of interest

The authors declare no conflict of interest.

Author details

Nilofar Yasmin¹, Khusboo Kaur² and Kaushal Sood^{3*}

¹ Center for Biotechnology and Bioinformatics, Dibrugarh University, Dibrugarh, Assam, India

² Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam, India

³ Department of Zoology, Tinsukia College, Tinsukia, Assam, India

*Address all correspondence to: kaushalsood@gmail.com

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