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Recent updates of probiotic dairy-based beverages

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There is a rapid paradigm shift in the food consumption habits of consumers globally. The interest in healthier, safer, minimally processed and nature-identical foods is the driving force of this paradigm shift. Although the roots of this consumer trend go back further, especially the Covid-19 pandemic has contributed to the acceleration of this process. The effects of probiotics on human health have been known for many years. The commercial success of some probiotic microorganism strains, supported by clinical studies, is also evident. Probiotic microorganisms can be found in commercial products in a wide range of forms including powder, tablets or incorporated into liquid or solid food matrices. Milk and dairy products are suitable vehicles for the delivery of probiotics into the human body. Apart from well-established dairy-based probiotic foods including yogurt and yogurt-type beverages, in recent years some dairy products supplemented or enhanced with postbiotics and paraprobiotics are gaining popularity. The incorporation of next-generation probiotics in probiotic beverage formulations has also attracted the attention of researchers. The current state-of-the art for the utilization of next-generation probiotics, postbiotics and paraprobiotics in dairy-based probiotic beverages is the main focus of this review. Conventional milk-, whey- and buttermilk-based probiotic beverages are also covered.

1. Introduction

The International Scientific Association for Probiotics and Prebiotics (ISAPP) describes probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host".[1,2] According to this definition, for the therapeutic and prophylactic effects of probiotics to occur, they must be present at a certain level in the final product and this limit is at least 10^6 cfu g^{-1} or mL^{-1}.[3] Probiotics have been demonstrated to exert positive health impacts, but there are a number of conditions that must be met before these benefits emerge. For example, preserving the stability of probiotics and maintaining their viability during food production or storage are some of the challenges faced by the industry. Variable interactions with the host, strain-dependent activity, low colonization, and poor dosage control can be listed as limiting factors of probiotic application on food.[4] In addition to these limiting factors, negative individual responses to the consumption of probiotics may also occur. For instance, uncontrolled or excessive intake of probiotics might possibly cause adverse

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side effects in immunocompromised individuals and horizontal gene transfer of virulence genes, including antibiotic resistance genes, to bacteria in the gut microbiota has been specified to be a significant issue.[5]

To date, many studies have been done on probiotics and many commercial probiotic foods in various forms have been launched into global markets. Among them, dairy products have a distinct place. Yogurt seems to be the most convenient vehicle for the transfer of probiotic microorganisms into the human body.[6] Dairy-based beverages are also suitable media for the incorporation of probiotics and many commercial probiotic beverages are available in the markets. The probiotic dairy-based beverages market size was valued at USD 39.74 billion and forecasted to reach USD 95.52 billion by 2033.[7] Vast majority of these products contain *Lactobacillus* and/or *Bifidobacterium* strains with clinically proven probiotic properties, such as *Lactobacillus acidophilus* La-5, *Bifidobacterium animalis* subsp. *lactis* BB-12, *Lacticaseibacillus paracasei* Shirota and *Lacticaseibacillus rhamnosus* G (LGG).[8] The dairy-based probiotic beverages market consists of three sub-segments: (a) milk-based beverages (fermented or non-fermented), (b) whey-based beverages and (c) buttermilk- or buttermilk serum-based beverages. Milk and whey-based beverages have been well researched, and many commercial products have been released during the last two decades. In contrast, buttermilk-based probiotic beverages constitute a relatively new category of probiotic beverages.

Although the theory that lactic acid bacteria prolong human life was first developed by Elie Metchnikoff in 1907, Lilly and Stillwell were the first researchers to define probiotics in 1965.[9] Subsequently, the effects of prebiotics on probiotic microorganisms were identified and synbiotic products started to appear in global markets in the early 2000s.[10] The presence of conventional probiotic/synbiotic milk-based beverages in the global probiotics market remains strong. Although the functional effects of some probiotic strains on human health have been clinically proven, the incorporation of these strains into food matrices remains a significant challenge for the food industry. In particular, low stability of probiotic microorganisms against food processing and human gastric digestion conditions is an important problem for the food industry. Although many processing strategies have been developed in this regard, few probiotic strains have been able to reach the microbial counts required for the probiotic effect (*i.e.*, 10^6–10^8 cfu mL^{-1} or g^{-1}). The scientific data accumulated during the last 10–15 years have shown that apart from live probiotic microorganisms, metabolites synthesised by these microorganisms and/or dead probiotic cells can also have positive impacts on human health. Based on this, novel functional products containing metabolites and/or inanimate probiotic cells may have strong potential to gradually replace classical probiotic milk-based beverages. Bacterial metabolites with health-promoting effects, also called postbiotics (refer to section 2), are known to be stable against environmental conditions such as temperature, gastric acidity or digestive enzymes.[10] The presence of metabolites derived from probiotic strains in the food matrix instead of live probiotic microorganisms will provide a significant operational advantage for the dairy beverage industry. Microorganisms that have functional characteristics through their metabolites/cell fragments but have low stability against processing technologies and/or GIT conditions will also be utilized in industrial applications.

This review mainly focuses on next-generation probiotic dairy-based beverages and on the state-of-the art of personalised probiotic milk-based beverages. Conventional dairy-based probiotic beverages that have achieved commercial success will be briefly mentioned below.

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1.1. Milk-based probiotic beverages

A great number of commercial probiotic milk-based beverages have been enjoying market success for many decades. Among the well-known probiotic milk-based beverages are Yakult (*Lb. casei* Shirota), Actimel (*Lb. casei* strain Danone®), *Acidophilus* milk (a generic product, *Lb. acidophilus*), Proviva (*Lb. plantarum* strain 299v), Gefilus (*Lb. rhamnosus* GG), YoPlait (*Bifidobacterium animalis* subsp. *lactis* Bb-12), Bifighurt (*B. longum* CKL 1969 or DSM 2054), Biomild (*Lb. acidophilus* + *Bifidobacterium* spp.), CHAMYTO (*Lb. casei* or *Lb. paracasei*), and Cultura or A/B milk (*Lb. acidophilus* La-5 and *B. animalis* Bb-12). Conventional milk-based probiotic beverages are beyond the scope of this review. A comprehensive evaluation of conventional probiotic milk-based beverages is available in Eminoglu *et al.*, Turkmen *et al.* and Jan *et al.*[8,11,12]

1.2. Whey-based probiotic beverages

Whey is a byproduct of cheese-making. For a very long time, whey has been considered as a raw material for high value-added products rather than a cheese-making waste. Whey contains high levels of lactose, soluble protein, mineral substances, and small amounts of fat and its composition varies based on factors such as the milk source, cheese type, or thermal treatment.[13–15] Numerous commercial whey-derived or whey-based products have been developed so far and many of them have been in a strong position in global probiotic dairy beverages markets. Apart from the conventional whey products such as whey-based powders (whey powder, whey protein powder, whey protein concentrate powder, whey protein isolate, hydrolysed whey protein powder, *etc*.), whey ingredients (whey proteins, lactose, minerals, *etc*.) are also produced from whey with high purity. The latter products are widely utilized by the pharmaceutical industry. There are many clinical studies regarding positive health impacts of whey and whey ingredients, and whey proteins are recognized with high digestibility capacity. Apart from the favourable health impacts of whey sourcing from milk serum proteins (*i.e.* α-lactalbumin and β-lactoglobulin), it provides a suitable medium for the growth of probiotics. In the last decade, there has been a significant increase in research on whey-based functional beverages including probiotics. Whey is suitably used in probiotic beverage formulations alone or in combination with other dairy (*i.e.*, buttermilk) or non-dairy (*i.e.*, fruit juices) liquids or pulps.[16–18] The use of prebiotics in probiotic whey beverage formulations is a common practice. However, the selection of the prebiotic agent is of critical importance for the stimulation of probiotic microorganisms. For example, inulin, oligofructose or polydextrose stimulated the growth and viability of *Lb. acidophilus* La-5 and *Str. thermophilus* St-36 in sweet whey beverages, but the growth and viability of *B. animalis* Bb-12 was not influenced by these prebiotics.[19] Similarly, addition of lactulose to sweet probiotic whey beverages was ineffective on the viability and growth of *Lb. acidophilus* La-5, as reported by Matijević *et al.*[20] Resistant starch has a positive impact on the viability of *Bifidobacterium pseudocatenulatum* in probiotic whey beverages.

Since sensory problems are often encountered with whey-based beverages, the combinations of whey with fruit juice and/or skimmed milk may offer a solution for this problem. Overall, if the whey ratio exceeds 50% in the formulation of probiotic beverages, the possibility of sensory problems is likely to increase.[21] De-proteinized whey,[22] UF-whey permeates and retentates,[23] and UF-permeate enriched with whey retentate (10%)[24] are also suitable matrices for the development of probiotic whey-based beverages. Microencapsulation of probiotic microorganisms is a well-known technology to protect probiotics from harsh environmental conditions. In the selection of the microencapsulation agent, care should be taken to protect the probiotic microorganism as well as not to cause sensory and physical problems in the product. Obradović *et al.* reported that chitosan did not affect the viability of probiotic cells during fermentation but the physical properties of the final beverage was improved.[25] It was shown that the use of Ca-alginate as a coating material increased the physical stability of the probiotic Doogh beverage (a traditional Iranian dairy-based beverage) and triggered the development of off-flavours during cold storage.[26]

There are a limited number of *in vitro* and *in vivo* studies targeting the health impacts of probiotic whey beverages. Increased body weight and body mass index,[27] decreased acetate concentration and increased butyrate levels,[28] decreased allergenicity,[29] increased bioactive peptide levels,[30] and decreased colitis symptoms[31] are among the health effects associated with probiotic whey beverages. Rosa *et al.* demonstrated that probiotic whey-milk beverages fermented by *Lb. casei* 01, *Lb. acidophilus* La-5, *B. animalis* Bb-12 or *Lb. acidophilus* La-3 positively affected the production of bioactive peptides and phenolic compounds.[32] While *Lb. casei* 01 produced antihypertensive peptides at higher concentrations, *Lb. acidophilus* La-3 promoted the formation of phenolic compounds more than the other probiotic strains. Some of the outputs of the scientific studies carried out on whey-based probiotic beverages are summarized in Table 1.

1.3. Buttermilk-based probiotic beverages

Buttermilk is a by-product of butter-making and contains mainly milk fat globule membrane (MFGM) materials and water-soluble milk components (lactose, minerals and proteins).[50] MFGM materials contain polar lipids such as phospho- and sphingolipids as well as neutral lipid fractions such as triglycerides, diglycerides, monoglycerides, cholesterol and their esters.[51] In addition to lipid fractions, MFGM also contains proteins integral, peripheral or weakly bound to the MFGM surface such as mucin, xanthine oxidase/dehydrogenase, CD36, PAS 6/7, adipophilin and butyrophilin.[52–54] Due to its rich chemical composition, it can be used as an emulsifier and a stabiliser in food systems and also shows positive health effects.[55] However, compared to whey, both scientific studies dedicated to the development of buttermilk-based probiotic beverages and the commercial examples of functional buttermilk beverages are limited. Antunes *et al.* formulated a probiotic buttermilk beverage enriched with sucrose or sucralose. The end product had a probiotic bacteria count high enough for a therapeutic effect (>107 cfu mL^{-1}) after four weeks of cold storage.[56] The stimulated growth of *B. animalis* subsp. *lactis* in buttermilk was demonstrated by Antunes *et al.*[57] A symbiotic buttermilk beverage developed by Hashem contained red beetroot puree, carboxymethyl cellulose and *Lb. acidophilus* as probiotic species.[58] The formulated product had high sensory scores as well as high probiotic counts at the end of a 3-week storage period under cold conditions. MilkyMist – an Indian dairy innovator – has launched the world's first UHT probiotic buttermilk beverage recently. Akshayakalpa – an Indian dairy company – produces organic probiotic buttermilk commercially.

2. Postbiotics and paraprobiotics in dairy-based beverages

Recent studies have demonstrated the health benefits beyond the inherent viability of probiotics.[59–61] Therefore, the viability of microorganisms for a probiotic effect may no longer be necessary since clinical benefits are not directly linked to viable bacteria.[60] The view that some of the health benefits attributed

Table 1 Main outputs of some recent studies on whey-based probiotic beverages

Strain	Additives/combinations	Main outputs	Ref.
Technological outputs			
Lb. acidophilus La-5, *Lb. casei* LBC-81	Soy isoflavones and phytosterols	• Beverage supplemented with phytosterols received higher sensory scores.	33
Kefir starter culture	Prebiotic fructo-oligosaccharides (FOS) and refined sugar	• Antimicrobial and antioxidant activities were evident.	34
Kefir starter culture		• Antagonistic effect against *Salmonella* spp. *enteritidis* was demonstrated.	35
B. animalis subsp. *lactis* Bb-12	Carbonated whey beverage	• A slight time-dependent sedimentation was observed.	36
Lb. acidophilus La-5	Channa whey and pineapple juice mixture (0 : 100, 15 : 85, 25 : 75 and 35 : 65%)	• The survivability rate of the probiotic strain used was >80% during 56 days of storage.	37
Lb. rhamnosus ATCC 7469	Milk + whey	• The probiotic counts were above >10^7 cfu mL^{-1} after three weeks of cold storage.	38
Lb. acidophilus, B. animalis subsp. *lactis* and *Streptococcus thermophilus*	Milk + whey	• At higher whey concentrations in the formula (>50%), sensory acceptance of the beverage decreased.	21
Lb. rhamnosus and *Lb. acidophilus*	Fermented milk (40.2), sucrose (5.6%) and fruit preparation (5.2%)	• The counts of probiotic microorganisms were >10^6 cfu mL^{-1} at the time of consumption.	39
Lb. acidophilus, Lb. bulgaricus and *Str. thermophilus*		• The growth of probiotic strain was stimulated by yogurt starter bacteria.	40 and 41
Lb. rhamnosus NCDO 243, *B. bifidum* NCDO 2715 and *Propionibacterium freudenreichii* subsp. *shermanii*	De-proteinized whey	• Both organoleptical properties and the probiotic counts of the final product were acceptable.	22
Lb. acidophilus, Lb. casei and *Lb. rhamnosus*	UF-whey retentate and UF-whey permeate	• Overall physical and sensory properties of the formulated beverage were satisfactory after 14 days of cold storage.	23
Lb. acidophilus M92, *Lb. plantarum* L4 and *Enterococcus faecium* L3	UF-permeate enriched with whey retentate (10%)	• Probiotic strains successfully acidified the milk and colony counts of the probiotics were *ca.* 8 $\log_{10}$.	24
B. animalis Bb-12, *Lb. acidophilus* La-5 and *Str. thermophilus* St-36	Prebiotics (inulin, oligofructose and polydextrose)	• Prebiotics stimulated the growth of probiotics other than *B. animalis* Bb-12.	19
B. animalis subsp. *lactis*	UHT-goat milk plus cheese whey	• Mixed beverage containing 45% goat cheese whey and 6% oligofructose yielded the highest probiotic colony count after 28 days of cold storage.	42
Str. thermophilus and B. animalis subsp. *lactis*	Kiwi powder (1%)	• Antioxidant capacities of the beverages made from reconstituted cow, sheep and goat WPC were superior.	43
Bifidobacterium spp.	Inulin or resistant starch	• Resistant starch positively affected the growth and viability of *Bifidobacterium pseudocatenulatum*.	44
Probiotic strains	Microencapsulated probiotics	• Chitosan was ineffective on the viability of probiotic microorganisms.	25
Microencapsulated *Lb. acidophilus* La-5 and *B. animalis* subsp. *lactis* Bb-12	Peppermint essence nanoliposomes (1–2%)	• Beverage supplemented with 2% nanoliposome received the highest sensory scores.	45
Lb. acidophilus	Pineapple juice	• Combination of cheese whey (65%) and pineapple juice (35%) yielded a better product.	46
Xylooligosaccharide (prebiotic)	Cold-plasma-treated whey beverage	• Cold plasma treated beverage had higher levels of bioactive compounds.	47
Health effects			
Str. thermophilus and *Lb. bulgaricus*	Umbu (*Spondias tuberosa*) fruit pulp (10%)	• Increased body weight and albumin level and decreased cholesterol level in malnourished animals. • Increased body weight and body mass index in malnourished children.	27
Lb. rhamnosus RC007		• Increased anti- and pro-inflammatory cytokines IL-10 and TNF-α, goblet cells, and intraepithelial lymphocytes in intestinal fluids in mice.	48
Str. thermophilus 2 K, *Lb. bulgaricus* BK, *Lb. bulgaricus* K, *Lb. plantarum* W42 and *B. lactis* Bi30		• Reduction in the levels of allergy markers interleukin-4 (IL-4), immunoglobulin E, and specific immunoglobulin G1. • Secretion of major regulators of IL-10 and TGF-β enhanced.	29
Spontaneous fermentation or natural whey starter		• 49 bioactive and 21 ACE-inhibitor peptides were released during fermentation. • Spontaneous fermentation resulted in a higher ACE-inhibitory and DPP-IV-inhibitory activities.	30

Table 1 *(Contd.)*

Strain	Additives/combinations	Main outputs	Ref.
Lb. rhamnosus (MTCC-5897)		• Severity of colitis and emergence of clinical symptoms reduced. • Improved immune homeostasis and barrier integrity were observed.	31
Lb. casei, *Lb. paracasei* and *Lb. brevis*	Inulin (5%), vitamin A (0.5 mg L^{-1}), vitamin C (50 mg L^{-1}) and potassium iodide (0.5%)	• The acute score toxicity and *in vivo* evaluation of allergenic properties proved the safety of the whey-based probiotic beverage.	49

to probiotic microorganisms are provided by the non-viable probiotic cells or metabolites synthesized by or released after inanimation of probiotic microorganisms has triggered the development of concepts of postbiotics and paraprobiotics. Today, potential health benefits of postbiotics and paraprobiotics are being studied widely in comparison with probiotics.[62]

Postbiotics are described as "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" by ISAAP.[63] According to this definition, a postbiotic must contain inactivated cells or cell components. However, the presence of cell metabolites is not a prerequisite for postbiotic identification. Paraprobiotics are defined as "non-viable probiotic or non-probiotic cells with intact structure or crude cell fragments, which, when administered in adequate amounts, confer a benefit on human or animal consumers".[64] Both definitions need to be clarified by international scientific and regulatory bodies in order to harmonise the terminologies and to eliminate uncertainties in the global functional food trade.

ISAAP claims that metabolites purified from inactive cells such that no cell biomass and/or components remain are not postbiotics, regardless of their possible health benefits.[65] Therefore, it is stated that when metabolites are purified, each will be considered as a separate component and may have a synergistic effect with cell cytoplasm substances, rather than these health-promoting components being purified separately. However, this definition does not fully satisfy some part of the scientific community and regulatory bodies. For example, the methodology employed to distinguish defined molecular markers from undefined matrices released from microbial cells is unclear. Additionally, the lack of well-defined markers of efficacy in products containing inanimate microorganisms is another point that needs to be focused on. On the other hand, no risk of translocation from the gut lumen to the blood, targeting the oral cavity, skin, genitourinary tract and nasopharynx as well as the intestine, easier standardization, higher stability under a wide range of pH and temperature conditions and little or no interaction with food matrix components are among the advantages of postbiotics and paraprobiotic over probiotics. The health effects attributed to postbiotics are explained by four dissimilar underlying mechanisms that can affect independently or in combination, and these mechanisms are as follows: enhancing the gut microbiota, modulating systemic metabolism and immune response, improving the epithelial barrier function and signalling through the nervous system.[63–66]

The production of postbiotics and paraprobiotics essentially consists of three stages: choosing the appropriate microbial strain, producing biomass and separating (for postbiotics) or not separating (for paraprobiotics) the resultant biomass using an appropriate method such as sonication, high pressure application, heat treatment or radiation treatments. Postbiotics generally cover exopolysaccharides, cell-wall fragments such as teichoic acids and lipoteichoic acids, supernatants, bacterial lysates, short-chain fatty acids (SCFAs), enzymes, vitamins and phenols.[67] In most cases, separation of soluble fractions and intact cells by an appropriate technique such as membrane filtration or centrifugation is necessary to produce postbiotics.

Readers are recommended to refer to Cuevas-González *et al.* and Monteiro *et al.* for more details on health-promoting mechanisms of postbiotics and paraprobiotics.[60,68]

There are a number of commercial products containing postbiotics and paraprobiotics available in the markets. Majority of these products are designed for pharmaceutical purposes (*i.e.*, Hylak®Forte, CytoFlora®, Zakofalk®, Bactistatin, Pro-Symbioflor®, PostbiotiX, Totipro PE0401, EpiCor®, Lacteol Fort® and Del-ImmuneV®).[60] Fermented foods such as yogurt, kefir, kombucha and pickled vegetables naturally contain postbiotics.[69] The incorporation of postbiotics into functional dairy beverages is also an exciting new development in the beverage industry. *In situ* production of postbiotics or their subsequent addition to the product are thought to be two different strategies.[70] It has been reported that *in situ* production of postbiotics by lactic acid bacteria rather than their subsequent addition to the product resulted in significant anti-listerial activity in milk stored under cold conditions.[71] In a recent study, the laxative effects of milk fermented with *Lacticaseibacillus paracasei* and its postbiotics in BALB/c mice in a loperamide hydrochloride-induced constipation model were examined.[72] The mice were fed with fermented milk at concentrations of 4.86, 9.71, 14.58, and 48.60 mL kg^{-1} day^{-1}. Constipation symptoms were relieved in mice consuming fermented milk at 14.58 mL kg^{-1} and 48.60 mL kg^{-1} concentrations. An alleviation in colon inflammation, decreasing defecation time, and increasing levels of *Firmicutes* and *Actinobacteriota* species in the intestinal flora were also reported.

Postbiotics and paraprobiotics are more resistant to processing conditions compared to probiotics. Additionally, paraprobiotics and postbiotics facilitate minimization of the risk of bacteraemia because they contain inanimate microorganisms or cell metabolites.[60,73,74] It was reported that consumption of fermented milk containing non-viable intact *Lactobacillus gasseri* (CP2305) reduced intestinal disorders[75] such as irritable bowel syndrome, regulated the gut environment and function,[76] reduced stress-related symptoms, and improved intestinal patterns and quality of sleep during stressful situations.[61,77,78] Canani *et al.* reported that consumption of fermented milk containing *Lactobacillus paracasei* CBAL74 as a paraprobiotic resulted in modulation of the intestinal microbiota in children.[79] Some of the potential health benefits of postbiotics and paraprobiotics in dairy-based beverage matrices are presented in Table 2. Despite the promising scientific findings on the positive health impacts of postbiotics and paraprobiotics, their inclusion in dairy-based beverage formulations requires a more concrete scientific background. To date, majority of the research on postbiotics and paraprobiotics has focused on the characterization and isolation/purification of bioactive compounds. There is a need to have a deeper knowledge on the characteristics of newer postbiotic compounds and set the ideal blend of these postbiotics in the selected food matrices for the expected health benefits. Also, the method of preparation, lack of *in vivo* and clinical trials, dose–response status and mode of administration are the major restrictions of postbiotics and paraprobiotics for commercial applications. From the regulatory point of view, there is a complexity as postbiotics can fit within various regulatory categories. Currently, the Food and Drug Administration (FDA) is yet to establish specific regulation regarding postbiotics. In the EU, postbiotics are subject to novel food regulation and health claim regulation as with probiotics and prebiotics.[65] Therefore, a comprehensive safety and toxicological evaluation is required. In this context, safety evaluation of inanimate microorganisms (postbiotics or paraprobiotics) is expected to be simpler than that of live microorganisms (probiotics). The

Table 2 Some recent studies on *in vitro* and *in vivo* health and anti-bacterial effects of postbiotics and paraprobiotics

Postbiotics/paraprobiotics	Food matrices	Bioactivity	Main outputs	Ref.
***In vitro* studies**				
Intracellular and extracellular contents of *Lactobacillus satsumensis* LPBF1, *Leuconostoc mesenteroides* LPBF2 and *S. cerevisiae* LPBF3	Honey-based kefir	Antioxidant activity ranging from 20 to 28% of (DPPH) inhibition	• Preventive effect on oxidative DNA damage and cellular oxidation	100
Cell-free supernatant and paraprobiotics of *Lb. reuteri* PTCC 1655		Anti-proliferative and anti-metastatic effects	• Inhibitory effects on colon cancer stem-like cells (HT29-ShE cells)	101
Postbiotics of *Lb. rhamnosus*: surface layer protein, genomic DNA, and unmethylated cytosine–phosphate–guanine containing oligodeoxynucleotides		Immunomodulatory effect	• Inhibitory effect of most postbiotic fractions on the activity of toll-like receptor, mitogen-activated protein kinases, extracellular regulated protein kinases, and nuclear factor-kappa B signalling pathways	102
Exopolysaccharide (R-5-EPS) isolated from fermented milk of *Lb. helveticus* LZ-R-5	Tibetan kefir	Immunomodulatory effect	• Significant immunomodulatory effect by R-5-EPS	103
Fraction with Mw <6000 in the supernatant produced from *Lb. paracasei* FX-6	Kefir	Anti-microbial effect	• A remarkable inhibitory effect on the growth of *Pseudomonas putida*	104
Supernatant of kefir and kefir-like products	Kefir-like beverages	Antibacterial, antioxidant and cytotoxic effects on Caco-2 cells	• Radical scavenging activities of skim milk kefir and buttermilk kefir-like supernatants were found to be higher than the Trolox standard	105
Exopolysaccharides from *Lb.paracasei* VL8	Viili (Nordig fermented dairy beverage)	Immunoregulatory effect	• EPS derived from *Lb. paracasei* VL8 exerted an immunomodulatory effect	106
Lb. acidophilus supernatant	Fermented milk	Antimicrobial effect	• *Lb. acidophilus* supernatant showed an anti-microbial effect on *S. enteritidis*	107
***In vivo* studies**				
Intracellular content of *Lb.casei* CRL 431		Antioxidant effect	• Aflatoxin-induced lipid peroxidation decreased with increased antioxidant capacity	108
100H DSF commercial culture	Fermented milk	Protecting mice against *Salmonella* infection	• High survivability of mice fed with the cell-free supernatant of skim milk fermented by 100H DSF culture	109
Heat-killed *Lb. helveticus* strain MCC1848		Anxiolytic- or antidepressant-like effects	• MCC1848 may alleviate anxiety or depression in mice subjected to social defeat stress	110
Paraprobiotic of *Lb. paracasei* PS23 (inactivated at 95 °C)	Fermented milk	Anti-collitis effect	• Reduced intestinal epithelial permeability, and enhanced resistance to pathogens in mice with colitis	111
Postbiotic *Lacticaseibacillus paracasei*	Fermented milk	Reduction of constipation and modulation of the intestinal flora structure in mice	• Relieved constipation symptoms in mice consuming fermented milk	72

EFSA Panel published its opinions on the safety of three model heat-killed postbiotics namely *Bacteroides xylanisolvens*,[80] *Akkermansia muciniphila*[81] and *Mycobacterium setense manresensis*[82] under the proposed conditions of usage. Heat-killed *Bacteroides xylanisolvens* and *Akkermansia muciniphila* were authorised by the EFSA in 2015 and 2022 as a novel food, respectively.[83,84] It should be noted that there is still no specific regulation covering postbiotics in the EU but they are subject to novel food regulation and health claim regulation.[65] It is clear that health claims associated with postbiotics must be approved by the EFSA before being commercialized.

3. Next generation probiotics and dairy-based beverages

The concept of next-generation probiotics (NGPs), also called designer probiotics and smart probiotics, is emerging in order to enhance the function of traditional probiotics and develop personalized treatment by increasing the expression of specific therapeutic components.[85,86] *Akkermansia muciniphila*, *Eubacterium hallii*, *Roseburia intestinalis*, *Faecalibacterium prausnitzii* and *Ruminococcus bromii* are given as examples of NGPs and are stated to have positive health-promoting effects on various diseases such as improvement of metabolic functions, diabetes, atherosclerosis, colorectal cancer and inflammatory bowel diseases, respectively.[87] Numerous clinical studies have been conducted to reveal the health effects of novel NGPs and their metabolites. For example, *Akkermansia muciniphila* (ATCC BAA-835 T) isolated from the human gut was reported to activate T cells by increasing acetate levels, thereby reducing the risk of ovarian cancer in mice.[88,89] It was suggested that specific metabolites or membrane proteins of the same microorganism may be effective in the prevention of obesity, diabetes, metabolic syndrome and neurodegenerative diseases.[90,91] Fengycin – a postbiotic secreted by *Bacillus subtilis* ZK3814 – effectively mitigates infections by *Staphylococcus aureus* by specifically targeting the quorum sensing mechanism in *S. aureus*.[92] Deconjugation of taurocholate into cholate by EcN-CbH – an engineered probiotic – suppresses the growth of spore-forming *Clostridioides difficile*, leading to inhibition of infections caused by *C. difficile* in mouse models.[93]

The FDA defines live biotherapeutic products (LBPs) as "a biological product that: contains live organisms, such as bacteria; is applicable to the prevention, treatment, or cure of a disease or condition of human beings; and is not a vaccine".[94] According to this definition, non-genetically modified NGPs are classified as live biotherapeutics, while recombinant probiotics represent a different group.[95] Therefore, NGPs differ from traditional probiotics because they are subject to different legal regulations and have the potential to contain genetically modified microorganisms.[95,96] In this context, it is important to design special recombinant probiotics developed through genetic engineering applications such as the CRISPR/Cas9 gene editing tool or plasmid-mediated recombination to eliminate various limiting factors such as the production of non-specific antimicrobials, the presence of mobile antimicrobial resistance genes, and the different levels of effectiveness in different hosts of traditional probiotics.[96–98] Effective colonization on the gastrointestinal tract, the production of specific therapeutic compounds, and the optimization of metabolic processes are made feasible by the development of genetically modified probiotics.[96,98,99]

On the other hand, the utilization of bioengineering solutions in the creation of NGPs capable of using prebiotics in order to increase the viability of NGPs is noteworthy.[112] Despite the technical advantages of NGPs, there are some constrains on the utilization of these organisms in foods including insufficient risk assessment on their safe use, limited isolation sources, difficulty in culturing conditions and, more importantly, legal restriction of the use of genetically modified organisms in many countries.[95]

4. Personalized nutrition and dairy-based probiotic beverages

Rapidly changing and fast lifestyle brings about various health problems. Metabolic syndrome, heart diseases, type 2 diabetes, irritable bowel syndrome, gastroesophageal reflux disease, and psychological disorders such as depression and anxiety are examples of health problems that have targeted many people in recent years.[113–118] This fact has directed consumers toward more nutritious foods, and countless diet options have been proposed to the consumers. The food industry has put more effort to meet this demand.[119] However, each diet applied causes different responses in individuals.[119–122] Since each person has a unique microbiota and metabolism, the idea of personalized nutrition becomes more popular today. Personalized nutrition describes a nutritional routine in which the nutritional order is recreated in accordance with the individual by examining complex diet–host microbiota interactions, considering that each individual's requirements are different.[123–125] At this point, due to the regulation of host-derived factors and their high diversity, shaping the microbiota with environmental factors such as dietary exposure is important in the control and prevention of diseases.[126] It has been suggested that probiotic supplementation in personalized diets can bring about modifications by influencing an individual's microbiota and, consequently, their metabolism. Phenotypic screening and a target-oriented bottom-up strategy have replaced the conventional, top-down approach in recent years, which involved assessing observational data and the findings of clinical trials involving humans and/or animals.[127] One of the topics that has gained interest recently is the development of tailored probiotics based on the needs of the individual by assessing the individual data and the parameters it influences collectively.[128] It is critical to modify foods and drinks that include probiotics to suit individual dietary needs and assess the results.

At this point, many dairy companies are developing specific fermented beverages and foods to meet individual needs.[74] The yogurt developed by Hori Nyugyo Dairy for women suffering from cold fingers that is claimed to regulate the temperature of the hands and feet of consumers and the triple yogurt offered by Morinaga Milk for consumption by individuals suffering from hypertension, containing the company's patented Met-Lys-Pro hydrolysed casein peptide, are listed as commercial examples of probiotic-based foods and beverages in personalized nutrition.[74,129,130]

Fermented milks supplemented with probiotics contribute to the regulation of intestinal flora by promoting the growth of beneficial bacteria in the host's microbiota and decreasing the generation of excess metabolites by harmful microorganisms.[131,132] Diet programs were tailored to meet the needs of individuals using a web-based program called RISTOMED. Consumers were presented diets supplemented with VSL#3 containing probiotic strains (*Bifidobacterium infantis* DSM 24737, *Bifidobacterium longum* DSM 24736, *Bifidobacterium breve* DSM 24732, *Lactobacillus acidophilus* DSM 24735, *Lactobacillus delbrueckii* ssp. *bulgaricus* DSM 24734, *Lacticaseibacillus paracasei* DSM 24733, *Lacticaseibacillus plantarum* DSM 24730, and *Streptococcus thermophilus* DSM 24731) for 8 weeks. Results showed that supplementation of VSL#3 increased the folate and vitamin B_{12} concentrations in individuals with reduced inflammation. Furthermore, supplementing with VSL#3 resulted in a decrease in homocysteine levels in individuals and an increase in colony counts of *Bifidobacterium* in the gut microbiome, suggesting that it may be protective against neurological and cardiovascular disorders.

Healey *et al.* demonstrated that healthy people who regularly consume fiber had a relatively stronger gut microbiota response to inulin-type fructan prebiotics than individuals whom consume fewer fiber-rich foods.[133] In this context, it is predicted that the consumption of probiotic-based dairy beverages containing inulin may cause a positive microbiota response in individuals. Modulation of faecal microbiota by probiotic species/strains such as *Lactobacillus* spp. is seen in some individuals,[134,135] but not in others.[125,136] The conflicting results are attributed to the unique microbiota of individuals, as well as the individual effect of personalized diets on host metabolism and the complexity of diet–host microbiota interactions.[125,137]

5. Future perspectives

The strain-dependent effects of probiotics, the metabolic and physiological factors of the host, and the balance of an individual's gut microbiota make probiotic beverage formulations complex. At this point, it is predicted that if individual-specific needs are taken into account in the development of probiotic-based dairy beverages, they can play an active role in the treatment and prevention of diseases, and thus a positive outcome can be obtained from the increasing interest in probiotic-based dairy beverages.

As discussed above, keeping probiotic microorganisms in sufficient numbers in milk-based beverages seems to be the most important challenge. This difficulty also limits the probiotic microorganism options that may be suitable for industrial applications. Although probiotic microorganisms are accepted in the GRAS status, they also carry the risk of weakening resistance to infections in individuals with weak immune systems. However, the risks of high consumption of postbiotics have not yet been clarified. It is essential to clarify a dose–response relationship for postbiotics. The accumulation of clinical data on the health effects of next-generation probiotic microorganisms and postbiotic/paraprobiotics and achieving a certain scientific satisfaction level will accelerate the adaptation of these functional components to industrial applications. In addition, the development of risk analysis methodologies for these products and understanding their behaviour against novel food processes (high hydrostatic pressure, pulsed electric field, ultrasonication, *etc.*) will be advantageous for industrial applications.

The difficulties in optimization of probiotic dairy-based beverages can be overcome with the adaptation of artificial intelligence and 3D printing technologies into personalized nutrition formulations.[124,138,139] Daily meals and physical activity are collected in a database through mobile applications designed to increase individual access to personalized nutrition. The data collected are stated to be very useful for developing a personalized diet plan that takes into account each person's needs and dietary goals.[140]

Author contributions

ŞT, CA and HBÖ: conceptualization. ŞT and CA: writing – original draft. HBÖ: writing – reviewing and editing of the final draft and handling of the revisions. All authors read and approved the final manuscript.

Visualization

All the tables used in the text have been prepared by HBÖ, ŞT and CA.

Data availability

There is no data availability in this review article.

Conflicts of interest

We have no competing interests to declare in this review.

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