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Review

Use of Characterized Microorganisms in Fermentation of Non-Dairy-Based Substrates to Produce Probiotic Food for Gut-Health and Nutrition

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Abstract: Most fermented foods are dairy-based products; however, foods prepared using non-dairy-based materials such as grains, cereals, vegetables, and fruits can meet the dietary requirements of consumers following different food practices, including vegans and consumers that have dietary issues with dairy-based products. Traditional food fermentations have been conducted by the functioning of bacterial and yeast cultures using the inoculum of uncharacterized microorganisms isolated from naturally fermenting foods. However, pure viable strains of microorganisms characterized as probiotic cultures have the potential for their application in the fermentation process. Such fermented foods can be labeled as probiotic products, displaying the names of strains and their viable number contained in the portion size of that specific product. The significance of the development of probiotic functional food is that they can be used as a source of nutrition; in addition, their consumption helps in the recovery of healthy gut microbiota. In a fermented food, two components—the fermented substrate and the microorganism(s)—are in a synergistic relationship and contribute to healthy gut microbiota. The intake of probiotic foods for sustainability of a healthy gut can manipulate the functioning of gut–brain axis. The aim of this article is to present a review of published research conducted with specific strains characterized as probiotics, which have been studied to perform the fermentation growing on the matrices of non-dairy-based substrates.

Keywords: fermentation; cereal; vegetables; prebiotics; nutrition; gut; health; probiotics; synbiotics; microbiota

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1. Introduction

The subject of fermented foods is an essential part of the food industry and a traditional part of nutrition and dietary practices in many cultures such as in South-East Asian, Far-East, and African countries. Microbiological knowledge through recent research on fermented food is contributing significantly to innovations in traditional food customs. At the same time, it is recognizing new opportunities for innovation and development of consumer-friendly products to satisfy the broader necessities of customers for the purpose of nutrition and gut health. The food industry is one of the largest manufacturing sectors in several countries, it contributes to the local economy in terms of the provision of value-added products and the workforce associated with the food industry. In the last few decades, food exports have doubled within European countries, reaching over EUR 90 billion and contributing to a positive balance of almost EUR 30 billion [1].

Food items labeled as probiotics and synbiotics are in high demand by consumers with some health issues, which makes such items the most popular functional foods. The constituents of fermented food, including the substrate and fermenting cultures, influence the growth of individual microbial strains in the gastrointestinal tract. Therefore, it is sensible to consider the nutrients and bioactive components in fermented foods, which could be the regulators and main contributing factors affecting the composition and health of gut microbiota. The food products prepared in a controlled fermentation system with the activity of specific probiotic strains contain whole microbial cells and their metabolites; therefore, these can be considered as a natural formulation of synbiotic preparation containing the substrates and all biotics including pre-, pro-, post-, and parabiotics. There have been several studies on gut microbiota composition and its effect on health [2]. Research has confirmed that gut microbiota contributes to our general well-being significantly. Studies include the contribution of probiotics and prebiotics mainly due to their combination of health advantages in various systemic disorders by the maintenance of gut health [3].

Health Benefits from the Intake of Probiotic Foods

Beneficial gut microbiota (probiotics) in the host's gut system selectively utilize the prebiotics and oligosaccharide-based fibers as substrates, developing and sustaining their population [4]. Thus, prebiotics enhance the growth and colonization of a large number of beneficial gut bacteria. Such resident gut microbiota also act toward the exclusion of various infective microorganisms, provide health benefits such as immune-modulatory properties, and enhance the integrity of the gut barrier [5]. Research on fermented foods is focused on several projects to study their impact on the alleviation of gastrointestinal infections, food allergies, type 2 diabetes, cardiovascular disease, and neurological disorders, as well as the impact of consumption of fermented food on immunity, personalized nutrition, and overall health [6]. The potential for consumption of functional foods has also been explored to help in the remediation of certain psychological issues. Studies have reported that alteration of the composition of the gut microbiota through the intake of bioactive components including prebiotics, postbiotics, and parabiotics in fermented foods can affect the intelligence, mood, autism, behavior, and psychology of its host through the gut–brain axis [7,8].

Studies have produced the interesting outcome that the gut microbiota can be targeted and manipulated by suitable dietary means [9–11]. Research findings have confirmed that the unhealthy gut microbiome, disturbed due to several reasons, can be improved by the intake of appropriate functional foods or probiotic supplements [12,13]. Foods prepared with the use of probiotic cultures are generally considered safe. Such cultures have been widely used in food fermentation. The probiotic strains are easily available from standard culture collections. The microbial strains that are widely used in the food fermentation industry are mostly lactic acid bacteria (LAB) [14]. Their characteristics include the competitive ability to create a low pH due to lactic acid production and other primary and secondary metabolites. Their metabolites and extracellular polysaccharides can play a role in the competition of LAB with other microorganisms during food fermentation [15].

Most food fermentations utilize seasonal raw materials available from local agriculture practices. Although some fermented products are used as food accompaniment as the side dish providing delicacies to main meals, several preparations also provide sources of nutrition. Current practices of fermented food are through designed fermentations to produce products as natural synbiotics, rather than just a dietary supplement prepared by combining separate sources of prebiotic substrates and freeze-dried cells of probiotic strains. The additional benefit of food fermentation lies in the provision of a fermented product where both the components are in a synergistic relationship, which is an important factor for the sustainability of probiotic cultures contributing to healthy gut microbiota [3,15]. The following sections present published information on specific microbial strains characterized as probiotic cultures, which have been studied in the fermentation process growing on the matrices of non-dairy substrates.

2. Microbiological Status of Fermented vs. Probiotic Food

Fermented foods are very popular and consumed on a regular basis in several societies and cultures, where such products are prepared using family recipes and ingredients available in that geographical region. Several food products have been prepared using a natural fermentation process initiated and controlled by lactic acid bacteria. Such processes have been conducted without needing information on the contributing microorganisms, as long as the fermented food was produced with desired familiar flavors and appearance of a particular product.

All fermented foods cannot be termed as functional food, as there is an obvious microbiological difference between probiotic food and fermented food [7]. Some fermented foods might contain live microbes at the time of consumption; however, they may not fit the specific definition of probiotics, which should only contain characterized strains of organisms and must contribute to clinically tested health benefits. Some fermented food products may not even contain live cultures because the operation factors during production steps and downstream processing might have inactivated live probiotic cultures [7,8]. If the fermentation for the preparation of products has not been performed using standard pure strains characterized as probiotic cultures, the fermented foods will only be categorized as potential biotic food [6].

Even though the traditional foods prepared under uncontrolled fermentation make an addition to any diet, it is difficult to know the exact probiotic strains present in these products at the time of consumption; hence, such items cannot be labeled as probiotic foods. Therefore, it is important to know the identity of microorganisms for use as inoculum and their suitability for a specific food product before they are employed to perform any fermentation process. Strains used in fermentation mostly belong to bacteria, mainly *Lactobacillus*, *Bacillus*, and *Bifidobacterium*, while some strains of yeast *Saccharomyces* genera are also identified as probiotic cultures.

The expanding usage of microorganisms in food production requires the implementation of uniform standards for their categorization as probiotic strains. Therefore, a common agreement for the terminology must be followed between nutritionists, dieticians, food-manufacturers, and regulators. Before the employment of a specific microbial strain in the fermentation or formulation of a probiotic-labeled product, the selected strain should satisfy certain criteria. Since not all species of a particular genus could be probiotic in nature, each strain intended for use in the preparation process should be characterized as next-generation probiotics in comparison to traditional microorganisms, as discussed by Martin and Langella [3]. The strain(s) contained in the final product must remain in viable numbers after all stages of product manufacturing and downstream processing, transportation, and the shelving period. Other than that, the product must have demonstrated one proven beneficial effect on consumers' health.

Permitted Microorganisms for Probiotic-Labeled Functional Food

The European Food Safety Authority (EFSA) has maintained a list of microbial strains and particular species presumed to be safe for human consumption in foods under the certification of "Qualified Presumption of Safety" (QPS). The official definition of probiotics according to the Food and Agriculture Organization/World Health Organization is "Live microorganisms which when administered in adequate amounts confer a health benefit on the host", mainly through the process of replacing or including the beneficial strains of bacteria in the gastrointestinal tract. Probiotic microorganisms are included under the "Generally Recognized As Safe (GRAS)" category by the US Food and Drug Administration (FDA) [16,17].

Recently, three main classes of probiotics have been proposed: 1—'True Probiotic' (TP) refers to viable and active probiotic cells; 2—'Pseudo Probiotic' (PP) refers to viable and inactive cells, in the form of vegetative or spore (PPV or PPS); 3—'Postbiotic' (PP) refers to dead/nonviable cells, in the form of intact or ruptured (GPI or GPR). Each class is further classified into two groups based on their site of action: 'Internal' (in vivo) or 'External' (in vitro) [16,18].

The statement given by the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus panel reports that probiotic activities can only be produced by certain strains of a particular species of bacteria or yeast, for example, *Lactobacillus casei* or *Bifidobacterium bifidum* [19]. For the microbial strains to be considered as efficient probiotics for their use in food preparation, it is important that such products after consumption must have demonstrated their health benefit to consumers. The properties making probiotics eligible for their consumption through food or supplements include their role in immune system function and eliminating unwanted pathogenic microorganisms from GIT. Probiotic strains also help with the digestion of certain fibers, resulting in the production of health-enhancing fragments and short-chain fatty acids [20,21].

Based on positive research outcomes, several strains of lactic acid bacteria are accepted for use in the food industry to benefit from their valuable properties. These strains have been granted QPS status in the EU for food applications [22]. The most common probiotic bacteria used for food applications are from the Lactobacillaceae family or *Bifidobacterium* genus [23,24].

3. Need for Non-Dairy Probiotic Products

Probiotic cultures have been mainly isolated and studied in products of milk origin; therefore, the most widely used means for supplying probiotic microorganisms are through dairy-based fermented foods such as yogurt, fermented milk, kefir beverage, and cheese [10,11]. However, non-dairy-based foods are progressively being considered suitable carriers of probiotic strains because a section of the global population suffers from high levels of intolerance for lactose and have health problems with the consumption of dairy products. In addition, some consumers are increasingly selecting diets free from animal sources and dairy products. Thus, cereals, grains, and vegetables are suitable and economical non-dairy substances for the preparation of probiotic products, and as diet options for vegetarians and vegans.

Substrates/materials from plant origin also provide a beneficial environment that protects the viability of probiotic cultures from stress factors during the shelf-life of the product. The health problems and the dietary option of veganism have promoted a requirement for non-dairy foods, for example, probiotic-fermented cereals and vegetables, and non-dairy milk substitutes [25,26]. Hence, there has been an increasing demand for non-dairy products, which can meet the needs of people with dietary restrictions to dairy foods [27,28]. With proven benefits of health and food options for vegans, non-dairy-based products present them globally as suitable alternatives to milk-derived preparations; therefore, these have taken the advantage of their frequent acceptance in large-scale applications [29]. The global market for dairy alternatives is estimated to be valued at USD 27.3 billion in 2022 and is projected to reach USD 44.8 billion by 2027, recording a CAGR of 10.4% in terms of value [30].

4. Selection of Non-Dairy Substrates for Probiotic Foods

Products based on non-dairy substrates have been widely studied in several projects. The examples of specific raw materials used in the fermentation process can be divided into four groups. The fermented products as the source of nutrition also act as prebiotics, contributing to the fiber component in food products (Table 1).

Table 1. Variety of non-dairy substrates used in fermentation for probiotic food.

Group—1	Group—2	Group—3	Group—4
Cereals and Grains: Oats, Maize, Sorghum, Red Rice, Wheat, Rye, Pearl Millet, Glutinous Rice, Black Gram	*Vegetables*: Leafy vegetables Root-vegetables *Regional-Fruits*: Olives, Gherkins, Beet-root	*Beans, Nuts, Legumes*: Soya Beans Peanut Press Cake Locust Bean Soya Bean Curd	*Animal-sourced*: Pork Chicken Beef Fish

The first group of substrates used in food fermentation include cereals and grains as the main raw materials, which yield staple-food delicacies. The most commonly used materials are oats, maize, sorghum, red rice, wheat, rye, pearl millet, glutinous rice, and black gram [7].

The second group includes fresh agricultural materials such as vegetables, fruits, and leafy and root vegetables. Although it was envisaged that non-dairy-based materials could be problematic in fermentation for the introduction of the traditional lactic acid bacteria, which preferably grow in lactose media such as *Lactobacillus acidophilus* and *Bifidobacterium* probiotic bacteria. However, the probiotic strains *L. rhamnosus*, *L. casei*, and *L. plantarum* are found to be better adapted to the non-dairy matrices during fermentation. The most popular materials of cabbage, olives, and cucumbers are now used for popular fermented products such as sauerkraut, kimchi, pickled olives, and gherkins as potential biotics [6,7]. The use of vegetables in fermentation has been known since ancient times, and it is generally accepted that fermented vegetables can offer a suitable medium to propagate and deliver probiotics through their fibrous structures [31].

The substrates placed in the third group include beans, nuts, and legumes used in the fermentation. Some examples of popular substrates are soya beans, peanut press cake, locust bean, soya bean curd, etc. Additionally, the soya bean has received global attention due to its high protein content and its amino acid profile. Soya curd is suitable for the growth of lactic acid bacteria and Bifidobacteria. In soya-based probiotic products, fermentation by probiotics has the potential to (1) reduce the levels of some carbohydrates possibly responsible for gas production in the intestinal system; (2) increase the levels of free isoflavones, which have many beneficial effects on human health; and (3) favor desirable changes in bacterial populations in the gastrointestinal tract. Supplementing soymilk with prebiotics, such as fructo-oligosaccharides, mannitol, maltodextrin, and pectin, was found to be a suitable medium for the viability of probiotic bacteria. Some foods prepared by fermenting substrates placed in group four are derived from animals, such as pork, chicken, fish, etc.

The variety of abovementioned non-dairy materials have demonstrated potential as carriers for probiotic strains in the process of immobilization, by encapsulation or entrapment in the matrices of the substrate used in fermentation [32]. For nutritional and health-beneficial effects, cereals and grains have been widely used in fermented foods as a source of functional probiotic microorganisms [33]. Cereal-based fermented products contain health-benefiting probiotic microbes and fibers as potential prebiotics. The development of new functional foods that can combine the beneficial effects of prebiotic substrates and health-promoting probiotics is a challenging topic of research. Nevertheless, cereal-based substrates offer many possibilities; their fermentation requires lactic acid fermentation, often in co-fermentation with yeast culture [34].

The consumption of fermented probiotic food should have viable probiotic cells in the recommended population. A guideline of the WGO (World Gastroenterology Organisation) presented in 2017 suggested a concentration of viable probiotic cells of about 10^8 to 10^9 CFU/g of fermented food [35]; however, later reports in 2019 and 2020 recommended that foods with a lower concentration could also be effective in the provision of beneficial impact on gut health [36,37].

4.1. Probiotic Strains for Fermentation of Plant-Based Substrates

Table 2 summarizes some of the studies for non-dairy food products prepared using characterized strains of probiotic cultures fermenting grains, cereals, vegetables, fruits, etc.

Table 2. Probiotic strains * used in non-dairy food for vegans and lactose-intolerant vegetarians.

Microbial Strains * Recognized as Probiotics	Non-Dairy Functional Products	Reference
Bacillus coagulans BC4	Freeze-dried strawberries incorporated with probiotic strain	[24]
Lactobacillus paracasei KUKPS6201, *L. acidophilus KUKPS6107*, *L. reuteri KUKPS6103*, *L. rhamnosus KUKPS6007*, *L. salivarius KUKPS6202*, *Bacillus coagulans KPSTF02*, *Saccharomyces boulardii KUKPS600*	Formulated in probiotic-supplemented Thai-pigmented rice grains (cultivar Riceberry, Luem Pua and Black Jasmine), and rice bran oil	[38]
Lactobacillus plantarum TISTR 2075	Spray-dried fermented cereal extracts	[39]
Lactobacillus rhamnosus GR-1	Functional food with fermented rice, oats, and inulin	[40]
Two lactic acid bacteria *Lactobacillus plantarum* TK9, *Bifidobacterium animalis* subsp. *lactis* V9	A synbiotic food with whole oats	[41]
A commercial thermophilic starter culture FD-DVS YC-180 Yo-Flex®	Germinated brown rice	[42]
Diverse strains of Lactic acid bacteria	Slow sourdough fermentation	[43]
Saccharomyces cerevisiae and Lactic acid bacteria	Sourdough fermentation	[44]
Co-cultures *Bacillus coagulans* KPS-TF02, *Lacticaseibacillus rhamnosus* KPS-VE9	Novel probiotic products using Thai-pigmented rice (purple, red, and yellow color) as a carrier of strains	[45]
Lactobacillus casei	Synbiotic edible film based on cassava starch and inulin	[46]
Four probiotic strains (*Lactobacillus acidophilus*, *L. casei*, *L. rhamnosus*, and *Bifidobacterium bifidum*)	Edible films based on carboxymethyl cellulose with immobilized probiotic strains	[47]
Lactobacillus delbrueckii subsp. *bulgaricus* CIDCA 333, *Lactobacillus plantarum* CIDCA 83114	Edible methylcellulose films with two strains of lactobacilli for the development of functional foods	[48]
Lactic acid bacteria and yeast cultures	Sourdough starter, as a natural leavening agent	[49]
Mixed cultures *Kluyveromyces marxianus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, or *Lactobacillus helveticus*	Sourdough bread with enhanced aroma volatiles	[50]
Kluyveromyces marxianus, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus helveticus*	Sourdough bread with enhanced texture and digestibility	[51]
Kefir and *Lactobacillus casei* immobilized on brewery-spent grains	Sourdough wheat bread	[52]
Novel kefir grains as starter cultures	Baking products with probiotic strains	[53]
Lactobacillus paracasei subsp. *paracasei* E6, and *L. paraplantarum* B1, isolated from mature Melichloro cheese	Microencapsulated strains in biopolymer-based coacervate with enhanced cell viability for food products	[54]
Lactobacillus amylovorus TISTR1110	Glutinous rice probiotic product	[55]
Bifidobacterium longum BB536 (ATCC BAA-999), *B. bifidum* Bb-12, *Lacticaseibacillus rhamnosus* GG (ATCC 52103), Cryofast SST 31 (*Streptococcus thermophilus*), Lyofast SY 1 (*S. thermophilus* + *Lactobacillus delbrueckii* ssp. *bulgaricus*), YoFlex®YF-L02DA thermophilic LAB	Four formulations of germinated brown rice fermented products functionalized by probiotics, with enhanced γ-aminobutyric acid, oryzanol, and neutralized phytic acid.	[56]
Bacillus coagulans, *Lactobacillus acidophilus*	Non-dairy snacking product of probiotic-loaded banana leathers (sheets), using banana puree, polymer-digestible cassava starch, and non-digestible bacterial cellulose	[57]
Lactobacillus plantarum	Spray-dried probiotic Sohiong fruit powder	[58]
Lactobacillus salivarius spp. *salivarius* encapsulated	Probiotic culture incorporated into a fruit matrix	[59]
Bifidobacterium animalis Bb-12® or *Lactobacillus casei*-01.	Edible coatings and films with probiotic strain	[60]

* Although a newly revised nomenclature is available, the names of cultures given in this table are the same as those used in the relevant reference cited in each row of the table.

4.2. Probiotic Strains for Fermentation of Animal-Sourced Substrates

A variety of fermented food has been studied for their strains and their role in the sensory qualities prepared using animal-derived materials for non-vegan consumers with lactose intolerance. Table 3 summarizes some of the studies for non-dairy food products prepared using characterized strains of probiotic cultures fermenting animal-derived substrates such as pork, beef, poultry, etc.

Table 3. Probiotic strains * used for the preparation of non-dairy food for lactose-intolerant non-vegans.

Microbial Strains Recognized as Probiotics *	Non-Dairy Animal-Sourced Products	Reference
Lactobacillus acidophilus, *Bifidobacterium lactis*	Sausages—Italian salami style	[61]
Enterococcus faecium UAM1	Sausages with probiotic cells encapsulated in prebiotic apple flour, pectin gels	[62]
Lactobacillus acidophilus CCDM 476, *Bifidobacterium animalis* 241a	Fermented mutton sausage	[63]
Lactobacillus rhamnosus LOCK900	Sausages from pork meat	[64]
Bifidobacterium animalis subsp. *lactis* BB-12: DSM15954, *Lactobacillus rhamnosus* LOCK900: CP005484	Fermented dry-cured pork meat sausages	[65]
Lactobacillus casei/paracasei CTC1677, *L. casei/paracasei* CTC1678, *L. rhamnosus* CTC1679, *L. plantarum* 299v, *L. rhamnosus* GG, *L. casei* Shirota	Sausages from pork meat	[66]
Commercial probiotic strains: *Lactobacillus paracasei* BGP1, *L. rhamnosus* GG	Low-fat fermented sausage with added prebiotic fructo-oligosaccharides NutraFlora® P95	[67]
Lactobacillus lactis ssp. *lactis* strain 340, *L. lactis* ssp. *lactis* strain 16, *L. casei* ssp. *casei* strain 208, *Enterococcus faecium* UBEF-41	Salami fermented and matured at low temperature without adding preservatives nitrates and nitrites,	[68]
Lactobacillus plantarum TN8	Meat from minced beef	[69]
Lactobacillus sakei BAS0117	Fermented Pork sausages	[70]
Commercial probiotic strain: *Lactobacillus plantarum* Bioflora™	Dry-fermented sausage with added prebiotic chestnut flour	[71]
Lactobacillus acidophilus FERM P-15119, *L. rhamnosus* FERM P-15120, *L. paracasei* subsp. *paracasei* FERM P-15121 *L. sakei* (Chr. Hansen's)	Fermented pork sausages	[72]
Lactobacillus acidophilus CRL 1014	Chicken burger with okara flour prebiotic added	[73]
Lactobacillus plantarum 299v, *L. plantarum* DSM 9843, *L. rhamnosus* LbGG, or ATCC 53103, *L. casei* Shirota YIT 9029, *L. reuteri* DSM 17938, *L. casei* ATCC 393	Fermented salami made from beef	[74]
Lactobacillus rhamnosus R0011, *L. helveticus* R0052, *L. rhamnosus* Lr- 32, *L. paracasei* Lpc-37, *L. casei* Shirota, *L. reuteri* DSM17938, *L. reuteri* DSM17918, *Enterococcus faecium* MXVK29	Dry fermented sausage	[75]
Enterococcus faecium ATCC 8459 Strain resistant to curing salts	Cured sausages	[76]
Lactobacillus sakei	Fermented pork sausages	[77]

* Although a newly revised nomenclature is available, the names of cultures given in this table are the same as those used in the relevant reference cited in each row of the table.

4.3. Probiotic Strains in Traditional Non-Beverage Food

Traditional foods have been prepared using starter cultures from fermented products. Table 4 includes some non-dairy fermented products containing probiotic strains.

Table 4. Traditional foods with probiotic properties from fermentation of non-dairy substrates.

Non-Dairy Substrates	Fermented Food (Region)	Probiotic Strains Used for Fermentation	Reference
White or Red Sorghum, Tef, Wheat, Barley, Finger Millet, or Maize	Injera Pancake-type (Africa)	*Pullaria* sp., *Aspergillus* sp., *Penicillium* sp., *Rhodotorula* sp., *Hormodendrum* sp., *Candida* sp., *L. bulgaricus*	[78,79]
Maize	Kenkey Sourdough Dumpling (Ghana)	*L. fermentum*, *L. reuteri*	[78,80]
Wheat	Khambir Flat Bread (West Himalayas)	Yeast, mold, lactic acid bacteria, *Bifidobacterium* sp.	[81]
Sorghum	Kisra Pancake-type (Sudan)	*Lactobacillus cellobiosus*, *L. brevis*, *L. fermentum*, *L. amylovorus*, *Lactobacillus reuteri*, *Candida intermedia*, *Debaryomyces hansenii*, *S. cerevisiae*	[78]
Maize, Sorghum, Millet	Mawe—Dough (S. Africa, Togo)	Lactic acid bacteria	[78,79]
Maize	Mutwiwa—Porridge (Zimbabwe)	Lactic acid bacteria	[80]
Maize, Millet, Sorghum	Ogi, Ogi-Baba—Pudding (Nigeria, W. Africa)	*L. plantarum*	[78,80]
Fish	Plaa-som (Thailand)	LAB isolates as *Pediococcus pentosaceus*, *Lactobacillus alimentarius/farciminis*, *Weisella confusa*, *L. plantarum*, *Lactococcus garviae*	[82]
Vegetables—Cabbage, Radish, Cucumber	Kimchi (traditional Korean food)	*Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *L. sakei*, *Weissella koreensis*, *W. cibaria*	[83,84]
White Cabbage	Sauerkraut	*Lactobacillus plantarum* L4, *Leuconostoc mesenteroides* LMG 7954	[85,86]

5. Controls in Probiotics Growth on Matrices of Non-Dairy Substrates

The studies presented in the tables above show various non-dairy substrates with varying matrices, which have been used to grow live probiotic microorganisms in fermentation. The composition of each substrate and its structural matrix has unique properties and disadvantages; therefore, non-dairy materials may cause some technological barriers to the growth of those strains, which usually grow effectively on dairy substrates. Normally, most probiotic strains have been isolated from dairy-based naturally fermenting products such as sour milk, kefir, soft cheese, etc. Therefore, there is a possibility that some of these strains may not find viable conditions in non-dairy substrates other than milk and may not produce the desired results. The application of probiotic cultures in fermentation to manufacture non-dairy products needs experimentation in the selection of suitable strains for growth on specific substrates [87]. Therefore, the essential factors for the development of functional foods supporting gut-microbiota for vegans and lactose-intolerant non-vegans are the suitable substrate matrix and selection of the probiotic strain, and its co-culture(s) with the capability to ferment that specific substrate (Table 1). This research and development strategy is necessary to ensure the success of the production of functional food products [88–90], which are new and attractive, and also fulfill the dietary requirement of consumers.

6. Conclusions and Future Perspectives

The content of this article has been summarized in Figure 1, indicating the potential of probiotic strains for non-dairy-based probiotic products, providing dietary options for vegans, lactose-intolerant vegetarians, and non-vegans.

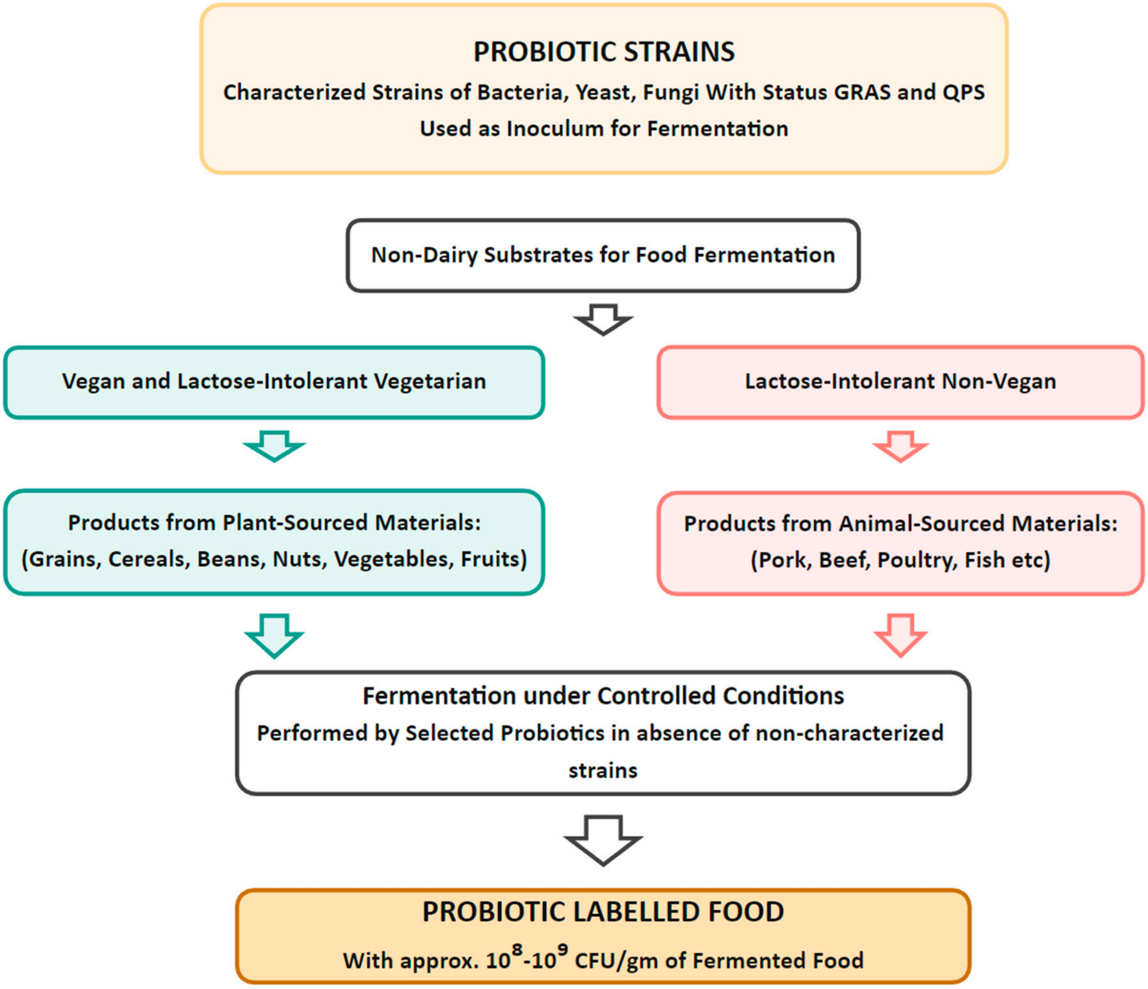

Figure 1. Probiotic strains for non-dairy-based fermented food for vegans and lactose-intolerant vegetarians or non-vegans. GRAS—generally regarded as safe; QPS—qualified presumption of safety. CFU/gm—colony forming unit or viable cells of probiotic strain present in each gram (weight of product).

Although Figure 1 states the selection of probiotic strains for fermentation of non-dairy substrates for the development of functional food, supporting gut-microbiota for vegans and lactose-intolerant non-vegans, the process will initially be started from a collection of GRAS/QPS strains. In addition, the specific food safety of the selected strain should be further evaluated following the criteria recognized by the scientific community and the WHO. Due to the benefits of the reduction in cost and time needed for complete sequencing of a bacterial genome, in the near future, genome sequencing of any new probiotic strain introduced on the food market should be mandatory. That would be useful in avoiding problems related to the possible transfer of antibiotic resistance genes or virulence factors in products.

In the last few decades, there has been a rise in the number of studies on the effect of probiotic food, beverages, and fermented foods as potential synbiotics, consisting of pre-, pro-, post-, and parabiotics on the gut microbiome [91–95]. The interest of research has moved towards clinical studies to understand how the gut microbiome can be manipulated for establishing and maintaining a healthy gut. The intake of food prepared with probiotic microbial strains is recommended through the outcome of several studies, where they have been reported to influence human health and show effectiveness in the relief of several diseases. The probiotic and synbiotic foods can be prepared using non-dairy substrates to meet the requirement of vegans and the population allergic to, or with low digestibility of dairy products. A variety of probiotic items have been developed and marketed, including fruit and vegetable juices, dried fruits, fermented vegetables, and desserts for vegetarians. However, studies that show the feasibility of incorporating probiotic bacteria in fruits and vegetables found their stability in these foods to be highly dependent on several factors. Plant-based substrates are potential carriers of probiotic strains; for this reason, the tissue matrices of fruits and vegetables provide strong adhesion of bacterial cells. An example of such a substrate is olive, which is used in large-scale industrial fermentation. Green olives have been studied as a source of plant-derived substrate for fermentation that started with the inoculation of probiotic strains such as *Lactobacillus pentosus* and *L. plantarum* [96–98]. Fermented olives are widely available as table olives in the market.

With the wealth of knowledge available on QPS probiotic strains, regionally available, low-cost, seasonal-agriculture-sourced materials can be explored for the preparation of probiotic and synbiotic food. The fermented foods prepared from non-dairy plant-based materials and the integration of viable numbers of probiotics in products at an industrial scale is not a smoothly regulated process. It involves a team of several workers with skills in dealing with microbiological, technological, and economical responsibilities.

There are prospects for further research on the design of appropriate technologies for the utilization of a variety of seasonal agricultural materials available regionally at a low cost. For the proper usage of selected bacterial or yeast probiotic strains in the manufacturing process, the parameters of temperature, osmotic, and oxygen levels must be regulated. Further studies are necessary on the survival, improved viability, and delivery of enhanced health benefits during their passage through the gastrointestinal tract. The colonization of probiotics on prebiotic oligosaccharides present in fermented food is important for their sustainability as a criteria for healthy gut microbiota. It is anticipated that the use of resources available from local agricultural practices for fermentation will be within adequate limits to remain competitive in the globalized market of expensive probiotic supplements.

Note that the names of probiotic cultures in tables are kept the same as those used in the publications cited for each item. However, a revised nomenclature has been reported by Zheng et al. [99].

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