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From probiotics to fermentation: A review of microbes in food and supplements

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Abstract

Microorganisms have a crucial role in processing food and preservation, as well as enhancing the nutritional and sensory qualities of food products. Microorganisms are utilized as additives for altering the nature of a substance and have a significant role in fermentation. Molds (e.g., *Penicillium roqueforti* and *Geotrichum candidum*), yeasts (e.g., *Saccharomyces cerevisiae*), and lactic acid bacteria are used to increase the shelf life of foodstuffs and the nutritional quality of processed foods in the fermentation process. Acetic acid bacteria and lactic acid bacteria are used in applied microbiology to increase the flavor of different manufactured foods. Further, bacteria, in the form of probiotics, are involved in the processing of food. Various strains of different bacteria are used as probiotics to increase the quality and preservation of foodstuffs. *Bifidobacterium* and *Lactobacillus* are used to produce prebiotics and postbiotics as well. This article discusses the many ways that microorganisms may be added to food and supplements such as probiotics, prebiotics, postbiotics, and symbiotics in order to fulfill the world's food demand and make up for the scarcity of arable land. It also discusses the future prospects and challenges of this field.

Keywords: Prebiotics, Postbiotics, Synbiotics, Technological hurdles, Global regulations

1. Introduction

Microorganisms play a great role in the quantity and quality of manufactured food. For instance, yogurt and cheese are fermented products that are acquired from milk. For yogurt production, a specific culture of microorganisms is inoculated in pasteurized milk for fermentation. Microorganisms are utilized to alter the nature of one thing to another, which is then used as food. For instance, bread is made from wheat flour and wine from grapes, while yogurt and cheese are produced from milk. In this way, microorganisms are very important in the food industry (Kalsoom et al., 2020) and in our daily diet as well. There is a significant role of microorganisms in producing aromas and flavors in our lives (Román et al., 2017). A wide range of microorganisms, such as *Yarrowia lipolytica* (Braga & Belo, 2022), *Pseudomonas taiwanensis VLB120* (Maurya et al., 2022), *Aspergillus, Penicillium, Trichoderma, and Rhizopus* (M. Muradova et al., 2023), as well as vanillin and coumarin (Gupta et al., 2015), are utilized in the pharmaceutical, perfume, and food industries. Vanilla beans have about 200 volatile compounds that are used for flavoring in these industries and vanillin is the most important flavoring compound used in these industries. Previous research revealed that vanilla contains antioxidant, nutraceutical, and anti-mutagenic characteristics due to volatile compounds (Ayseli & İpek Ayseli, 2016; Zakaria & Kamal, 2015).

The metabolic activity of microbes can result in the synthesis of aroma, but microorganisms are not directly flavoring agents. Microorganisms are utilized for the preservation and modification of vinegar, meat, pickles, soybeans, milk, fermented vegetables, beer, and wine (Dan et al., 2019; Shojaei Zinjanab et al., 2021; Wang et al., 2020). Food is prepared and preserved in a common way called fermentation (Dan et al., 2019; Paulino et al., 2021).

Fermented foods as well as beverages have been involved in the diet of humans since the arrival of the human race (Hutkins, 2006).

The most commonly used bacteria in applied microbiology are *Lactobacillus* and *Lactococcus* bacteria (lactic acid bacteria), while other microbes such as yeast and molds are also widely used (Guerra et al., 2018; Lemos Junior et al., 2019; Mota de Carvalho et al., 2018). Generally, cereal foods are known as the main component in the diet of humans all over the world, as they provide a significant amount of energy and other essential ingredients (Bondia-Pons et al., 2011). Sourdough has two basic constituents: water and flour, and its sour properties are developed due to these constituents during microbial bioconversion. Bread sourdough is considered a lacto-fermented flour containing acidic characteristics. The manufacturing of sourdough and fermentation of cereal are very significant sections of processing, almost for every cereal. The preparation of sourdough is attained by spontaneous fermentation, which is also a prime method of sourdough preparation (Bartkiene et al., 2022).

Numerous varieties of fermented cereal drinks are produced and utilized in a variety of contexts in Southern America, Asia, Africa, and several European countries, e.g., boza in Bulgaria (Marsh et al., 2014). Cereals belong to edible grains of the grass family. Cereals are edible seeds that are highly nutritious and very important food sources (McKevith, 2004). The market for global functional beverages is a developing unit in the food industry because modern consumers are very health conscious and accept foods that improve their health and decrease disease probability. Fermented milks, particularly yogurt-style products, are widely used functional beverages in Denmark, North America, and Western Europe. According to industry estimates, the global functional beverage market was valued at approximately USD 75 billion in 2003 and increased to around USD 113 billion by 2010, reflecting a 1.5-fold growth (Marsh et al., 2014). Recent projections estimate the market to surpass USD 200 billion by 2025 (FMI, 2025). Concerning future food requirements and supply, many researchers acknowledge the obligation to enhance food preparation by more than 60% in the upcoming 30 years to compensate for the requirements of the world's growing population (Pikaar et al., 2018).

Microorganisms do not require much space and grow very fast. Microorganisms contain a high protein content. For example, the protein content is about 40–50% in the case of bacteria. Therefore, microorganisms are considered to have a high yielding ability (Mueed et al., 2022). Microorganisms contribute beneficially and detrimentally to food production, as the flavor and stability of food products have been examined for centuries with the help of microorganisms (Kim et al., 2011).

Consequently, this review article is aimed at providing the readers with the history and types of microorganisms as well as their use as probiotics, prebiotics, postbiotics, and synbiotics. Additionally, this article outlines key areas for future probiotic research by highlighting significant challenges in the probiotic industry, such as exploring the mechanisms of action of probiotics, investigating their therapeutic applications for chronic infections, studying their safety and efficacy, developing new formulation and delivery methods, and analyzing global regulatory frameworks. Moreover, this article identifies several problems within the probiotic industry, such as inconsistent global regulations, technological hurdles in maintaining probiotic viability, insufficient clinical trials to support health claims, and the spread of misleading marketing information.

2. Microorganisms in Food Supplements: Beyond Probiotics

A food that is healthy and has medicinal benefits is termed as functional food. Functional foods consist of bioactive constituents, which can be chemical compounds or probiotics (Tomasik & Tomasik, 2020). About 10 years ago, probiotics were investigated as those viable microorganisms that have very useful effects on the host health by promoting its intestinal microbial balance. Fermented milk is considered as the first food with living microorganisms that are recorded in the Old Testament. There are evidences from 2500 B.C. that the Sumarians also used milk fermentation (Rosenstock et al., 2021).

2.1. Prebiotic Compounds

Flavonoids and triglycerides could be involved in this group simultaneously, with a few peptides decomposed by bacteria into active constituents. Even inorganic materials (i.e., bacteria need small nutrients for their growth) utilized outside and inside could be treated as prebiotics (Tomasik & Tomasik, 2020).

2.2. Prebiotics involved in Cereals and Derivatives

Prebiotics involve a new group of cereals (Das et al., 2012). Cereals are full of insoluble/soluble fibers (β-glucans and arabinoxylane), oligogalactosyllactose (OGLs), oligofructose, and resistant starch (Reque et al., 2019). Cereals that are gained from lactic acid fermentation are very healthy, have increased nutritional value, and are safe. Lactic acid fermentation enhances the palate and is also helpful in the preservation of food (Kalui et al., 2010).

2.3. Postbiotics compounds

Postbiotics is a new term that has been introduced recently (Aguilar-Toalá et al., 2018). It is a mixture of all bioactive ingredients produced by bacteria. The immune system can be stimulated by postbiotics. Fermentation is the most general origin of postbiotics in medical technology as well as the food industry (Arora et al., 2011; Capozzi et al., 2012).

2.4. Synbiotics

A dietary supplement having a blend of probiotic and prebiotic is called a synbiotic, which acts in synergy to synthesize a dual supplement of advantageous effects (e.g., on the medical condition, immunity, and protection of fish and shrimp against disease-causing pathogens) (Huynh et al., 2017). The diversity of microorganisms and soil microbial health are improved due to prebiotics because the growth of microorganisms living in the soil is promoted by these prebiotics. Prebiotics consist of natural products, generally waste from agro-industry, leaf mold, sewage sludge, waste from animals, and waste containing chitin (Baker et al., 2011; Strachel et al., 2017; Vassilev et al., 2013).

Prebiotics, postbiotics, and synbiotics, in short, are a range of strategies to alter the gut microbiota and enhance human health (Li et al., 2021). Probiotics provide helpful live bacteria, postbiotics supply non-living microbial metabolites with possible health benefits, and prebiotics provide the substrate for beneficial microbial development (Vera-Santander et al., 2023). Synbiotics increase the survival and effectiveness of probiotics by combining them with prebiotics for a synergistic impact (Saghir & Suede, 2024). When combined together, all these elements are essential for preparing supplements as well as functional food which in turn help improve immunity, digestive health, and general wellbeing of humans (Markowiak & Śliżewska, 2017).

3. Microorganisms in Food and Supplements: An Overview

It is a major problem to meet the demand for nutritional supplements for the world's increasing population. Protein is a vital part of our diet. In the absence of a sufficient supply of protein, our body is unable to nourish itself well (Adedayo et al., 2011). A lot of efforts have been made to find an alternative source of protein with the help of unconventional or modern techniques. Protein products were produced with the help of new techniques from algae, yeasts, bacteria, and fungi (Ageitos et al., 2011). Microorganisms are used to upgrade organic materials from low-protein to high-protein foods and, therefore, are used in industry. Microorganisms are, on a large scale, a source of single-cell protein (Adedayo et al., 2011). So, these microorganisms have been utilized for producing different food supplements.

3.1. Acetic Acid Bacteria (AAB)

AAB are called gram-negative bacteria. They develop in the presence of oxygen and are non-spore-forming bacteria. AAB range in size from 0.8 to 4.5 μm in length and 0.4 to 1 μm in width. AAB can develop at 5–6.5 pH (optimum pH), while they can also survive at lower pH (3–4) (Yassunaka Hata et al., 2023). AAB was once split into two primary genera: *Gluconobacter* and *Acetobacter* (Malimas et al., 2009; Yamada & Yukphan, 2008).

Acetic acid bacteria (such as *Komagataeibacter*, *Gluconobacter*, and *Acetobacter* used in the synthesis of vinegar) selectively favor specific strains based on their ability to withstand acidic environments, and they cannot grow on every medium equally. It is very difficult to investigate AAB because AAB require pure culture for maintenance and cultivation, particularly those strains that are isolated from high acetic acid sources (Gullo et al., 2006). So, various types of media were utilized for separation, and physiological ability-based phenotypic techniques were used for recognition. Additionally, there are a few limitations for plating, such as the required time and the inability to locate viable but non-culturable (VBNC) cells. New methods with molecular approaches have emerged to deal with these disadvantages of cultivation (Gonzalez et al., 2006).

Sugars and alcohol are oxidized with the help of AAB, producing the accumulation of organic acids as a byproduct. Alcohols are converted to sugars by the oxidation process of AAB, such as fruit sugar (fructose), which is made from mannitol ($C_6H_{14}O_6$); sorbose ($C_6H_{12}O_6$), which is made from D-sorbitol; and erythrulose, which is made from erythritol. With the aid of these microorganisms, high concentrations of acetic acid can be synthesized from ethanol, so they are very important in the vinegar industry (Sengun & Karabiyikli, 2011). Sometimes AAB is involved as a harmful bacterium in beverages and the food industry, like in wine (Bartowsky & Henschke, 2008).

3.2. Lactic Acid Bacteria (LAB)

LAB are gram-positive, non-spore-forming bacteria that can withstand oxygen presence (aerotolerant) and retain violet crystals (Hayek et al., 2019; Hayek & Ibrahim, 2013; Quinto et al., 2014; Sadishkumar & Jeevaratnam, 2016). Lactic acid bacteria (LAB) are very significant in producing lactic acid, which is a byproduct of metabolic activities.

Lactic acid bacteria play various roles in the food, clinical, and agricultural sectors. Lactic acid bacteria are used in the fermentation of many foods, and fermentation utilizing these bacteria is the most traditional and acceptable art in the preservation of food (Bintsis, 2018). Lactic acid bacteria are very crucial in many food functions; therefore, the food industry is always looking for strains with remarkable properties and aspects to increase sensory and product quality (Bintsis, 2018). The initial pH is also very important, as 4.5–6.5 pH is required for the optimization of LAB to begin the lactic acid fermentation. LAB is used to enhance the shelf life and safety of fermented food products. This is achieved by the metabolism of LAB, as LAB produces lactic acid, which is utilized to decrease the environmental pH and reduce the growth of spoilage-causing microbes. In this way, the product's shelf life is enhanced (Rachwał & Gustaw, 2024). *Streptococcus thermophiles*, *Lactobacillus bulgaricus*, and *Streptococcus salivarius* are combined to convert lactose (milk sugar) to lactic acid and are called lactic acid bacteria (Oyeleke, 2009). One thing that must be taken into consideration here is that not all lactic acid bacteria are considered probiotics. Each probiotic strain contains a significant genetic trait that may support various phenotypes (Azais-Braesco et al., 2010).

3.3. Yeast

People nowadays are particularly worried about human health and avoid using items that include a large number of food additives. The majority of consumers choose natural meals that are devoid of artificial preservatives. Food producers have encountered several challenges in satisfying people's desires for higher-quality food items, such as the utilization of more natural food ingredients in food making, during the last several decades. Hence, safety and quality of food are the main problems for producers, users, and governments (Settanni & Corsetti, 2008). *P. kudriavzevii* is the fourth most used non-Saccharomyces yeast extracted from most samples in 2016, a year of port wine with recognized good quality. Malic acid is detrimental to the quality of wine when available in extreme amounts in grapes. Malic acid is degraded by *P. Kudriavzevii,* which is a non-Saccharomyces yeast (Chu et al., 2023).

So, food manufacturers have found yeast to be a natural source for making bread, pastries, hot dogs, buns, crackers, cookies, biscuits, and cakes. Moreover, yeast is also famously used in making alcoholic drinks and non-alcoholic soft drinks.

The overall comparative characteristics of fermentation microorganisms (AAB, LAB, and yeast) are given in Table 1.

Table 1. Comparison of fermentation microorganisms based on their fermentation characteristics

Fermentation Microorganism	Application	Acid Tolerance	Key Function	Reference
AAB	Vinegar production	High	Ethanol oxidation to acetic acid	Hua et al. (2024)
LAB	Yogurt, pickles	Moderate	Lactic acid production and preservation	Aguirre-Garcia et al. (2024)
Yeast	Bread, wine, beer	Variable	Alcohol fermentation, Aroma formation	Romano et al. (2022)

4. Probiotics: The Beneficial Microorganisms

Probiotics are real beneficial microorganisms (particularly bacteria) that may reside in the human digestive tract and may offer various health advantages. Fermented foods that are rich in probiotics include yogurt, soft cheese, fermented cabbage, soybeans, and fermented tea. The concept of probiotics was suggested by Nobel Prize winner Eli Metchnikoff in 1908 by explaining how the use of fermented milk products contributed to the long lives of Bulgarian peasants (Metchnikoff & Chalmers-Mitchell, 1908). Lilly and Stillwell used the term "probiotics" in 1965. They used this word while explaining a substance released by one organism that is capable of provoking the development of another organism (Lilly & Stillwell, 1965).

In close association with humans, a large number of microorganisms are present in the gastrointestinal tract, on the skin, and in the mouth of human beings. More than 500 species of bacteria are found in the gastrointestinal tract of humans; a few of them play important functions in health, including stimulation of the body's defense system, aiding the digestive system, and protecting the host from invading viruses and bacteria (Macfarlane & Macfarlane, 1997; McGhee, 1999).

The gut normal flora, which is quickly established after birth, remains constant throughout life. It has a significant role in the maintenance of human homeostasis (Macfarlane & Macfarlane, 1997). The composition of normal flora can be altered due to the immense use of irradiation, immunosuppressive therapy, and antibiotics during treatment. Stress is a very important and well-recognized factor in many intestinal diseases, and intestinal dysfunction is a stress-related disease. There are a few studies that have revealed the direct effects of severe stress on healthy gut microbes' function and composition. Furthermore, in-vitro studies revealed that the gut microbiome can mix with stress hormones produced by mammals and synthesize a large variety of neuroactive compounds (Ahmed et al., 2022). So, beneficial bacterial species are introduced into the GI tract for the establishment of microbial equilibrium and to avoid disease (Leigh et al., 2023).

Probiotics are "live microorganisms" that, when used in suitable doses, impart health benefits to the host, according to a committee of specialists recognized by the World Health Organization (WHO) and Food and Agriculture Organization (FAO) (Gupta & Garg, 2009). *Streptococcus, Bacillus, Enterococcus, Escherichia, Bifidobacterium,* and *Lactobacillus* are some of the bacterial genera that are usually used in the preparation of probiotics (Alvarez-Olmos & Oberhelman, 2001; Jin et al., 2000).

The first probiotic was *Lactobacillus rhamnosus GG* (LGG) (Gorbach, 2000). It has been proven that *Lactobacillus rhamnosus* strain GG is very effective on the immunity of the intestine, as it enhances the number of IgA and other immunoglobulin-releasing cells in the intestinal mucosa (Reid et al., 2003). Some important microorganisms that are used as probiotics are listed in Table 2.

Table 2. List of microorganisms utilized as Probiotics (Alvarez-Olmos & Oberhelman, 2001; Gibson & Roberfroid, 1995; Jin et al., 2000)

Saccharomyces sps.	**Streptococcus sps.**	**Bifidobacterium sps.**	**Lactobacillus sps.**	**Others**
S. boulardii	*S. thermophilus*	*B. adolescentis*	*L. acidophilus*	*Propionibacterium freudenreichii*
		B. breve	*L. bulgaricus*	*Enterococcus*
		B. bifidum	*L. casei (rhamnosus)*	*Escherichia coli*
		B. infantis	*L. fermentum*	*Bacillus cereus*
		B. lactis	*L. gasseri*	
		B. longum	*L. johnsonii*	
			L. lactis	
			L. paracasei	
			L. plantarum	
			L. reuteri	
			L. salivarius	

4.1. The Mechanisms of Action of Probiotics

The sector of probiotics is full of new, outstanding advancements, but there are still improvements needed in their mechanisms of action. These are a few mechanisms by which probiotics exert positive potential on the body of humans: competition, synthesis of neurotransmitters, advancement in barrier functions of the intestine, and modulation of the immune system in the body of the host. It is very difficult for pathogens to survive in the gut because probiotics compete with these pathogens for the sites of receptor binding and nutrition (Plaza-Diaz et al., 2019). The production of bacteriocins and H_2O_2 helps probiotics act as antimicrobial agents (Ahire et al., 2021). Probiotics stimulate the synthesis of mucin protein, which is utilized to enhance the barrier function of the intestine (Chang et al., 2021). Probiotics involve the modulation of T lymphocytes, dendritic cells, and macrophage B, as well as the regulation of adaptive and innate immune responses. The synthesis of cytokines that are anti-inflammatory in nature is enhanced by probiotics while interacting with epithelial cells of the intestine (Petruzziello et al., 2023). The modulation of dopamine, gamma-aminobutyric acid (GABA), and serotonin is also attained by specific probiotic strains (Gangaraju et al., 2022). The overall mechanism of action of probiotics is shown in Fig. 1.

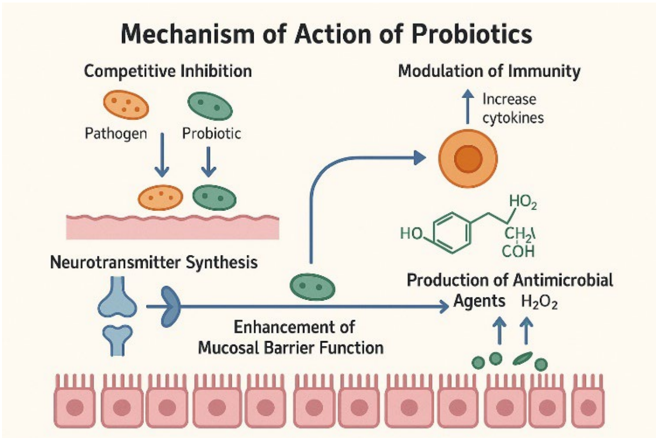

Fig. 1. Mechanism of action of probiotics (that involves competitive inhibition, modulation of immunity (e.g., increase in cytokines), neurotransmitter synthesis (e.g., GABA), enhancement of mucosal barrier function, and production of antimicrobial agents such as H_2O_2 and bacteriocins).

4.2. Therapeutic Applications of Probiotics for Chronic Diseases

There are many therapeutic applications of probiotics in different chronic diseases (Fig. 2), and some of the important diseases are discussed below.

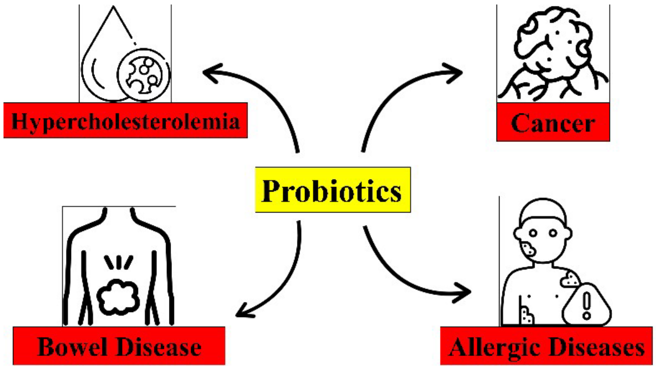

Fig. 2. Health attributes of probiotics. Source: (Latif et al., 2023).

4.2.1. Probiotics Activity as Cancer Suppressors

Probiotics utilized as adjuvants in different types of cancer depend upon their capability to regulate enteric flora and increase systemic as well as local immunity. Probiotics are involved in the prevention of progression, initiation, and tumors induced by chemicals (Samanta, 2022). The involvement of probiotics in the suppression of intestinal cancer was also observed (So et al., 2017). Many researchers revealed that the interaction of probiotics with critical metabolic pathways includes inflammation, angiogenesis, apoptosis, cellular proliferation, and metastasis. In addition, carcinogenesis is inhibited by probiotics by enhancing the production of short-chain fatty acids and increasing apoptosis (Latif et al., 2023). Apoptosis is induced by L. reuteri (a probiotic) in human myeloid leukemia-derived cells by modulating NF-kappa B (Saber et al., 2017). The apoptosis of cancer cells induced by heat killed S. cerevisiae (Shamekhi et al., 2020).

4.2.2. Effect of Probiotics on Hypercholesterolemia

Probiotics are used to maintain levels of cholesterol in the blood. Probiotics are used directly or indirectly to decrease the levels of cholesterol in the body. The inhibition of de novo synthesis of cholesterol by hypocholesterolemia factors, including lactose, orotic acid, and uric acid, is a direct mechanism (Thakkar et al., 2016). The hypocholesterolemic characteristics of Levilactobacillus brevis MT950194 and L. brevis MW365351 were recognized in vitro as well as in vivo in a study. In lipid metabolism, the potential of the probiotic complexes Lactobacillus, Bifidobacteria, and Pediococcus was also examined (Galli et al., 2020).

4.3. Safety and Efficacy of Probiotics

In the USA, probiotics are considered an additive in food that the FDA has approved on the basis of efficacy and safety data, or they can be considered "generally recognized as safe" (GRAS). Probiotics are considered GRAS, or GRAS status is provided to a probiotic when its usage history seems safe. The assessment tests for the safety and efficacy of probiotics must be executed using animal models or in vitro assays. Knowledge about the probiotic strains and their mechanisms are attained by in-vitro assays. There are two phases for clinical evaluations, including phase 1 (safety assessment) and phase 2 (efficacy assessment), as shown in Fig. 3. If these assessments prove that the probiotic strain is safe, then this probiotic strain is marketed as a probiotic food (Ezendam & van Loveren, 2006).

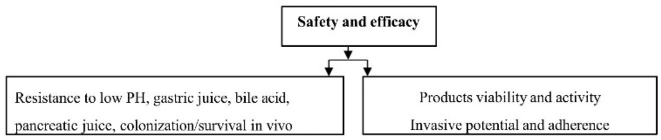

Fig. 3. Safety and efficacy parameters of probiotics

4.4. Developing New Formulation and Delivery Methods

Probiotics experienced severe physicochemical stresses, including an increase in temperatures and acidity during processing, that could reduce their viability. So, researchers used various encapsulating methods to reduce these stresses and increase the viability of probiotics in the body (Luo et al., 2022). Microencapsulation is the most commonly used and traditional method. Microencapsulation is divided into four techniques: freeze-drying, extrusion, spray-drying, and emulsification. The capability of probiotics to survive in the extreme environment of processing and in the body is improved by these techniques (Razavi et al., 2021). Recently, the nanoencapsulation method has been mostly used to increase the probiotic's capability to face acute processing and in vitro stresses. Targeted delivery and control of the release of probiotics in the intestine are facilitated by these methods (Xu et al., 2022). The unique biological properties of nanocapsules, including higher surface areas and small particle sizes, bring improvements in the efficacy of encapsulated probiotics (Singh et al., 2022). L. rhamnosus stability is observed after loading them into chitosan-gelatin-coated nanoliposomes (Hosseini et al., 2022).

4.5. Analyzing Global Regulatory Frameworks

Different countries have their own framework for fermented foods and probiotics. Here we discuss some of the important global regulatory frameworks established for probiotic regulation.

Microorganisms utilized in food synthesis, such as fermented foods, are called food cultures and are considered a category of food ingredients in Europe. There are some novel foods that are produced by microbes, also called fermented products, that are approved and used in the EU according to the EU regulation for novel foods. UV-treated baker's yeast (Saccharomyces cerevisiae) and fermented soybean extract are the most commonly used fermented products in Europe. There are other regulatory frameworks in the world that are given in Table 3.

Table 3. List of food regulatory authorities in the world (Mukherjee et al., 2022)

Name	Country
The European union	Europe
The Russian Federation	Russia
US Food & Drug Administration (FDA)	USA
Canadian Food Inspection Agency (CFIA)	Canada
Food Safety & Standards Authority of India (FSSAI)	India
Ministry of Food & Drug Safety (MFDS)	South Korea
Food Standards Australia New Zealand (FSANZ)	Australia and New Zealand
Codigo Alimentario Argentino (CAA)	Argentina

4.6. Problems within the Probiotic Industry

The probiotic industry is revolutionizing day by day and fulfilling the need for food required by the large population of the world, but there are challenges in the probiotic industry. The challenges in the probiotic industry are given below.

4.6.1. Inconsistent Global Regulations

There are few guidelines related to the choice of probiotic strains and the assessment of their safety and effectiveness. Till now, there has been no international authoritative harmony, specifically regarding probiotic food products. There is insufficient data available related to the stability of probiotics in powdered milk (Senok et al., 2005). The microbiota of the gut can affect the drug metabolism directly and indirectly, with consequences for effectiveness and toxicity as well. Probiotics may transfer from the GIT and can cause invasive infections on very rare occasions (Merenstein et al., 2023).

4.6.2. Technological Hurdles in Maintaining Probiotic Viability

Probiotics must fulfill a few criteria before being used for the benefit of human health. Probiotics must contain better technological characteristics so that they can be synthesized and used in food products. There are key aspects like safety, technological characteristics, and functional characteristics that have to be considered before using microorganisms in the selection process of probiotics (Mattila-Sandholm et al., 2002). Technological factors that influence the functionality of probiotics are shown in Fig. 4.

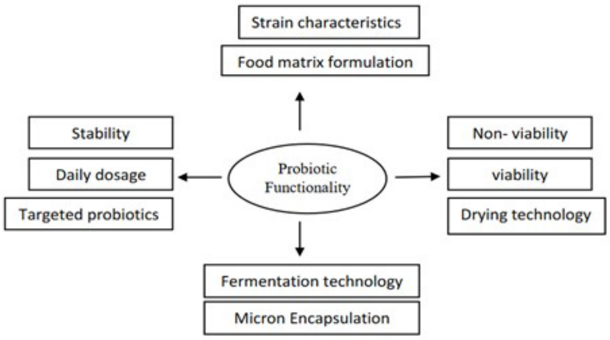

Fig. 4. Technological factors influencing the functionality of probiotics. Source: (Mattila-Sandholm et al., 2002).

4.6.3. Insufficient Clinical Trials to Support Health Claims

Previous studies revealed that the bacteria in GIT have a significant role in our health. The experiment on germ-free animals also showed that the microbiome of the intestine is responsible for providing vitamins and energy to the host through fermentation. It is also revealed that the normal flora of the GIT also has a significant role in the protection against pathogenic microorganisms and proper development of the immune system (Doron & Gorbach, 2006). Even though studies have been conducted, there is still a lack of evidence regarding the positive use of probiotics in clinical settings.

4.6.4. The Spread of Misleading Marketing Information

The most active area for the usage of functional foods is Europe, and probiotic yogurts and milks are mostly probiotics. The Japanese market is still dominated by soft drinks, which are the original functional foods. There are some important factors that are considered for the success of fermented food, which include acceptance and interest in a functional food. Fermented foods must comply with consumer demand and provide scientific evidence that food contains health benefits. A strong health claim or inference could be an important motivator to purchase something, but it does not guarantee the success of a product. The customers have to be motivated to purchase again, which is not going to happen if the product is not offering the benefits that consumers demand (Stanton et al., 2001).

5. Fermentation: The Microbial Transformation

Microbes like lactic acid bacteria (LAB) are applied in the food processing technique called traditional fermentation. Bacteria propagate using food as a substrate. This is an old-time technology of food preservation that is used for the preservation of food. In most emerging nations, this technology has become part of their cultural and traditional norm, especially in Africa. Fermented foods, with their edible taste, attractive color, and pleasant texture, are preferred over unfermented foods (Aderiye & Laleye, 2003; Mosha & Vicent, 2004). Various types of food fermentation have been recommended and also categorized according to the main yields during the fermentation process. Fermentation with ethyl alcohol as an end product and lactic acid fermentation (non-alcoholic) are mostly used in conjunction with other fermentations like peptide sauce fermentations, alkaline fermentation, and acetic acid fermentation (Anukam & Reid, 2009; Blandino et al., 2003). Fermentation's pH is always in the acid range (lower than 5) because lactic acid is the primary byproduct of fermentation (Mokoena et al., 2005). Cereals, dairy products, beverages, and meat products are processed utilizing fermentation techniques (Pswarayi & Gänzle, 2022).

5.1. Important Microorganisms used in Fermentation

A mixed colony of microorganisms like bacteria, molds, and yeast for indigenous natural fermentation is first isolated (Chen et al., 2022). The fermentation process's final products are determined by the bacteria utilized in the fermentation (Siddiqui et al., 2023). Bacteria have enzymes like lipases, proteases, and amylases that can break complex foods into simple and nontoxic products, as well as make these products edible for consumption by giving them an aroma and desirable texture (Agrawal, 2024). LABs such as *Streptococcus, Lactobacillus*, and *Enterococcus* are involved in the fermentation process (Min et al., 2022). The isolated yeast *Saccharomyces* are mainly used in fermentation. G. fermentans, *Geotrichum candidum,* and *Rhodotorula graminis* are extracted during the maize fermentation (Mishra & Panda, 2022). Molds such as Penicillium, Mucor, and Rhizopus are used in milk and cheese fermentation (Varga et al., 2005; Wouters et al., 2002).

5.2. The Importance of Fermentation

There are many reasons why fermented food is preferable to unfermented food, but some of them are discussed below.

5.2.1. Flavor Enhancement

Aroma and flavor are enhanced by fermentation. These organoleptic characteristics increase consumer acceptance of fermented food over unfermented food (Blandino et al., 2003). The specific mechanism of flavor generation is still under investigation. Moreover, the same desired results are not shown following the addition of citric acid to lemon juice to emulate the lower pH of fermented products (Leroy & De Vuyst, 2004).

5.2.2. Nutritional Quality

Cereal foods are of low nutritional quality. However, fermentation with the help of LAB increases the nutritional value of cereal foods (Nout, 2009). Fermentation also increases the amount of vitamin C (Santos et al., 2008; Slingerland et al., 2021). In the past, the nutritional merit of food also increased with the help of nutrient supplementation.

5.2.3. Preservative Properties

LAB is employed as a preservative in various fermented foods, including cereals. The growth of disease-causing microbes is inhibited by decreasing the pH to 4. These microorganisms cause the spoilage of food, which leads to food poisoning and disease (Ananou et al., 2007). For instance, antifungal activities are provided by LAB bacteria (Huwig et al., 2001; Lind et al., 2005; Magnusson et al., 2003; Schnürer & Magnusson, 2005). This act enhances the shelf life of fermented food.

5.2. Research Priorities and Emerging Technologies in Microbial Fermentation

Beyond conventional uses, microbial fermentation is still developing, with an increasing emphasis on enhancing safety, functional outputs, and efficiency (Graham & Ledesma-Amaro, 2023). Optimizing fermentation parameters, integrating new fermentation substrates including food and agricultural waste, and improving strains through metabolic and genetic engineering are current research objectives (Yadav et al., 2024). In order to improve synergistic effects during fermentation processes, researchers are increasingly giving priority to the investigation of microbial consortia.

Comprehensive knowledge of microbial communities and their metabolic pathways has been made possible by developments in omics technologies, including metagenomics, transcriptomics, and metabolomics (Ferrocino et al., 2023). These insights enable the selection and creation of strains with desirable characteristics, including improved nutritional absorption, superior fragrance profiles, and bioactive component synthesis (Sukaew, 2024). The key technologies of different microbial fermentation to enhance the pertinence of the research include artificial intelligence (AI) and machine learning, CRISPR-Cas9 and synthetic biology tools, encapsulation and immobilization, bioreactor engineering, and starter culture optimization. In summary, microbial fermentation is positioned as a key component in the creation of sustainable, health-promoting food systems by these new technologies and research goals.

6. Microorganisms' involvement in Fermented Food

Microorganisms are being used in fermented food for different purposes, which are discussed below.

6.1. Microorganisms used in the Flavoring of Food

Flavoring is important for fermented food products. Microorganisms primarily utilize amino acid metabolism, carbohydrate metabolism, fatty acid metabolism, nucleotide metabolism, and other metabolic pathways to accumulate flavor substances in fermented food (Han et al., 2020). Bacillus subtilis secretes an alkaline protease. Acid protease synthesized by Rhizopus, Aspergillus oryzae, Mucor, and other molds has high activity (Han et al., 2020). Lactobacillus, Pediococcus, and Streptococcus are lactic acid bacteria (LAB) that are involved in the flavoring of food by secreting lactic acid, acetic acid, ethanol, 2,3-butanediol, and acetone to the food (Hu et al., 2022).

6.2. Microorganisms Used in the Aroming of Food

Various microorganisms are used to enhance the aroma of food in different ways, like Aspergillus niger, Lentinus edodes, and Neurospora crassa, which release an enzyme called b-glucosidase (Mariam Muradova et al., 2023). Penicillium simplicissimum, Penicillium citrinum, and Penicillium candidum produce lipase and are used in aroma production (El-Shall et al., 2022).

6.3. Microorganisms Involved in Improving Nutritional Value and Decreasing Antinutritional Factors

In the different current scientific literature, the various studied antinutritional factors (ANFs) interfering with protein and mineral availability are phytic acid, phenolic compounds, particularly condensed tannins, and protease inhibitors (Kårlund et al., 2020). Lactobacillus plantarum strains are usually isolated from fermented food materials, and a lot of them release tannase enzymes, and these enzymes can degrade tannin compounds. The degradation of tannin compounds releases energy and reduces toxicity (de Las Rivas et al., 2019; Jiménez et al., 2014).

7. Applications of Microbial Enzymes in the Food Industry

The stability of microbial enzymes is much greater when compared to the enzymes of plants and animals. This stability is the major reason for the use of microorganisms in the food industry (Gurung et al., 2013). Table 4 lists some of the most significant microbial enzymes utilized in the food business, along with their uses.

Table 4. Some important microbial enzymes with their potential use in the food sector

Microbial Enzyme	Applications	References
Glucose Oxidase	Enhancement in the food's shelf life	(Hanft & Koehler, 2006; Zhu et al., 2006)
Lipase	Advancement in the taste of cheese	(Aravindan et al., 2007; Jooyandeh et al., 2009)
	Synthesis of American cheese	(Aravindan et al., 2007)
Glucoamylase	Production of beer	(Blanco et al., 2014)
	Enhancement in the quality of bread	(James & Simpson, 1996)
Cellulase	As feed of animals	(Sukumaran et al., 2005)
	Resolution of juice obtained from fruits	(Raveendran et al., 2018)
Peroxidase	Enhancement in the taste, chroma, and nutritional merit of food	(Regalado et al., 2004)

8. Future Trends and Research Directions

Microorganisms modify the food in such a way that consumers are attracted to it and make it palatable for them. Fermentation is the oldest but most effective way to use microorganisms in the food industry to increase the taste, quality, and nutritional value of foodstuffs. Microorganisms are used by every food industry for different purposes. In other words, microorganisms are very effective and have a crucial role in the food industry. Moreover, further research work (keeping the use of artificial intelligence or machine learning in perspective) is desired to explore more about the potential usage of microbes in the food industry.

There is a dire need to spread awareness about the usage of fermented food as well as the safety of food among people. However, fermented foods are generally very healthy, non-toxic, and safe to eat, as the presence of antimicrobial factors is involved, but the hygiene of people and the environment will determine the product's quality. Much importance is given to safety. Technology needs improvement to enhance the nutritional value and potential of food safety through advanced research. The challenge is that technology should ensure that the food's shelf life, the flavor of the food, and the packaging and labeling appeal to consumers. LAB probiotic organisms are not preserved by old ferments, as old ferments are not efficient for preservation.

The transformation of microbial biomass into food is one of the most challenging tasks. Besides that, nutritional value has enhanced taste and flavor and is competitive in terms of cost with animal-derived protein (milk, cheese, meat, and eggs). Many microorganisms, particularly fungal, yeast, and algal clades, contain cell walls that are thick in nature. This is a major contributor to fiber in the diet in many cases. Although some single-cell proteins (SCP) have thick cell walls, this can reduce the amount of nutrients that can be taken up and can itself be indigestible. So, it may be necessary to treat the SCP using heat and/or enzymatic processes to enhance nutrient bioavailability.

9. Conclusion

This article concludes that if there are progressive revolutions and microbial foods are composed with sustainability and ethics in mind, they have the potential to revolutionize present food systems. Probiotics play a very important role in our food as well as in our therapeutic revolutions, as infectious diseases are controlled by probiotics. This revolution in food microbiology could be a key to composing proof strategies in the future to face the health and environmental challenges of the future.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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