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Recent Innovations in Non-dairy Prebiotics and Probiotics: Physiological Potential, Applications, and Characterization

Fasiha Fayyaz Khan[1] · Asma Sohail[1] · Shakira Ghazanfar[2] · Asif Ahmad[1] · Aayesha Riaz[3] · Kashif Sarfraz Abbasi[1] · Muhammad Sohail Ibrahim[1] · Mohammad Uzair[4] · Muhammad Arshad[4]

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Abstract
Non-dairy sources of prebiotics and probiotics impart various physiological functions in the prevention and management of chronic metabolic disorders, therefore nutraceuticals emerged as a potential industry. Extraction of prebiotics from non-dairy sources is economical and easily implemented. Waste products during food processing, including fruit peels and fruit skins, can be utilized as a promising source of prebiotics and considered "Generally Recognized As Safe" for human consumption. Prebiotics from non-dairy sources have a significant impact on gut microbiota and reduce the population of pathogenic bacteria. Similarly, next-generation probiotics could also be isolated from non-dairy sources. These sources have considerable potential and can give novel strains of probiotics, which can be the replacement for dairy sources. Such strains isolated from non-dairy sources have good probiotic properties and can be used as therapeutic. This review will elaborate on the potential non-dairy sources of prebiotics and probiotics, their characterization, and significant physiological potential.

Keywords Immunomodulatory effects · Synbiotics · Bacterial strains · Physiological functions · Polysaccharides · SCFAs

Introduction

Prebiotics are non-digestible food constituents (such as inulin, and fructooligosaccharides), which have potential effects on the host's health, particularly enhancing the growth of specific bacteria (*Bifidobacteria*) in the gastrointestinal tract (GI) [1]. Gibson and Roberfroid [1] used the term prebiotic for the first time. Carbohydrates, which are used as dietary fibers, are considered potential prebiotics. Prebiotics and dietary fibers are exchangeable terms used for those food components, which are not digested in the GI tract [2]. The prebiotics is grouped as disaccharides, oligosaccharides, and polysaccharides. Fructooligosaccharide products, including inulin, and oligofructose, are potent prebiotics. Moreover, glucooligosaccharides, xylooligosaccharides, glycol oligosaccharides, maltooligosaccharides, trans-galactooligosaccharides, stachyose, and raffinose also have the prebiotic potential [3]. Various in-vitro and in-vivo studies evidence that oligosaccharides are promising prebiotic options, which include galactooligosaccharides, trans-galactooligosaccharides, xylo-oligosaccharides, fructooligosaccharides, soybean oligosaccharides, and maltooligosaccharide [4]. Several fruits and vegetables contain an appropriate amount of oligofructose and inulin, usually, leeks, onion, wheat, garlic, and banana are the common sources of prebiotics [5]. Lee and Salminen [6] categorized prebiotics as only partially digested or undigested in the GI tract; small intestine not absorbed them; bacteria in the oral cavity poorly ferment them; they are well fermented by useful intestinal microflora, or potential bowel pathogens poorly ferment them [7–9].

Conventionally, yogurt and dairy-based products are considered valuable sources of probiotics. Though, nondairy

✉ Fasiha Fayyaz Khan
fasihafayyazk@gmail.com

1 Institute of Food and Nutritional Sciences (IFNS), Department of Food Technology, Pir Mehr Ali Shah (PMAS), Arid Agriculture University, Rawalpindi 46000, Pakistan

2 Functional Genomics and Bioinformatics, National Institute of Genomics and Agriculture Biotechnology (NIGAB), National Agriculture Research Centre, Park Road, Islamabad 45500, Pakistan

3 Faculty of Veterinary & Animal Sciences, Department of Parasitology & Microbiology, Pir Mehr Ali Shah (PMAS), Arid Agriculture University, Rawalpindi 46000, Pakistan

4 Department of Biological Sciences, Faculty of Basic & Applied Sciences, International Islamic University Islamabad, Islamabad 44000, Pakistan

foods are alternative sources of probiotics and have potential probiotic characteristics. Fruits and vegetables lack lactose, allergens, and cholesterol, which have an adverse effect on a dietary-restricted group of individuals [10]. Fruits and vegetables have phytochemicals and sugar contents, which support the growth of probiotics [11]. After fruit processing, about 70% of the weight consists of peels, and seeds remain as waste, but fruit peels have a high amount of fructooligosaccharides, pectin, and insoluble dietary fiber, other than proteins, vitamins, phenolic compounds, and minerals [12]. Plant waste materials have valuable compounds like disaccharides, oligosaccharides, and non-digestible oligosaccharides, which are currently categorized as prebiotics due to their indigestibility inside the colon and fermented mostly by lactic acid bacteria (LAB) and Bifidobacteria, therefore promoting health [1]. This review paper aims to highlight the potential non-dairy sources of prebiotics and probiotics that are natural, cheap, and generally recognized as safe. This review paper also highlights non-dairy-based foods, alternates of dairy food, including fruits, vegetables, cereals, etc. for both probiotics and prebiotics potential.

Need for Prebiotics and Probiotics

Dairy-probiotic products carry some potential health risks, including lactose intolerance, milk protein allergies, and high fat/cholesterol levels. However, lactose intolerance is the main health problem of dairy probiotics [13]. Lactose intolerance has associated with the inability to break down lactose into glucose and galactose due to low levels of the lactase enzyme. It is feasible to create probiotic food products without dairy, enabling those with intolerances or allergies to milk components to consume the healthy microbes [14]. Numerous alternative non-dairy-based probiotic foods have been developed to alleviate the drawbacks of dairy-based foods. Alternatives to dairy-based probiotic products are still being researched, and consumers may prefer non-dairy-based probiotic products, particularly those that use fruit and/or vegetable as a primary ingredient. Fruit and vegetable-based, cereal-based, and soy-based prebiotics and probiotics are becoming more popular among non-dairy source foods [15]. Fruits, vegetables, and grains are naturally occurring sources of prebiotics. They are not the only source of energy but also provide health and well-being to the consumers. They are helpful to reduce the occurrence and frequency of diarrhea and curing inflammation and other bowel disorders [16]. Prebiotics also promote the uptake and bioavailability of minerals, also lowering the risk of cardiovascular disease and controlling appetite and weight loss [17]. Prebiotics has a direct, indirect, or collective effect on the immune system, as well as increase the gut microbiota and lower the population of pathogens (Fig. 1). It is documented in different studies that mannose monomers reduce the adhesion of *Salmonella* in GIT [18]. Fructo, oligo, di, monosaccharides, and polyols (FODMAPs) can target the gut flora to treat intestinal bowel syndrome [19]. Animal model studies have proven that inulin-type fructans help in modulating carbohydrates and lipids. Oligofructose (OFS) has an anti-diabetic effect in both high fat and streptozotocin-treated mice [20].

Prebiotics Potential of Non-Dairy Products

Fruits and vegetables being a great source of vitamins, antioxidants, minerals, and bioactive with hydrating qualities and tasty flavors, simultaneously offer an excellent dairy replacement and a good option for the human population, especially those who are lactose intolerant, allergic to milk proteins, and strict vegetarians.

Fruits

Mango

Mango (*Mangifera indica*) juices and concentrates are industrial products of mango, enriched with phenols and dietary fibers. Approximately 50—55% of the fruit is wasted as by-products, including seeds, peels, and paste. Mangoes peels contain about 40% of dietary fibers and have prebiotic potential. Mango peels have the potential to enhance the growth of *Bifidobacterium* and *Lactobacillus*. Indigestible ingredients of mango peels have the potential to be used as prebiotics. Mango peels are used in Kefir and promote the threefold higher growth of LAB [21]. Mango's pulp has potential prebiotic effects by increasing the total bacterial counts, *Lactobacilli*, and *Bifidobacteria* counts in flavored probiotic yogurt [22]. Mango's peels are enriched with nutrients and compounds having nutraceutical value that exerts antimicrobial, antioxidant, anti-inflammatory, and prebiotic properties [23]. Probiotic growth can be enhanced by using mango selective cultivars having superior prebiotics and antioxidant properties [24]. Polyphenols are potentially beneficial and have effects on the gut microbes significantly enhancing the proliferation of the *Bifidobacterium* spp. In vitro studies also indicate that mangoes have prebiotic benefit. Evidence shows that polyphenols are utilized by gut microbes and produce metabolites [25, 26]. Further research would be promising to evaluate the health effects of mangoes or their products, which might have phenolic properties or effects on gut microbiota.

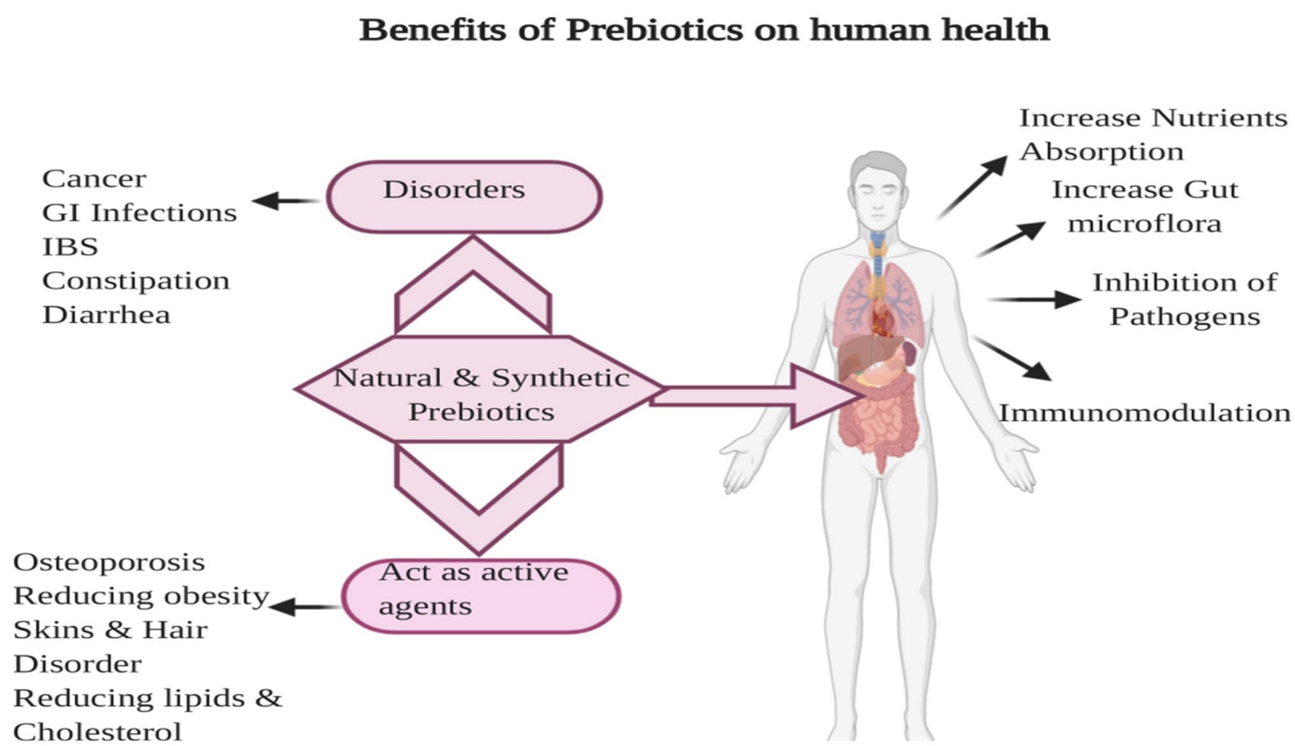

Fig. 1 Physiological benefits of prebiotics treating various disorders by acting as a bioactive component and regulating human health

Banana

Banana peels are also a good source of fructooligosaccharides (FOS) and generally recognized as safe (GRAS) status can be obtained from banana peels [26]. Banana is a source of prebiotic and oligosaccharides that promote the growth of *Lactobacillus paracasei* [27]. Green banana pulp, which contains resistant starch, has prebiotic potential. The synbiotic supplement prepared by green banana resistant starch and *Bacillus coagulans* spores exerts anti-inflammatory in mice models suffering from gut inflammation [28]. Banana has compounds categorized as prebiotic, for example, FOS and inulin [29]. Thus, the green banana pulp has shown prebiotic potential without affecting its physicochemical and sensorial properties [30].

Orange

Orange peel wastes produce pectin oligosaccharides (POS) through hydrothermal treatment, which promotes the growth of *Lactobacilli* and *Bifidobacteria* [31]. Prebiotic POS has emerged as a functional food ingredient, produced by hydrolyzing pectin-rich agricultural wastes to partially depolymerize them. Fruit wastes carry important components, mainly pectin that boosts the growth of Lactic acid bacteria (*Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum*), proving its prebiotic potential. Fruits peels of *Citrus lanatus*, *Citrus limetta*, and *Musa* spp. and rotten fruits of *Psidium guajava* and *Solanum lycopersicum are* used for extracting pectin [32]. POS significantly increases *Lactobacillus fermentum* compared to sugar, exerting great probiotic potential [33]. POS also exerts anti-microbial potential. Orange peels hydrolyzed using multi-enzyme complexes from *Aspergillus japonicas* also provide consistent POS release [34, 35]. Guava, passion fruit, and orange fruit's by-products promote bacterial growth during fermentation and enhance probiotic tolerance in simulated conditions of the GI tract. Such findings make the by-products of the fruits useful [36].

Dry Fruits and Nuts

Almonds and their skins also have prebiotic potential. Their intake increases the total fecal count of *Lactobacillus* spp. and *Bifidobacterium* spp. Almonds and their skins can also improve the gut microbiota population; therefore, exert considerable prebiotic potential [37]. The dietary fibers in Natural and blanched almond skins significantly promote Bifidobacteria and *Clostridium coccoides* growth [38], whereas raw and roasted almonds also increase *Lactobacillus acidophilus* and *Bifidobacterium breve*, decreasing *Enterococcus* spp. and *Escherichia coli* [39]. Similarly, walnuts also have prebiotic potential as it increases *Lactobacillus*, *Ruminococcaceae*, and *Roseburia*, while decreasing *Bacteroides*, *Anaerotruncus*, and *Alphaproteobacteria* [40]. A randomized controlled trial also shows that Butyrate-producing bacteria, *Ruminococcaceae* and *Bifidobacteria*, were increased, but *Clostridium* spp. decreased [41]. The cashew apple as an agro-industrial by-product has prebiotic properties and has shown a significant prebiotic impact on probiotic strains. It can be a value-added ingredient for the food industry [42]. Also, the chestnut extract has a substantial impact on lactobacilli's improvement in stomach tolerance [43]. The soluble crude polysaccharides from coconut kernel cake increase *Lactobacillus plantarum* and *Lactobacillus rhamnosus* CFU [44]. In addition, crude polysaccharides from defatted coconut residue exert positive effects on the growth of *Lactobacillus casei* Shirota and *Lactobacillus bulgaricus* [45].

Others

Different cultivars of the kiwifruit are used for their polyphenol and fiber contents to maintain microbial metabolism in the gut, confirming the prebiotic potential [46]. Strawberries have polyphenols, which have prebiotic activities that enhance the gut microbiota [47]. Strawberry supplements given into the diet of diabetic mice boost the amount of *Bifidobacterium* that plays a vital role in anthocyanin metabolism [48]. Ten percent Papaya pulp is used as prebiotics, while stevia leaves extract 1.0% used as a natural sweetener. The beverage is evaluated for sensory properties and viable count of probiotics during 10 days of cold storage. Such probiotic beverage, which is fortified with papaya pulp and stevia leaves extract, is healthy for consumption [49]. The peels of yellow watermelon, papaya, and honeydews significantly stimulate the growth of *Lactobacillus rhamnosus* and *Bifidobacterium bifidum* [50]. Thus, fruits and particularly their by-products exert considerable prebiotics and probiotics potential (Table 1).

Vegetables

Dairy products are inedible for several groups of people, including those who are lactose intolerant, allergic to milk proteins, and strict vegetarians. As a result, there is a need to investigate other non-dairy sources of prebiotics, such as vegetables, to provide consumers with an alternative to dairy products.

Gourd

Vegetables are a rich source of soluble and insoluble dietary fibers. Gourd family vegetables have prebiotic potential. Five gourd family vegetables *Trichosanthes anguina*, *Benincasa hispida*, *Momordica charantia*, and *Lagenaria siceraria* were blended with *Escherichia coli*, *Bifidobacterium breve*, *Lactobacillus fermentum*, and *Clostridium acetobutilicum* spp. During in vitro fermentation, all five gourd family vegetables produce short-chain fatty acid (SCFA). Among the five gourd vegetables, *B. hispida* produces the highest SCFA. Thus, gourd family vegetables have the prebiotic ability [51].

Leafy Green Vegetables

Leafy vegetables can also stimulate the growth of *Bifidobacteria* and lactobacilli in their pure culture. Such vegetables contain inulin, which is evident in their prebiotic potential [52]. Leafy vegetables are also known to promote the growth of *Bifidobacteria* and lactobacilli. They are grown in pure culture and supplemented with inulin. Such infusion might be tested as a potential prebiotic source using culture microorganisms or humans as host machinery [52].

Legumes

Many nations around the world rely heavily on legumes as a food source. Beans, chickpeas, peas, lentils, cowpeas, and soybeans are the most often consumed types. The overall composition of legumes is high in carbohydrates (30–60%), dietary fiber (9–25%), and protein (19–36%), which contains essential amino acids including lysine, leucine, and arginine. Bioactive chemicals found in legumes contribute significantly to human metabolism. Healthy components include dietary fiber, resistant starch, polyphenols, and phytosterols [53, 54]. One hundred grams of lentils (*Lens*

Table 1 Non-dairy sources of prebiotics

Prebiotics	Synbiotics with prebiotics and probiotics	Source	Reference
Inulin	Inulin + *Lactobacillus* genus bacteria	Agave, Artichoke Jerusalem, Banana, Chicory, Garlic, Onion & Wild yam	[75]
Fructooligosaccharides	Oligofructose + Bifidobacterium, *Lactobacillus* genus bacteria	Chicory, Onion, Artichoke, Banana, Garlic Asparagus	[76]
Xylooligosaccharides	FOS + Lactobacillus, Bifidobacterium genus bacteria and Streptococcus	Bamboo shoot, Vegetables, Fruits, Honey	[77]
Galactooligosaccharides	Oligosacchrides + Bifidobacterium, *Lactobacillus* genus bacteria	Human milk, Lentils Green pea, Kidney beans, Limabean, Chickpea/hummus	[78]

culinaris L.) typically contains 4071 mg of raffinose family oligosaccharides (RFOs), 1423 mg of sugar alcohol (SA), 62 mg of fructooligosaccharides (FOSs), and 7500 mg of resistant starch (RS), among other prebiotic carbohydrates [54, 55]. By encouraging the production of *Lactobacilli* and *Bifidobacteria* healthy for the intestines and preventing the growth of putrefactive *Enterobacteria* in the colon, these oligosaccharides and dietary fiber may have prebiotic effects. In vitro studies revealed that red kidney beans have prebiotic potential. Water extractable polysaccharides, obtained from red kidney beans, stimulated the growth of probiotic strains *Lactobacillus fermninetum* and *Lactobacillus plantarum*, inhibiting the formation of biofilm by *Escherichia coli*. Polysaccharides obtained from red kidney beans might be a novel addition to synbiotic product development [56]. By promoting the growth of the advantageous bacteria *Enterococcus*, *Ruminococcus*, *Blautia*, and *Bacteroides* and inhibiting the growth of the potentially pathogenic bacteria *Escherichia Shigella*, mung bean seed coat extract influenced the composition of the gut microbiota [57].

Sweet Potato

Orange fleshed sweet potato OFSP has prebiotic potential that promotes the growth of gut microbiota *Bifidobacterium* genus. It also triggers the production of short-chain fatty acids (SCFA), which is promising for human gut health [58]. White and purple peel sweet potato root flours promoted probiotics growth (*Lactobacillus acidophilus*, *L. casei*, and *Bifidobacterium animalis*) with enhanced metabolic activities (increased acetic, formic, lactic, malic, succinic, and tartaric acids and decreased fructose, glucose, and maltose) [59]. *Lactobacillus paracasei*, *Staphylococcus simulans*, and *Streptococcus thermophiles* survive and proliferate in potato peel supplemented media [60].

Carrot

Because carrots are simple to digest and have a low energy content per 100 g of edible components, they are very beneficial for newborns, elders, and those trying to lose weight. Carotenoids, a type of lipophilic antioxidant, and hydrophilic antioxidants are both abundant in carrots (phenolic compounds) [61]. A carrot-derived pectic polysaccharide, known as cRG-I, supplemented with rhamnogalacturonan-I (RG-I) significantly enhanced *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Bacteroides dorei*, *Bacteroides ovatus*, *Roseburia hominis*, *Faecalibacterium prausnitzii*, and *Eubacterium hallii* [62]. The cRG-I administered via SHIME® (Simulator of Human Intestinal Microbial Ecosystem) technology has shown a significant increase in *Bacteroides dorei* and *Prevotella* sp. [63]. Carrot juice, both straight and concentrated, fermented by *Lactobacillus gasseri* DSM 20,604 create a nutrient-rich beverage with fewer calories, increased carotenoid concentration, and prebiotic potential [64].

Cereals

Cereals are recognized as a novel group of prebiotics. Mostly dairy products, vegetables, and fruits have prebiotic potentials and are used as prebiotic carriers [65] (Table 1). Several different kinds of studies are in the process of finding and identifying new sources, which have prebiotic potential. Different cereals grains like wheat, corn, barley, oat, and rice can be used to develop prebiotics [66]. Grains are a promising source of potential prebiotics because cereals are enriched with resistant starch, soluble and insoluble fibers, Fructooligosaccharide (FOS), and galactooligosaccharides [67]. De-lignified wheat bran (DWB) has prebiotic properties and is used in fermented pomegranate juice. *Lactobacillus plantarum* ATCC 14917 was used as a probiotic strain. DWB gives the protection of probiotic cells. Synbiotic pomegranate juice provided a good sensory evaluation of functional pomegranate juice. DWB is also used as a synbiotic with *Lactobacillus paracasei*. DWB also exerts prebiotic potential by enhancing the viability of *Lactobacillus paracasei* [68, 69]. Hemicelluloses have important constituents such as arabinoxylans. They are found in endosperm and outer covering of cereals grains, including wheat, corn, rye, oat, rice, and barley. The enzymatic hydrolysis of arabinoxylans yields arabinoxylan oligosaccharides that have prebiotic potentials, antioxidant activities, and immunomodulatory properties [70]. Arabinogalactan peptide (AGP) found in wheat has shown prebiotic potential. In vitro study has shown that AGP has a potential prebiotic effect on *Eubacterium* and *Bifidobacterium* genera. It also increased the concentration of short-chain fatty acids (SCFAs) [71]. Immature wheat grains (IWG) can have an impact on probiotic strains that might be used to enhance the growth and survival of probiotic strains *Lactobacillus acidophilus* 20079 (L20079) and *Lactobacillus acidophilus* NCFM (LNCFM) in yogurt [72]. Non-polysaccharides such as β glucan and arabinoxylan (AX) are present in cereal grains. Arabinoxylan (AX) is found in rye and wheat, whereas β glucan is present in oat and barley. In vitro studies on β-glucan and arabinoxylan have shown prebiotic potential. Arabinoxylan (AX) exerts bifidogenic activity and boosts the concentration of SCFAs [71]. Fiber-based oat flour used in low oil salad emulsion supports the growth of *Lactobacillus paracasei* subsp. *Paracasei* DC 412. The oat flour enhances the viability of probiotic strain. Viable cell counts are 10^8 CFU/g under simulated gastrointestinal tract at 7.5 weeks of refrigeration storage [73]. Hard and soft wheat contains water-soluble extractable arabinoxylans (WE-AX). WE-AX has prebiotic potential. AX extracted from hard wheat have more prebiotic efficiency than those from soft wheat. It suppresses the growth of intestinal pathogens and promotes the production of SCFAs in the intestines [74].

Miscellaneous Sources

Flaxseeds (*Linum usitatissimum* L.) and their oil are considered a source of prebiotics, as it has antimicrobial activities but also stimulate LAB growth. Crude oil of flaxseed is used in in vitro study with *Lactobacillus plantarum* KCTC3105, *Lactobacillus brevis* KCT3102, and *Lactobacillus kefiranofaciens* DN1; *Lactobacillus bulgaricus* KCTC3635 shows a significant boost in the growth of these lactic acid bacteria [79]. Flaxseed comprises phenolic antioxidants and fiber gives it prebiotic potential. Synbiotic product with dark chocolate and flaxseed is fabricated with *Leuconostoc mesenteroides* and used as probiotics. Synbiotic dark chocolate gives numerous gut health effects as well as exceptional nutritional value [80]. The synbiotic yogurts are produced by starter cultures of probiotic strains. Synbiotic yogurt made with flaxseed and *Lactobacillus acidophilus* enhances the growth of *Lactobacillus acidophilus* [81]. Olive oil is enriched with unsaturated fatty acids and has a bifidogenic effect on gut microbiota to elevate the levels of *Bifidobacterium* [82].

Spices also have well-documented prebiotic potential. Seven spices investigated for their prebiotic potential includes cinnamon (Venturella), cayenne pepper (CAP), black pepper (BLP), ginger (GIN), turmeric (TUR), rosemary (ROS), and Mediterranean oregano (ORE). Cayenne pepper, black pepper, ginger, and oregano have prebiotic potential as well as antimicrobial effects and promote the intestinal microbiota and eliminate the pathogenic bacteria [83]. Consumption of mixed spices also has a strong influence on gut microbiota and is considered as prebiotic doses [84]. Turmeric (*Curcuma longa*) is an herbaceous medicinal plant, which has an anti-inflammatory effect. Turmeric extract has shown exceptional resistance again simulated gastrointestinal environment compared to control prebiotic inulin. It also enhances the growth of *Bifidobacterium animalis* BB12 and *Lactobacillus rhamnosus* GG (LGG). Ghiamati Yazdi and Soleimanian-Zad [85] first time reported that turmeric extract has prebiotic potential. Black pepper (*Piper nigrum*), Tulsi (*Ocimum sanctum*), and Ginger (*Zingiber officinale*) have prebiotic potential. Tulsi and ginger have high prebiotic activity as FOS with *Bifidobacterium* and *Lactobacillus*. Black pepper has similar prebiotic activity as FOS. Therefore, these herbs can be used to promote gut bacteria growth [86]. Turmeric and garlic have prebiotic properties by reducing the population of the intestinal pathogen and stabilizing the gut microbiota [87]. Dandelion roots (*Taraxacum officinale*) and Elecampane (*Inula helenium*) contain fructans. Dandelion roots 34% and Elecampane 44% fructans herbs have the prebiotic potential [88]. Cress seed gum (CSG) and coriander extract (CE) affect *Lactobacillus plantarum* LS5 growth and viability in yogurt drinks (Doogh). CE at 0.05% and CSG at 0.5% level give the highest probiotic count [89]. Fenugreek seeds have prebiotic potential as contain galactomannan. It is the first scientifically documented proof that galactomannan extracted from fenugreek seeds has prebiotic potential. It can play a vital role in the growth of gut microbiota and serve as a substrate for probiotic bacteria such as *Bacillus coagulans* MTCC [90]. Haghshenas [91] incorporated alginate-psyllium biopolymers with prebiotic fenugreek and inulin and the probiotic *Enterococcus durans* was microencapsulated. The study has shown that herbs fenugreek and psyllium might be used with alginate, as they exhibit prebiotic properties, and growth enhancers of probiotic bacteria in the harsh gastrointestinal environment [91].

Olive tree pruning biomass (OTPB) yields oligosaccharides by autohydrolysis that have prebiotic potential. Two fractions were obtained and used as in vitro fermentation of *Bifidobacterium longum* and *Bifidobacterium adolescentis*, increasing *Bifidobacteria* [92]. During the olive oil extraction process, olive mill wastewater is obtained, which is a good source of polysaccharides. It has prebiotic potential, and antioxidant properties, shows resistance under a simulated human GI environment, and also has the capacity to ferment by *Lactobacilli* strains [93]. Aloe vera is also used as a prebiotic source. Aloe vera is enriching with antibiotics activities. Aloe vera fructans can significantly enhance microbial growth compared to inulin [94]. Persian Gum has prebiotic effects and is used as encapsulation material with alginate. Probiotic strain *Lactococcus lactis* is encapsulated, and it increases the survival rate in the gastrointestinal environment, as observed by Nami et al. [95]. Recent research is focusing on introducing prebiotic chocolates, which served as functional foods. Various in vivo and in vitro studies were conducted to evaluate its pre and probiotic characteristics as in synbiotic chocolates [96]. Recent research documented that dark coffee beans roasted, brewed, and spent coffee grounds have a wide variety of oligosaccharides, which can be utilized as prebiotics [97]. Seaweed (*Gelidium sesquipedale*) and brown seaweed (*Ecklonia radiate*, *Osmundea pinnatifida*) are excellent prebiotic sources because they enhance gut microflora [98]. Polysaccharides and proteoglycans are found in mushrooms, which indicate prebiotic properties in mushrooms [99]. They can also induce antitumor and immunomodulation factors [100].

Physiological Potential of Probiotics

The outcomes of clinical trials are evident that probiotics have potential health benefits in humans (Table 2). In the modern era, probiotic captures public attention as playing a key role in promoting gut health and the supplement of

Table 2 Probiotic microorganisms and their impact on human health

Disease	Strains	Health impact	References
Cancer	*Lactobacillus casei Shirota*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Bifidobacterium* sp., and Propionibacterium spp.	Detoxifies the ingested carcinogens	[109]
Antibiotic therapy	*Lactobacillus plantram*, *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Lactobacillus brevis*, *Lactobacillus johnsonii*, and *Enterococcus mundtii*	Reduce the effect of antibiotics on ordinary bacterial flora	[110]
Inflammatory bowel disease (IBD)	*Lactobacillus plantram*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subsp. Bulgaricus, *Bifidobacterium breve*, *Bifidobacterium longum*, and *Streptococcus salivarius* subsp. thermophiles	Reduce Inflammatory disease	[111]
Diarrhea	*Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum*	Adhere to the population of pathogenic bacteria on epithelial cells	[112]
Urinary tract infection	*Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, and *Lactobacillus reuteri*	Reduce urinary tract diseases	[112]
Lactose intolerance	*Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus delrueckii*, and *Bifidobacterium lactis*	Break down of lactose	[112]

prebiotics and probiotics known as synbiotics [101]. Ingestion of probiotics has been recommended to enhance general health and the body's immune system [102]. All probiotic strains have specific genomic and microbiological properties, which influence human gut health [103]. The significant interest of customers in non-dairy probiotics is induced due to the vegetarian trend and lactose intolerance. The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the USA National Institutes of Health claimed that about 75% of the world population suffers from lactose intolerance. The milk allergens make dairy base probiotics unfit for consumers who have specific health issues. Probiotics obtained from vegetables and fruits may be considered more suitable as free from allergens, cholesterol, and disaccharide [104]. Though, all food products that contain potential probiotic strains should meet the established guidelines by FAO/WHO. Those guidelines are for commercial products as supplemented with probiotics strains: it should be safe from screening to isolation, correct identification, and have antimicrobial susceptibility; it should be stable in GI environment and keep intestinal epithelial adhesion characteristics; should be producing lactic acid and inhibitory potential against pathogens; should have a property to lower down cholesterol levels and boost up the immunity; and it should have anticarcinogenic and antimutagenic properties [105]. LAB probiotics exhibit anti-inflammatory, antimicrobial, anti-osteoporotic activities, and lactose intolerance. LAB can also weight, total cholesterol levels, and serum glucose [13, 106, 107]. The efficacy of probiotics depends on in vitro methods and under control conditions [108].

Probiotics microorganisms produce a wide range of inhibitory substances such as bacteriocins, peroxide, and organic acids. These compounds are helpful to inhibit the undesirable microbial population in the GI tract. Probiotics can provide immunity to encounter enteric diseases [113, 114]. LABs trigger the anticancer immune pathway (Fig. 2). LAB induce the production of different kind of immunity cells. Probiotics and pathogenic bacteria were grown in growing broth with supplementing carbon to promote the probiotics bacteria, indicating strong prebiotic potential and can be used as alternative commercial prebiotics [115].

Selection and Characterization of Probiotics

Isolation of potential probiotic strains has always been a keen interest for food technologists as well as pharmacists. Diverse sources for the isolation of LAB are taking the attention of researchers. LAB are widely distributed non-spore-forming rods or cocci, catalase-negative, anaerobic, gram-positive microbes that produce lactic acid by carbohydrate fermentation with other bioactive compounds, including organic acid and bacteriocins. Probiotics contribute to raised antioxidant, bioactive proteins, and exopolysaccharides levels, modulating oxidative stress and protecting cells from oxidative stress [116]. LAB cannot be only isolated from animal fecal and gut, mother's milkfishes, shrimps, milk, and milk products, but also from non-dairy sources, including, fruits, vegetables, oil-industrial residues, pickles, fermented meat products, and cereal (Fig. 3). An effective probiotic bacterium must possess features, such as survival in GIT low pH, adhesion ability with intestinal epithelial cells, antibiotic resistance, and antimicrobial activity. It is advisable to consume 10^6 to 10^7 CFU/mL of probiotics to get the targeted health benefits [117]. There are various probiotic properties that a LAB strain should pass through during the screening process, including simulated gastric environment (acid and bile tolerance), antibiotics, antimicrobial, antibacterial, auto-aggregation, and co-aggregation, surface hydrophobicity, and safety evaluation [118].

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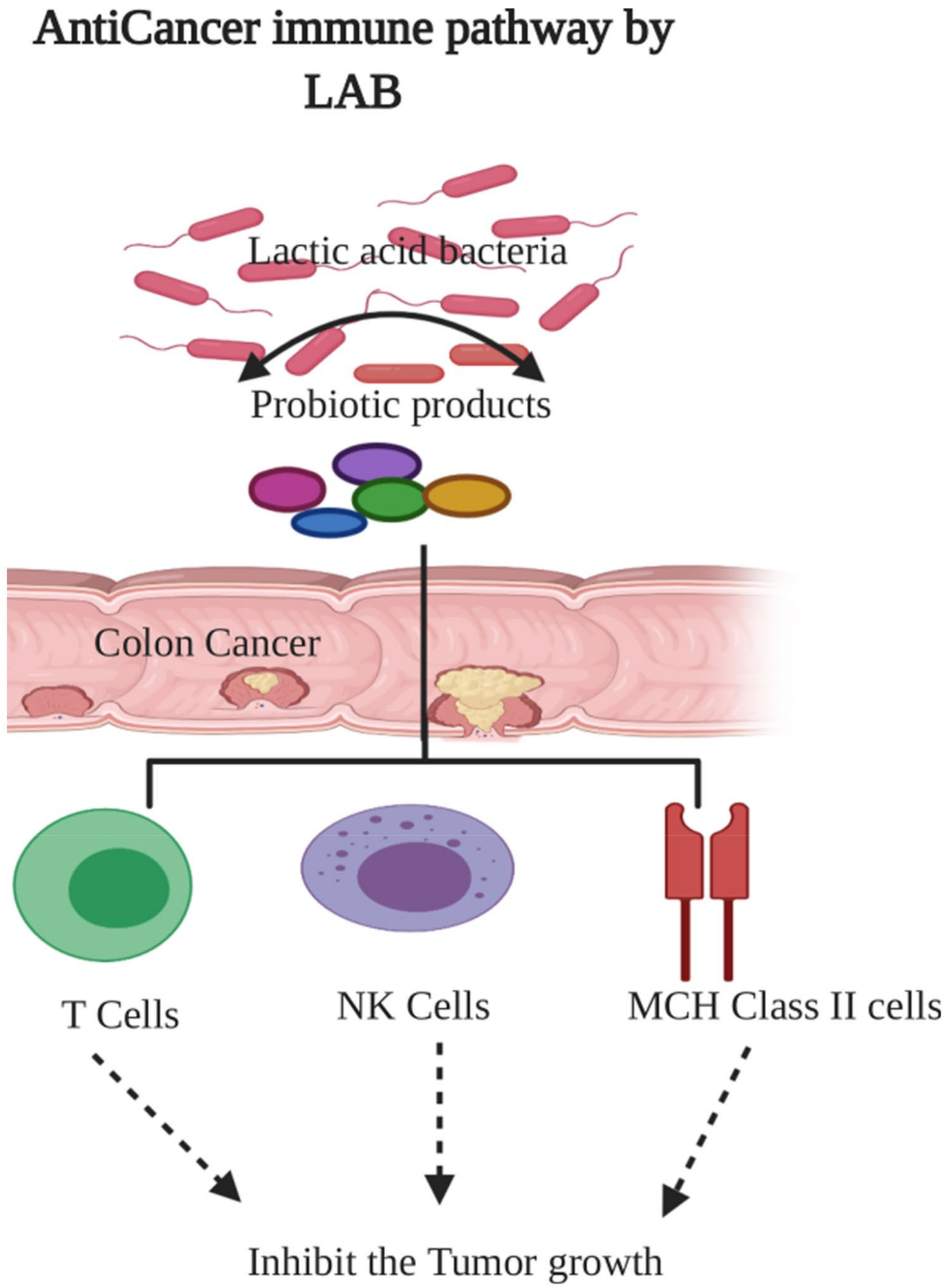

Fig. 2 Molecular role of probiotics in anti-cancer Immune pathway presenting immune cells and inhibiting the colon cancer cell growth

Molecular Characterization of Probiotics

Probiotics identification has a significant role in food-related industries, which cannot be ignored. Therefore, accurate taxonomic identification is essentially required for the precise identification of new probiotic candidate strains, also metabolic features, and genomic information. The typical phenotypic assays do not provide sufficient species-level identification of probiotic LAB or *Bifidobacterium* strains. Phenotypic characterizations are considered old-fashioned and may have many challenges, such as they can produce ambiguity in the results toward misidentifying a target and then limiting their applications. Therefore, species molecular techniques, such as pulsed-field gel electrophoresis and randomly amplified polymorphic DNA analysis, are most effective for strain differentiation. A variety of other molecular methods, either based on fingerprinting or on sequencing, has also been used for the identification of probiotic *Bifidobacteria* and LAB. These methods were prompted by the shortcomings of phenotypic approaches and the growing significance of molecular tools in the field of bacterial systematics. Molecular biology has contributed to our

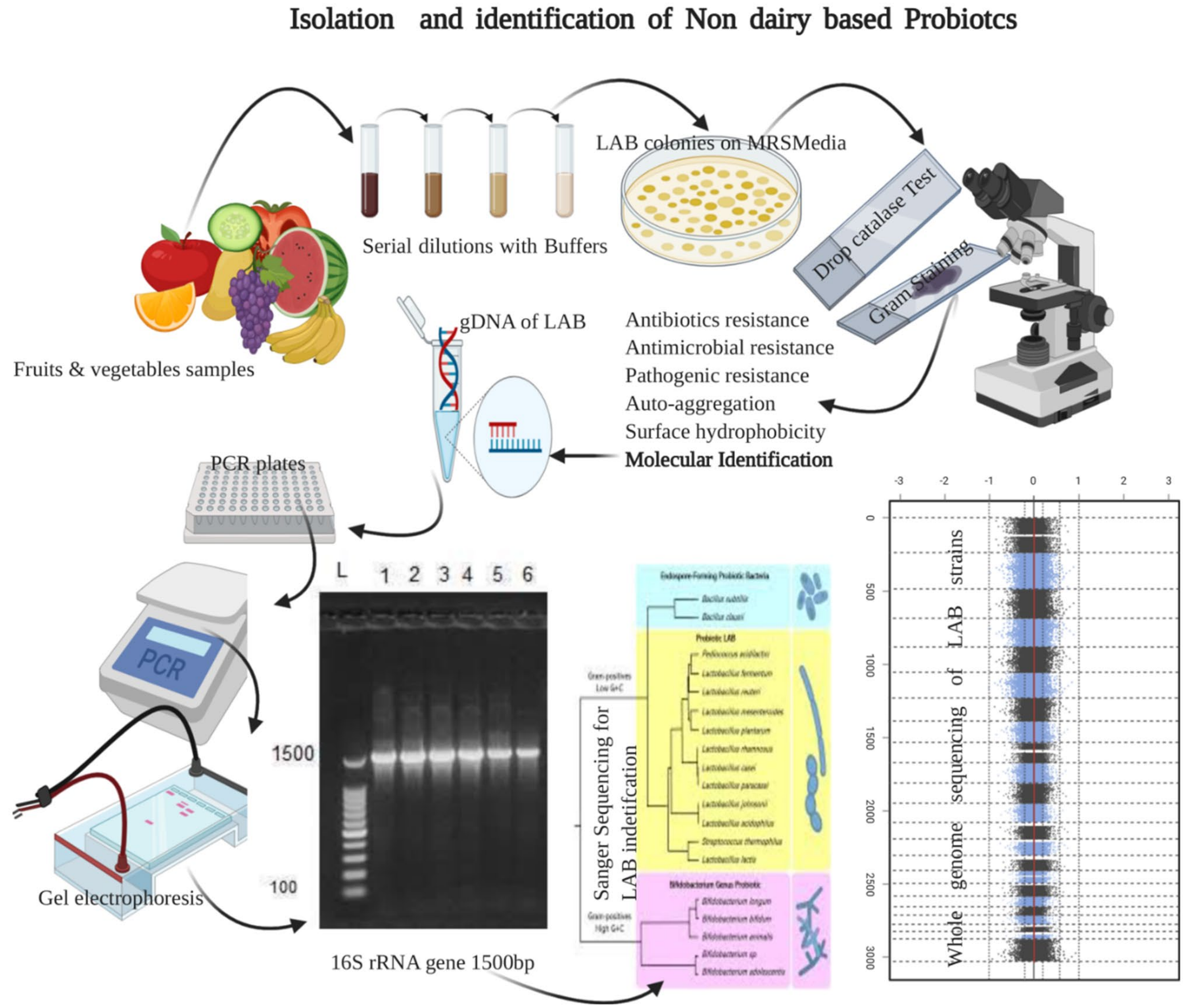

Fig. 3 Schematic presentation showing the process of isolation and identification of non-dairy-based probiotics

understanding of probiotics when comparing the microbial communities identified by conventional approaches with those characterized by molecular technologies [119]. Genotyping is a worldwide technique used for identification, and it differentiates among microorganisms. Using methods, such as DNA-DNA hybridization or DNA sequencing that encodes 16S rRNA, and strain identification methods like pulsed-field gel electrophoresis or randomly amplified polymorphic DNA, probiotic strains can be identified, characterized, particularly physiological characteristics, microbial phenotype, enzymatic activity, etc. [120]. The primers used in Ribotyping are universal to amplify the 16S rRNA gene (27 FYM 5′-AGAGTTTGATYMTGGCTCAG-3′ and 1429R 5′-GGTTACCTTGTTGTTACGACTT-3′) as reported by Behabahani et al. [118]. Currently, one of the most effective and reliable approaches for species identification is the comparison of rDNA gene sequences. The enormous quantity of sequences in the GeneBank database enables accurate strain comparison and identification when DNA genomic similarity is at least 70% [121]. As opposed to PCR and cloning/sequencing analyses, non-selective medium and anaerobic incubation have led to improved detection. Since whole genome sequencing is becoming more widely available, probiotic bacterial species will undergo extensive sequence-based identification research in the coming years. There are certain genotyping tools used for the LAB characterization and identification discussed in Table 3.

Table 3 Merits and demerits of various molecular tools involved in the identification of LAB

Molecular identification tools	Applied technique	Identified LAB strains	Advantage	Disadvantages	Reference
16S and 23S rDNA	Uses species-specific probes or PCR primers	*Lactobacillus* spp.	Improved phenotypic identification of bacteria Species-specific bacterial identification of 16S rRNA sequence	Not classify the analogous strains and intraspecies, i.e., the high similarity present between *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus delbrueckii* subsp. *lactis* led to failure in discrimination of these two subspecies	[119]
Amplified fragment length polymorphism (AFLP)	Target DNA digested UseRestriction endonucleases	*Lactobacillus plantarum*and *Lactobacillus pentosus*	Higher reproducibility and sensibility	Poor target DNA quality, complicated procedure with a large number of steps, expensive process, and the prerequisite of automated DNA sequencing tools	[122]
Amplified ribosomal DNA restriction analysis (ARDRA)	Digest the amplified rDNA Use particular restriction endonucleases	*Lactobacillus* spp.	Fast, easy, and cost-effective	The complexity of the banding profiles	[123]
Denaturing gradient gel electrophoresis (DGGE)	Separation of DNA fragments up to 500 bp in length by using a denaturing gradient gel Individual bands, separated by DGGE, can also be identified by direct cloning and sequencing, or by hybridization	*Lactobacillus* sp., *B. animalis* subsp. *lactis*	No cultivation of bacteria Analyze the complex microbial composition Strain-specific identification	Poor detection of bacteria that are present in insignificant numbers in a community and incorrect estimation of the bacterial diversity due to the detection of the heteroduplex formed by the heterogeneous rRNA operons Does not distinguish between viable and non-viable spores	[119]
Mass spectrometry (MS)	Fingerprinting is based on peptide analysis	*Lactobacillus*, *Leuconostoc*, and *Enterococcus*, *Lactobacillus*	Consistent, robust, and cheap, requires only a small amount of the sample, consumes less time, and manages data better	Database dependency, the requirement of trained staff, and the inability in identifying non-viable microbes	[124]
Multilocus sequence typing (MLST)	Sequencing of the housekeeping gene loci (seven in number) Alleles characterized strains of bacterial	*Lactobacillus brevis*	High discriminatory power, and provides obvious results	High cost and restricted accessibility Not adequately discriminating unrelated isolates	[125]
Pulse-field gel electrophoresis (PFGE)	DNA molecules are subjected to a periodical changing of the electric field polarity that causes the relaxing of DNA molecules in the gel upon the removal of the first field and their elongation to align with the following field	*L. fermentum*, *L. plantarum*, *L. gasseri*, and *L. paracasei*	Good discriminatory power, reproducibility, and type-ability	A labor-intensive and complicated procedure Prone to genetic instability, has minimal accessibility, and takes 3–4 days to complete	[119, 124]

Table 3 (continued)

Molecular identification tools	Applied technique	Identified LAB strains	Advantage	Disadvantages	Reference
Random Amplified Polymorphic DNA (RAPD)	Use of markers (20–25bps) Hybridization with cDNA sequences showing nearest homology Detect bacterial genome polymorphism	*Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus acidophilus*, and *Lactobacillus Plantarum*	Good discriminatory power, general applicability, and fast, inexpensive, and easy performance	Poor reproducibility Primer hybridization at non-specific locations	[122, 125, 126]
Real-time PCR	DNA amplification based on the fluorescence emission in real-time	*Lb. delbrueckii*, *Lb. helveticus*, *St. thermophilus*, and *Lb. fermentum*	Specific, sensitive, efficient, and reproducible. Accurate detection Precise quantification	Failure to differentiate dead bacteria from the living	[124]
Repetitive extragenic palindromic PCR (Rep-PCR)	Produces highly specific genomic fingerprints by linking primers that correspond to interspersed repetitive specific DNA elements	*Lb. brevis*, *Leu. mesenteroides*, and *Leu. citreum*	Short analysis time and high discriminatory power, require a small amount of DNA and is a procedure that is inexpensive and suitable for all LAB strains	The discriminatory power relies on the type of the primer and the number of repetitive sequences present in the LAB strain	[124]
Ribotyping	Study Ribosomal genes Bacterial genomic read using nucleic acid markers	*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus reuteri*, and *Lactobacillus sakei*	General applicability, reproducibility, and high discriminatory power	Expensive, labor-intensive, and time-consuming	[124]
Whole Genome Sequencing (WGS)	WGS potent method Highly accurate and interpret LAB at the genome level	All strains Identified	Characterize strains accurately and interpret functions of probiotics bacteria at the genome level Fast Accurate	Requires expensive equipment and a bioinformatician to handle the technical data	[127]

Safety Concerns

Probiotic strains must satisfy both safety and functioning criteria, as well as those related to their technological usefulness, as per recommendations of the World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), and European Food Safety Authority (EFSA) in their selection process [128]. The selective stimulation of intestinal bacteria's growth may be linked to wellness and health protection. The safety of probiotics is dependent on their intended usage, which takes into account the consumer's potential vulnerability, the dose, and length of consumption, as well as the method and frequency of administration. Probiotics are distinct from other food products or medicine that potentially spread infection or produce toxins in the body while being consumed. Since many kinds of bacteria can be employed as probiotics, safety is also closely related to the type of microbe that is being utilized. The safety evaluation of bacteria employed as probiotics includes factors such as antibiotic resistance expression and the transferability of antibiotic resistance determinants from fed probiotic strains to commensal microbiota in vivo. For instance, in vivo transfer of the vancomycin resistance gene, *vanA*, from an Enterococcus strain to *L. acidophilus* in mice model harboring enterococci-free human microbiota [129]. Moreover, Enterococci may also possess a wide range of virulence factors, such as hemolysin, gelatinase, or DNAse activity as well as the structural genes *cylL*, *ace*, *asal*, and *esp* [130]. Depending on the traits of the genus and species of the microbe being utilized, it is necessary to evaluate the probiotic's genetic stability over time, detrimental metabolic activities, and the potential for pathogenicity. Probiotic traits are not linked to a microorganism's genus or species, but rather to a few, carefully chosen strains of that species. The majority of human probiotic bacteria are from the genera *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Streptococcus*, and *Enterococcus*. The LAB and *Bifidobacteria* are probiotic microorganisms used essentially for human health. The origin of strain, lack of interaction with pathogenic strains, and antibiotic resistance profile all contribute to its safety [131]. For possible probiotic strains, the easily accessible and cost-effective genome sequencing methods would confirm the lack of genes of concern. Since entire bacterial genome sequences are becoming more widely available, sequence-based identification methods have been frequently utilized in probiotics identification and selection. It is anticipated that more probiotic candidate strains will be discovered by whole-genome sequencing as a result of improvements in DNA sequencing technologies that considerably increase capacity and speed at lower costs.

Bacteriocin Production and Probiotics

The production of specialized chemicals, antimicrobial peptides, which are active against foodborne pathogens may be a useful and optional factor for selecting probiotic strains. Despite the capacity to synthesize these antimicrobials and the health-promoting qualities, *Bifidobacterium* spp. is known to generate functional bacteriocins. Numerous bacteriocinogenic probiotic LAB, such as *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Lactobacillus plantarum*, *Lactobacillus lactis*, and *Enterococcus faecium*, are effective against foodborne pathogens [132, 133]. Probiotic bacteria can secrete a several bacteriocins, including short-chain fatty acids, hydrogen peroxide, and nitric oxide, which may improve their capacity to compete with other GI microbes and may suppress pathogenic bacteria. It has been suggested that *E. faecium* KH24 could be used as a probiotic and that bacteriocinogenic Enterococci might assist in beneficially controlling the local microbiota [134]. In the human GI tract, bacteriocin synthesis may aid in bacteria survival. *Bifidobacterium longum* subsp. *longum* DJO10A, an intestinal isolate, suggested that bacteriocin synthesis might be a crucial adaption for GI survival [135]. *Fusobacterium*, *Bacteroides*, and *Bifidobacterium* are a few of the significant gut genera that can be changed by bactofencin A-producing strains, *Staphylococcus aureus*, and *L. monocytogenes*, having a favorable impact on gut populations [136]. The *Lactobacillus salivarius* strains can produce certain bacteriocins under several stressful circumstances found in the gut, including Abp118 [137]. In several disease states, including the targeting of recently emerging pathobionts involved in a range of gastrointestinal illnesses, bacteriocins, and bacteriocin-producing probiotics could be a novel therapeutic approach [137].

Probiotic Potential of Non-Dairy Products

Fruits and vegetables are excellent sources of nutrients and sugar contents. These constituents have been considered considerable for the growth of probiotics, and the blended form provides a fast passage in harsh acidic stomach conditions [11]. As fruits and vegetables do not have inherent cholesterol, lactose, and other allergens, which have health hazards to a certain group of individuals [10]. Vegetables are considered a good source of minerals, sugars, vitamins, and other health-supporting compounds. Tomato, ginger, carrot root, cabbage, onion, and peanuts are used to isolate the probiotics. The lactic acid bacteria such as *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus*, and *Bifidobacterium longum* are mostly used for probiotic food based on vegetables [10].

Fruits

Several LABs have been isolated from mango brine and identified as PUFSTP38 *Bacillus amyloliquefaciens* (KT921420), PUFSTP35 *Bacillus licheniformis* (KT921419), PUFSTP *Bacillus subtilis* (KT921421), PUPUFSTP 70 *Enterobacter cloacae* (KU748633), PUFSTP *Enterobacter cloacae* (KU748634), and PUFSTP44 *Bacillus methylotrophicus* (KT921422). PUFSTP71 *Bacillus safensis* (KT92143), *Bacillus licheniformis* (KT921424) through 16 s rDNA gene sequencing. Most of these strains have antioxidant activity, hydroxyl radical scavenging ability, superoxide anion scavenging ability, plasma lipid peroxidation ability, and linoleic acid. The strain PUFSTP35 (*Bacillus licheniformis* KT921419) exhibits anticancer properties on the HT-29 colon cancer cell line [138].

Fruits and vegetables are used for the isolation of lactic acid bacteria *Lactobacillus pentosus* are isolated by chilly and Nagi Naspati (*Pyrus communis*), and *Lactobacillus fermentum* is isolated by PRSI Peru (*Pyrus pyraste*) using 16S rDNA [139]. Banana, cherry, orange, plum, and sapota fresh fruits are the vector for the isolation of *Weissella paramesenteroides*, which are valued by having bacteriocin and exopolysaccharide production ability. Five *Weissella paramesenteroides* strains are isolated from fruits. These strains are screened and selected based on tolerance at high acidic conditions, high cell surface hydrophobicity, bile-induced biofilm formation, and antimicrobial activity (AMA). Furthermore, these strains do not possess virulent traits, such as hemolysis, DNase production, and biogenic amine production [140]. Cardinal and red globe varieties, grown in Tunisia, were used to isolate the lactic acid bacteria. Screened lab strains have antifungal activities. *Aspergillus carbonarius* and *Aspergillus niger* are used fungal strains. The *Pediococcus pentosaceus* RG7B has the potential to detoxify ochratoxin A among all 18 strains. It can be considered a significant probiotic strain due to its stability in gastric juice and antimicrobial properties [141].

Vegetables

Lactic acid bacteria are isolated from fresh vegetables such as cauliflower, cluster beans, gherkins, fenugreek, bitter gourd, cowpea, French beans, tomatoes, cucumber, rigid gourd, and bottle gourd. Phenotypic and molecular characterization identified genera *Lactobacillus*, *Enterococcus*, and *Weissella*, which possess antibacterial properties against human and plant pathogens [142]. Vegetable juices are a good source of probiotics. Kale (*Brassica oleracea* L. var. *acephala* L.) vegetable juice is used to isolate LAB. Mainly *Lactobacillus coryniformis*, *Lactobacillus plantarum*, and *Lactobacillus sakei* are dominant strains. Among them, *Lactobacillus plantarum* has shown broad-spectrum antimicrobial activities. It also exhibits acid tolerance and bile tolerance characteristics. This study reveals that probiotics obtained from such vegetable sources can be considered a good probiotic potential source that can use as a starter culture [143]. Carrot juice inhibits the growth of *Bifidobacterium catenulatum*. Without affecting the probiotic species, carrots selectively prevented the growth of undesired intestinal bacteria [61]. Carrot juice increases both *Lactobacillus rhamnosus* and *Lactobacillus bulgaricus* probiotic bacterial strains up to levels of almost 5×10^9 CFU [144].

Cereals and Legumes

Lactic acid bacteria from legumes were isolated from Sweden, and found that *Enterococcus* and *Pediococcus* were predominant and closely associated with *Bacillus*. Tolerance toward low pH, bile salt hydrolase activity, and phenol are analyzed. Enterococcus strains were tested for resistance to antibiotics, whereas *Pediococcus* were evaluated for fermentation of bean beverages. Among 25 strains, five have the potential to resist at low pH, phenol, and bile also can survive even in GIT [145]. Leguminous fermented foods may be one of the probiotic bacteria carriers that are good for persons who cannot consume dairy products. Red kidney and green mung bean-based beverage KB (7:3) significantly improved the growth of probiotic *Lactobacillus casei* ATCC 335 and prevented the growth of intestinal *Escherichia coli*. Additionally, it had a significant amount of total reducing sugars and total carbs, which gave probiotics an energy source [146]. Prebiotics include the well-known soybean oligosaccharides, raffinose, and stachyose can be found in soygerm powder, which is also high in isoflavones [147]. Moreover, an enzyme, endogalactanases, produces arabinogalactan-oligosaccharides. The inclusion of *Lactobacillus reuteri* in a synbiotic with soygerm powder became more bile salt-resistant after being exposed to soygerm powder (4 g/l) [148]. *L. rhamnosus* GG, a probiotic strain, could proliferate and perform metabolic processes during the fermentation of leguminous porridges. Except for red bean porridge, where a drop in cell counts after 19 days was noticed, *L. rhamnosus* GG's cell counts remained consistent during storage [149]. The porridges have the potential to produce lactic acid during the storage period, i.e., LAB could be proliferated in porridges. However, soybean and speckled beans cannot facilitate the production of lactic acid. Chickpea porridge produced the most lactic acid during storage at a rate of 6.54 mg kg^{-1} h^{-1}. Amaranth flour and whole buckwheat flour showed lactic acid production. Also, no significant changes in lactic acid levels during storage were found in cereal and pseudocereal porridges fermented with *L. rhamnosus* GG [150]. Furthermore, the stability of the lactic acid bacteria was greatly improved by encapsulating in microparticles made of soybean protein concentrate

[151]. The solid-state fermentation of the whole soybean with LAB mixed with *B. subtilis* natto facilitates the growth of LAB, i.e., the viable counts of *B. animalis*, *L. casei*, and *L. plantarum* were 1.41 × 108 CFU/g, 1.74 × 1010 CFU/g, and 2.19 × 1010 CFU/g, respectively [152]. The combination of soy with probiotics considerably reduces cholesterol levels, whereas soy enriched with prebiotics reduces total and low-density lipoprotein cholesterol in hypercholesterolemic patients [153]. The combination of extra-virgin olive oil with probiotics also improves dyspeptic symptoms [154]. In addition to having a higher nutritional value than other conventional fermented foods, olives are a promising functional food that transports probiotic microorganisms. The probiotic LAB strains included *L. plantarum*, *L. pentosus*, *L. paracasei*, *L. rhamnosus*, and *L. coryniformis*, which were largely isolated from diverse fermented olive varietals [155]. The vitality of probiotics that have been encapsulated and their resilience to environmental stresses, such as simulated gastrointestinal conditions, are improved by multi-layered fish oil microcapsules [156].

Meat Products

Fresh and raw meat, cooked, matured, dried, or fermented meat products are used to isolate LAB-producing bacteriocins. The five strains are identified as *Lactococcus lactis* subsp. *hordinae* CTC 484, *Lactococcus lactis* subsp. *cremoris* CTC 204 and CTC 483, and *Lactobacillus plantarum* CTC 368 and CTC 469 based on morphological, biochemical, and physiological characteristics. Whole-cell proteins (SDS-PAGE) and random amplified polymorphic (RAPD-PCR) analysis have shown the similarity among *Lactobacillus* strains and variation between *Lactococcus* strains. Among these, all CTC 469 have shown more acid bile tolerance even at 0.1%, whereas all other strains have shown a good survival rate at 4 ℃, 25 ℃, and 37 ℃. Due to the lower level of biogenic amine production by CTC368 and CTC 204 can have the greatest potential to use as probiotics [96]. In a study from Serbia, 21 *Enterococcus faecium* strains were analyzed for the simulated gastrointestinal environment, antibiotics, and antimicrobial resistance. The study observed that all 21 strains showed tolerance in the simulated gastrointestinal environment and susceptibility against ofloxacin, amoxicillin, and penicillin antibiotics. The study revealed that *Enterococcus faecium* strains obtained from fermented sausage have shown potential to study further probiotic properties and might be a possible probiotic [157]. In a study from Germany, the lactic acid bacteria were isolated from fermented sausages from traditional and industrial processing lines. Lactic acid bacteria have antibacterial properties against *Listeria innocua* DSM 20649, *Pseudomonas aeruginosa* DSM 939, *Escherichia coli* DSM 1103, *Listeria monocytogenes* DSM 19094, *Salmonella typhimurium* DSM 19,587, and *Staphylococcus aureus* DAM 799. Real-time PCR has also performed the confirmation of the structural genes for bacteriocin. Thus, German fermented meat products have potential strains of lactic acid bacteria with bacteriocin production ability [158]. The lactic acid bacteria can reduce nitrite production in fermented meat products. Thai fermented sausage, locally known as E-sarn sausages, was used for the isolation and molecularly identification of lactic acid bacteria. These strains were identified as *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Enterococcus durans*, and *Lactobacillus brevis*. The identified strains have great potential to reduce and degrade nitrite, therefore used as starter culture in Thai meat fermented products [159]. Raw shrimps were used to isolate *Enterococcus lactis* Q1 & 4CP3. *E. lactis* can form biofilms against the strains of methicillin-resistant *Staphylococcus aureus* (MRSA). *Enterococcus lactis* Q1 & 4CP3 have significant potential to form biofilm alone as well as in combination [160].

Vegan Food

Vegetarian and/or vegan lifestyles are on the rise as more people choose to reject items made from animals. An increasing number of consumers rely on plant-based milk substitutes due to concerns about sustainability, health, lifestyle, and diet, which has led to an abundance of products made from nuts, seeds, or beans [161, 162]. The food items with added value and/or nutritional balance are of significant interest. Probiotics could a potential option due to their effectiveness, significant physiological properties, and the flexibility of the probiotic cultures to varied dietary matrices. The most common vegan probiotic carriers are fermented and non-fermented beverages, in which the survival of probiotics in vegan goods is influenced by the food matrix and processing. Vegan probiotic products may strengthen the immune system and lipid metabolism [162, 163]. The viability of *Lactobacillus* sp., *L. acidophilus*, *L. casei*, *Bifidobacterium*, and *B. longum* exceeded 7 $\log^{10}$ CFU/mL in the presence of FOS, inulin, mannitol, maltodextrin, and pectin. Maltodextrin, pectin, mannitol, and FOS supplementation considerably boosted the growth of LAB, also increasing lactic acid concentrations. Maltodextrin and FOS supplementation also raised the α-galactosidase activity of probiotics, resulting in improved soy oligosaccharide hydrolysis and utilization [164]. In another study, various strains of *L. acidophilus* (FTDC 1331, FTDC 2631, FTDC 2333, FTDC 1331) and *L. bulgaricus* FTCC 0411 were immobilized on dried and ground wastes of mangosteen, durian, jackfruit, and inoculated into soy milk. LAB that had been immobilized demonstrated improved growth, and enhanced reduction of sucrose and glucose, with higher lactic and acetic acid concentrations, resulting in lower pH in soy milk. Therefore, agro wastes might be used to immobilize probiotics with improved growth, substrate utilization, and organic acid generation [165]. Non-dairy plant-based milk, which is mostly made from fruits and seeds, such as soy, coconut, almond, rice, peanut, and cashew, can be used to substitute conventional milk products. The formation of lactic acid and other physiologically active substances contribute toward nutritional and physiological value results from the starter cultures' bacterial activity, giving fermented plant-based milk its distinctive flavor [166]. Soy milk also significantly increases the probiotics' ability to tolerate bile and acid [167]. With cell viability of 7.55 ± 0.07 log CFU/mL, coconut milk served as an acceptable substrate for *L. reuteri* growth without the need for additional nutrients. After storage, *L. reuteri* showed a significant pH decline and post-acidification. However, due to poor cell viability preservation, coconut milk may not be accepted as a temporary transport medium [168].

Innovative Probiotic Functional Foods

Day by day, there is an increase in vegetarianism (or vegetarian or veganism) and consumers are in search of alternative plant-based food that is loaded with nutrition and functional values, such as probiotics. Currently, probiotic products make up between 60 and 70% of the whole functional food industry [169]. As fortification of food products can enhance the nutraceutical value, such fortified products provide health benefits as well as possess excellent organoleptic values (Table 4). Functional foods have considerable nutritious values that positively affect human health and at the same time the state of mind satisfied and the well-being of consumers [170]. Fruits, vegetables, seeds, nuts, spices, and herbs are considered improved functional foods [171]. On the other hand, modified functional foods are those food products having additional bioactive components, including minerals, vitamins, fibers, probiotics, etc. to improve health conditions. Modified functional food, also known as fortified food, has a higher amount of nutrients compared to natural food. Various fortified food products have been developed, such as fortified juices, fortified grains, fortified cereal, fortified milk alternatives, etc., to combat malnutrition, hunger, and poverty worldwide [172, 173]. Plant-based fortified foods have become progressively prevalent over the last decade [174].

The probiotic juice's sensory qualities can vary depending on the type of fruit, probiotic organism, temperature at which they are stored, and whether prebiotics and protectants are included. It has been demonstrated that the probiotics' survival and stability during the preparation and storage of probiotic fruit, vegetable, and cereal foods depend on the food matrix, water activity, pH of the finished goods, and choice of the probiotic strains. Fruit and vegetable juices may provide some necessary nutrients, but other circumstances, such as low pH, which is linked to high quantities of organic acids and dissolved oxygen, may limit the survival of probiotics [185]. Non-dairy probiotics food is the healthy food component with high fiber content and causes no allergy. However, the low pH of non-dairy foods and the presence of antimicrobial compounds might affect the viability of LAB. The probiotic juice's sensory qualities can vary depending on the type of fruit, probiotic organism, temperature at which they are stored, and whether prebiotics and protectants are included. By adding a pleasant aroma

Table 4 Emerging innovative probiotic products

Innovative functional foods Products	Probiotic strains	References
Pine apple juice and apricot	*Lactobacillus*, *Bifidoacterium*, and *Lactobacillus plantarum* ATCC 14,917	[175]
Pomegranate juice	*Lactobacillus plantarum*, *Lactobacillus bulgaricus*	[176]
Cherry juice	*Pediococcus pentosaeus* and *Enterococcus gallinarum*	[177]
Broccoli juice	*Leuconostoc mesenteroides*, *Weissella* spp., *Enterococcus gallinarum*, and *Lactococcus* spp.	[177]
Cucumber juice	*Weissella confusa*, *Enterococcus durans*, *Bacillus coagulans*	[177]
Cucumber pickle	*Lactobacillus* spp., Leuconostoc spp. and *Pediococcus* spp.	[178]
Green curly kale juice	*Lactobacillus plantarum*, *Lactobacillus coryniformis*, *Lactobacillus sakei*	[143]
Wheat (buckwheat, rye corn, oat, maize) soup	*Lactobacillus casei*, *Lactobacillus plantarum Lactobacillus bulgaricus*, *Lactobacillus lactis*, *Lactobacillus acidophilus*	[179]
Maize, millet, sorghum drink	*Lactobacillus* casei, *Lactococcus cremoris*, *Lactococcus diacetylactis*	[10]
Rice and millet drink	Streptococcus and *Lactobacillus* spp.	[180]
Bread	*Lactobacillus fermentum*, *Lactobacillus plantarum*	[181]
Bakery products	*Lactobacillus rhamnosus* GG and *Lactobacillus casei*, *Lactobacillus acidophilus* LA-5	[182]
Dark chocolates	*Lactobacillus plantarum* and *Lactobacillus acidophilus*	[183]
Jelly desserts	*Lactobacillus* bulgaricus and *Lactobacillus acidophil*us	[184]

and volatile chemicals that can hide (or mask) the presence of probiotics, it may be feasible to lessen the perception of unpleasant flavors and odors in non-dairy probiotic diets. Particularly, the aroma and flavor of the finished product may benefit from the addition of tropical fruit juices like pineapple, mango, or passion fruit [186].

Synbiotics

Synbiotics are the blended products of prebiotics and probiotics that aid in survival and anchor microbes into the GI tract (Fig. 4). The term synbiotic, which suggests synergy, describes a combination of prebiotics and probiotics in which the prebiotic component favors the probiotic bacteria's ability to survive in the gastrointestinal tract [187]. Synbiotics have typically 10 g/kg of prebiotics. Different prebiotics will promote the development of various native gut microbiota, but these changes take place at the level of specific strains and species. A synbiotic containing *Bifidobacterium longum* and oligofructose increased feces bifidobacteria by 1.4 CFU/g, whereas oligofructose alone caused a 1.6 CFU/g rise in fecal bifidobacteria [188]. The combination of prebiotic and probiotic exerts more promising synergistic benefits in the living system of the host. Synbiotic association between probiotics and prebiotics leads to numerous health benefits. The functional foods comprising synbiotics have gained commercial interest due to their beneficial outcomes such as a healthy gut, prevention, and therapy of diseases [189]. Prebiotics also affects the immunological functions of several organisms. Various studies conducted on fishes demonstrated that prebiotics stimulates epithelial cells to secrete cytokines that modulate immune cells. Synbiotics also stimulate the degranulation of hemocytes in shrimps [190]. Synbiotics were developed to address potential issues with the survival of probiotics in the digestive tract since they have both probiotic and prebiotic qualities. Lactic acid production is boosted by the synergistic impact of synbiotics to reduce internal inflammation. LAB must pass through the digestive tract after being consumed, enter the colon in sufficient numbers (109 CFU/day), and then cling to and colonize the gut epithelium [191]. The probiotic bacteria's capacity to agglutinate might enhance their chances of interacting with the host. Autoagglutination and co-agglutination causes brief colonization and extends the time to reach the gut, exerting desired beneficial effects. This temporary colonization favors the local action of probiotic metabolites, such as SCFA. However, prebiotic oligosaccharides may also improve probiotics' ability to

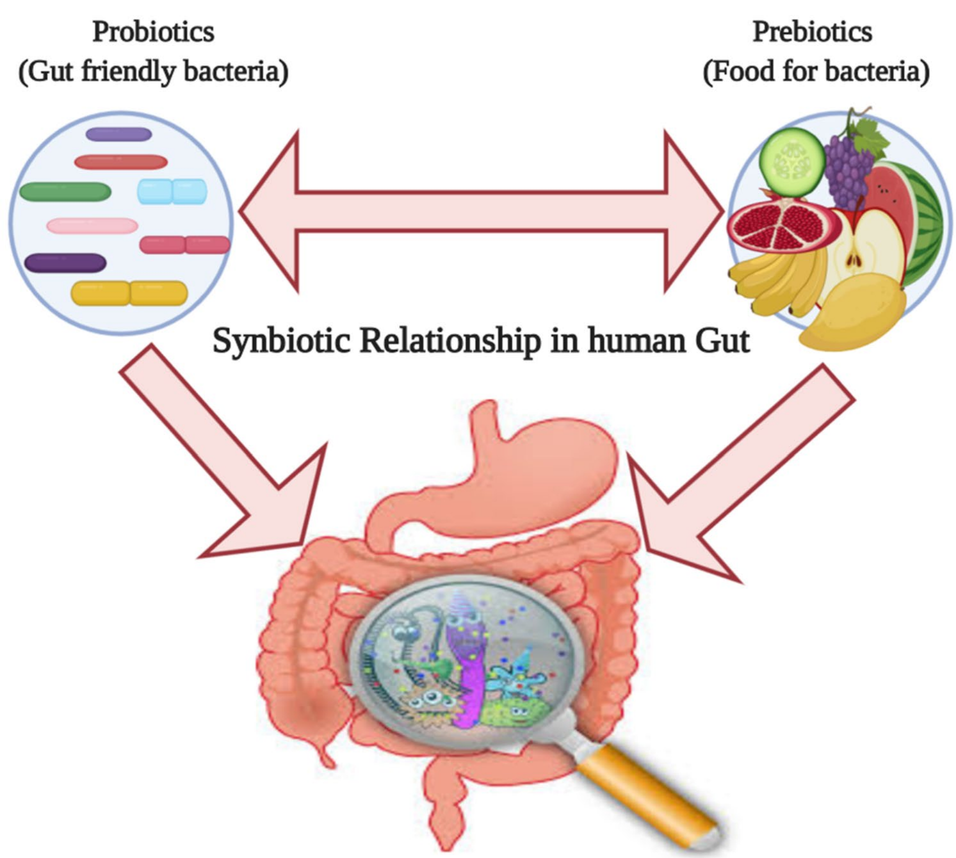

Fig. 4 The synbiotic relationship in human gut. Prebiotics aid in survival and anchor probiotics into the GI tract

adhere to surfaces, indicating that development of new synbiotic products may extend the time of probiotic bacteria in the gut [192]. In synbiotics, combination of prebiotics and probiotics, prebiotics amounts diminish during storage because probiotics consume prebiotics as a source of carbon. The viability of probiotics in a product during consumption is critical; therefore, probiotics must withstand the mechanical stress during food processing and storage. By choosing effective probiotic strains, physically coating them, and adding the right prebiotics, the viability can be preserved [193]. Retaining cells in a protective membrane through the process of microencapsulation helps to prevent damage and death. The probiotics are preserved by encapsulation, which also increases their viability throughout processing and long-term storage and maintains their metabolic activity intact both before and after intake. Additionally, encapsulation enhances and maintains the sensory qualities of food and aids in the uniform distribution of probiotics throughout the final product [193]. For long-term storage, drying the synbiotics product creates an ideal condition for the preservation of probiotics. After intake, the cells begin to divide once more as regular cells, which is advantageous to the host.

Prebiotics Encapsulation

Several prebiotics, such as FOS, inulin, and GOS, are used as coating materials for the microencapsulation of probiotics. As inulin combined with biopolymer alginate capsules elevates viability levels of *Lactobacillus acidophilus* at storage [194]. Similarly, FOS is used in the encapsulation of *Bifidobacterium bifidum* to give protection during encapsulation [195]. *Bifidobacterium adolescentis* successfully encapsulated with FOS and pea protein isolate-alginate matrix [196]. *Bifidobacterium longum*, *Lactobacillus reuteri*, and *Lactobacillus acidophilus* have protective capacity due to supplementation of alginate matrix with FOS, pectic-oligosaccharides, and inulin [197]. The encapsulation matrices pectin + inulin and pectin + rice bran give the maximum encapsulation efficiency, i.e., 90.59% and 91.24%, respectively. Thus, exerting the highest protection to the encapsulated microorganisms when placed in the simulated gastrointestinal tract [198, 199] developed a synbiotic formula, which has beads of inulin and alginate mixture. The most researched technique to improve probiotics' capacity to survive under challenging circumstances, such as low pH, oxygen, and extreme temperatures during manufacture, storage, and gastrointestinal transit, is microencapsulation. Additionally, microencapsulation might be a fascinating substitute to lessen the undesirable effects that probiotic microorganisms might have on food goods [185].

Bacterial Aggregation

Many bacteria have the capacity to bind to themselves in addition to binding to host cells, the extracellular matrix of host tissues, or inorganic surfaces, known as autoaggregation or autoagglutination [200]. Autoaggregation, a self-recognition process, is one of the initial steps in the development of biofilms, along with surface colonization. Similar types of bacteria, such as those in pure culture, create similar clumps during autoaggregation [201]. However, in co-aggregation, bacteria of different strains or even distinct species clump together [202]. Depending on the bacteria, the autoaggregative phenotype may be constitutive or may be brought about by specific circumstances, such as stress, oxygen supply, or a change in temperature [201]. Both environmental and pathogenic bacteria may benefit from autoaggregation since it typically defends against external pressures, especially in situations like food deprivation or oxidative stress. Surface proteins typically can act as autoagglutinins and mediate autoaggregation through homotypic interactions. In some circumstances, autoaggregation can also be induced by carbohydrates, especially exopolysaccharides [201].

Postbiotics

Any probiotic-produced, soluble metabolite due to the metabolic activity or fragment of LAB that has a positive impact on the host, either directly or indirectly, is referred to as a postbiotics [203]. Postbiotics can be cell-free supernatants, exopolysaccharides, enzymes, cell-wall fragments, SCFAs, bacterial lysates, vitamins, amino acids, etc. Postbiotics have pleotropic effects ranging from immunomodulatory effects, antitumor effects, antiatherosclerotic effects, wound healing to broad-spectrum antimicrobial effects [204]. Postbiotics may have a great potential to signal various organs and tissues in the host, inducing a variety of biological reactions, given their advantageous absorption, metabolism, distribution, and excretion properties. Additionally, postbiotics can replicate probiotics' health benefits without requiring the introduction of live microbes [205]. Although *Streptococcus* and *Faecalibacterium* sp. have also been described as sources of postbiotics, *Lactobacillus* and *Bifidobacterium* strains are the most common sources of postbiotics [203]. The cell-free supernatants of different *L. plantarum* strains have potential to inhibit pathogenic bacterial growth, including *Listeria monocytogenes*, *Salmonella enterica*, Escher*ichia coli*, and vancomycin-resistant *Enterococci* [206]. The interaction of postbiotics and probiotics may exerts protective effects and improves host health by delivering improved particular physiological effects [205]. Physiological protective effects of postbiotics may include antioxidant, anti-inflammatory, anti-proliferative, anti-hypertensive properties.

Consumer Awareness and Adaptation of Probiotics

As there is tremendous research done on probiotics and their therapeutic effect on human and animal health, but still, they are not globally standardized for their safety evaluation. Safety regularizations have diversity from country to country or region [207]. The FAO/WHO has been endorsed that probiotic products should contain this information on their labels: genus, species, and isolation source of strain; the dosage of viable cell count of probiotic strain; shelf life at storage conditions; and cooperate contact information [208]. Probiotic products often mention the viable cells counts as dose but found are significantly having lesser than on labels. The major threat for probiotic strains is that during food processing and handling, they might lose their functional characteristics and get contaminated [209]. Certain probiotics species may create a problem as they can transmit their antibiotics gene to pathogenic microbes and produce biogenic amine [210]. Antimicrobial resistance has been considered as a safety concern about probiotic consumption. As antimicrobial resistance horizontal gene can be transferred from probiotics to pathogenic microbes in the gastrointestinal tract [211].

Nowadays, consumers are becoming well aware of the food and its nutritional value. Thus, this enforces the manufacturers to focus on the advancement of functional foods. Hence, the successful marketing depends upon the acceptance of novelty in food products that have food quality and also value food functionalities [212]. Probiotic products have diverse food matrices. The international probiotic market is divided into different segments like probiotic dietary supplements, probiotic animal feeds, probiotic beverages, and probiotic foods. It can be further categorized into probiotics cereals, bakery products, probiotic baby food, and infant formula. Probiotics drinks are divided into fruit-based probiotic drinks, vegetable-based probiotic drinks, and dairy-based probiotic drinks. A diverse group of consumers are attracted to probiotic products, including children, by producing probiotic chocolates, jellies, and jams. Probiotic product markets capture the consumers of all age groups, particularly health issues and restricted diet individual to a child. Versatility in probiotic products also serves the vegan market. Various studies revealed that supplementing with probiotic cultures did not affect the organoleptic acceptance of probiotic products [213, 214]. Commercial non-dairy products contain more probiotic health-promoting microorganisms as a result of consumer demand for nutritious food options that enhance digestion, intestinal function, and general health. A significant portion of the global consumer population today consumes fruit juice, which is promoted as a nutritious food product. Additionally, juice does not include any dairy allergies (such as lactose) that can deter some population groups from consuming it. The consumer's sensory acceptance of non-dairy probiotic products during their development may depends upon appearance, aroma, texture, and taste.

Challenges

Non-dairy probiotic-potent products are becoming a necessity due to the considerable attention from consumers. However, technological feasibility and the development of technologies that may change with progress are not compatible. Although current research is progressing at a phenomenal rate, non-dairy probiotics/prebiotics still lack credibility when measured against the output of dairy sources [215]. Fruit and vegetable juices are good substrates and provide certain necessary nutrients, but other factors, such as low pH, which is linked to high quantities of organic acids and dissolved oxygen, may restrict the survival of probiotics [216]. Therefore, the survival of probiotics during processing and storage depends on the choice of the food matrix, pH, temperature, antimicrobial components, preservatives, natural/artificial flavors, and dyes, etc. [14, 217]. Fermentation, encapsulation, drying, rehydration, and storage are recent technologies that significantly protect some non-dairy probiotics from environmental factors. Several disadvantages of using such methods to develop probiotic non-dairy meals still need to be addressed. For instance, during fermentation, increasing temperature, lowering pH, or changing O_2/CO_2 concentrations could have adverse effects on the integrity of prebiotics/probiotics [218]. Moreover, oxidative stress, osmotic pressure, or mechanical stress can pose serious problems for probiotic survival. In addition, the survivability of microorganisms in various food products can be impacted by water activity. Many food additives, such as salt and sugar, may generate dry conditions by binding with water, improving the survival of microbes [219]. Moreover, the probiotic cell viability is probably assured for the duration of the product's shelf life. Non-dairy probiotics are generally stored at lowered temperatures, such as 5 °C. However, storage or preservation at room temperature may also affects the growth and survival of probiotic bacterial species [220]. There are still numerous challenges that need to be resolved for effectively developing and preserving non-dairy probiotic foods. When developing new probiotic products, technological concerns that affect the survival of probiotic strains during the manufacturing process and storage period should also be taken into consideration. Moreover, probiotic bacteria strains used for the preparation as well as the cultivar of the fruit or vegetable should be chosen selectively for significant production of probiotics and prebiotics. However, in comparison to dairy products, non-dairy sources make it harder to sustain the vitality of probiotic microorganisms. To ensure probiotic viability and produce acceptable organoleptic qualities (mostly scent and flavor) that can be altered by fermentation, the physicochemical parameters need to be closely monitored [221].

Conclusion and Future Conclusion

Non-dairy sources of prebiotic have not been given much attention. Fruits, vegetables, herbs, culinary spices have great prebiotic potential. Certain agro-industrial wastes products, which are produced during food processing, such as fruit peels, cereal bran/husk, have also prebiotic potential and contain polysaccharides, and oligosaccharides, which increase the level of SCFAs by probiotics. They are considered a growth promoter of probiotic bacteria and flourish in the population of gut microbiota in the gastrointestinal tract. Certain herbs are used as encapsulation matrices of lactic acid bacteria with splendid prebiotic properties. Recent trends in the food industry also give the diversity in probiotics world. The sources which acquire the novel strains gained attention from the researchers. In previous ages, dairy products were used as the source of probiotics, but nowadays, there are diverse sources that produce novel strains of probiotics, having countless health benefits like it can be effective even for the control of colon cancer. The strains isolated from fruits, vegetables, pickles, cereals, meat, etc. have great antimicrobial potential by producing bacteriocins, acid bile tolerance, possessing antifungal properties, and strong adhesion with intestinal epithelium. The prebiotics and probiotics obtained from non-dairy sources have significant gut health scope. Furthermore, the research gap exists; future research is of utmost demand in this field.

To find new possible probiotic microbes and new components as potential substrates, research on non-dairy probiotic beverages is still in its early stages and focuses on conventional non-dairy probiotics from around the world. The development of creative, economic, and technological matrices is absolutely necessary to develop non-dairy probiotic foods as healthy alternatives to dairy probiotic meals. The development of creative, economic, and technological matrices is an absolute necessity. Research is also required to find solutions to several issues with probiotic bacterial survival in harsh non-dairy substrates, such as the low pH of fruit juices, etc. Researchers must therefore search for probiotic microbes in numerous substrates to see which might be most suitable for global health. The development of creative, economic, and technological matrices is necessary. Functionality, stability, and sensory acceptance, particularly flavor, appeal, and pricing have to be considered while developing as these aspects play a significant influence in their successful commercialization. The development of new non-dairy-based probiotic products with enhanced flavor and taste will have substantial nutritional value.

Declarations

Conflict of Interest The authors declare no conflict of interest.

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