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Feature Review

Shaping the Future of Probiotics and Prebiotics

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Recent and ongoing developments in microbiome science are enabling new frontiers of research for probiotics and prebiotics. Novel types, mechanisms, and applications currently under study have the potential to change scientific understanding as well as nutritional and healthcare applications of these interventions. The expansion of related fields of microbiome-targeted interventions, and an evolving landscape for implementation across regulatory, policy, prescriber, and consumer spheres, portends an era of significant change. In this review we examine recent, emerging, and anticipated trends in probiotic and prebiotic science, and create a vision for broad areas of developing influence in the field.

Background and Current State

Probiotics (see Glossary) and **prebiotics** have received escalating attention in recent years in the scientific, healthcare, and public arenas. Publicity around microbiome research has also broadened the public perception of microorganisms, beyond disease-causing agents that should be avoided, to a more rational view integrating an understanding of the beneficial roles of microorganisms in human health. In line with these advances, public awareness and acceptance of probiotics and prebiotics continues to expand [1], with probiotic industry growth estimated at 7% annually [2], and prebiotic growth forecast at 12.7% over the next 8 years [3].

While there is a general consumer view that probiotics and prebiotics are beneficial, there is still a gap in understanding on definitions of the terms 'probiotics' and 'prebiotics', their benefits to health, how they function, and where to find the best sources in food and healthcare products [1,4]. Both probiotics and prebiotics are increasingly incorporated into a wide range of foods, beverages, and topical products (even toilet paper), in some cases with questionable or no scientific validation of any health benefit to the host, as is the requirement of existing consensus definitions.

In this scientific field, definitions for both are clearly established, with the International Scientific Association for Probiotics and Prebiotics (ISAPP) having convened consensus panels whereby experts reviewed and published the science behind probiotics [5] and prebiotics [6]. The conclusions of these panels highlighted that they play an integral role in health status. Some key mechanisms have been elucidated (Box 1) and both have been used in a variety of health states, prophylactically and therapeutically.

Currently, multiple spheres of influence are acting on the probiotic and prebiotic fields (Figure 1). Broad technological advances in data collection and analytical tools are enabling the exploration of new candidate probiotics and prebiotics as well as providing deeper insights into their interactions with the microbiome and host. Interest continues to grow into new applications of probiotics and prebiotics across health conditions, body sites, population subgroups, and delivery formats.

Highlights

An expanding range of candidate probiotic species and prebiotic substrates is emerging to address newly elucidated data-driven microbial niches and host targets.

Overlapping with, and adjacent to, the probiotic and prebiotic fields, new variants of microbiome-modulating interventions are developing, including synbiotics, postbiotics, microbial consortia, live biotherapeutic products, and genetically modified organisms, with renewed interest in polyphenols, fibres, and fermented foods.

Personalised nutrition and precision medicine are beginning to influence the application of probiotics and prebiotics, with growing interest in modulation of microbial signatures of health and disease.

Demand for probiotics and prebiotics across divergent product formats is driving innovation in quality assurance techniques to measure dose, viability, and structural and functional integrity.

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Box 1. Mechanisms of Action of Probiotics and Prebiotics

Mechanisms of action of probiotics and prebiotics are complex, diverse, heterogeneous, and often strain- and compound-specific. While many have been described, there remain calls for increased understanding, especially structure–function explanations of observed health effects and long-term influences [134,152,153].

Probiotics interact with both the host and the microbiome via molecular effectors present on the cell structure or secreted as metabolic products. Probiotic metabolites can act on the microbiota by crossfeeding interactions, changes in the gastrointestinal microenvironment (e.g., pH lowering), competition for nutrients and binding sites, and inhibition of growth via the production of strain-specific antibacterial compounds including bacteriocins [133,152,153]. Such microbiota-directed effects contribute to the ability of probiotics to mediate health benefits in pathogen overgrowth states such as vaginal and oral dysbioses [153].

With regard to host cells, probiotic effector molecules can interact directly with receptors in intestinal epithelial, enteroendocrine, and immune cells as well as vagal afferent fibres. These interactions produce local gut effects, such as enhancement of intestinal barrier integrity and inflammation (e.g., via Toll-like receptors), as well as systemic effects via host immune, endocrine, and nervous system mediators [133,152,153]. Probiotics can also perform enzymatic metabolism of host compounds such as bile salts and ingested xenobiotics [152]. Specific probiotic surface-associated effector molecules include pili, lipoteichoic acids, exopolysaccharides, and various surface-layer proteins, many of which are strain-specific and therefore mediate the delivery of strain-specific effects [133,153].

Classical prebiotic effects are mediated through consumption of the substrate by specific groups within the microbiota, promoting their growth and metabolic activity. Provision of substrate to select group/s of bacteria can also indirectly influence other bacterial groups within the microbiome – promoting growth through crossfeeding interactions as well as inhibitory effects via pathogen displacement. Resulting changes in microbial composition and metabolite concentrations from prebiotic administration impact host epithelial, immune, nervous, and endocrine signalling and mediate health benefits such as improvements in bowel function, immune response, glucose and lipid metabolism, bone health, and regulation of appetite and satiety [6]. Chief by-products of bacterial prebiotic metabolism are the SCFAs acetate, butyrate, and propionate, which are well recognised to interact with these host systems and facilitate many prebiotic effects [10].

In addition to nutritive effects on microbes, prebiotic molecules are also recognised to interact directly with host receptors, modulating immune and gut epithelial cell signalling with local effects on inflammation and barrier function [154].

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Furthermore, evolution in regulatory frameworks, clinical guidelines and industry trends is influencing the implementation of probiotics and prebiotics into nutrition and healthcare. As our knowledge continues to expand in each of these fields, a broad and integrated review of trends shaping the future of probiotics and prebiotics is timely.

Probiotics – Novel Species, Health Targets, and Evaluation Frameworks

Traditionally, lactobacilli, bifidobacteria, and other lactic acid-producing bacteria (LAB) have been used as probiotics, primarily isolated from fermented dairy products and the faecal microbiome. As knowledge of the breadth of the human microbiome and its functions has expanded, the future holds a range of potential new discovery approaches [7] as well as new potential probiotic taxa. Developments in affordable complete genome sequencing and powerful cultivation methods have allowed isolation and characterisation of a new range of microorganisms from human microbiomes with potential health benefits and the opportunity to be developed as **next-generation probiotics** [8] (Figure 2). Various bacteria, such as *Roseburia intestinalis*, *Faecalibacterium prausnitzii, Eubacterium* spp., *Bacteroides* spp. and *Akkermansia muciniphila*, have been isolated from the human gut with growing interest in their probiotic potential [8,9]. These candidates represent a significant proportion of the currently cultivable human gut microbiome and offer physiological functions that are not always directly conferred by bifidobacteria or lactobacilli, such as the production of butyrate, propionate, and other bioactives [10]. Converting these species into industrially viable probiotics presents challenges as their requirement for rich growth media and anaerobic conditions adds cost and complexity, as well as investment in determining optimal fermentation and manufacturing processes over time. Despite these difficulties, *A. muciniphila* is one of the more promising candidates. Isolated in 2004 [11], it has been tested in preclinical animal models and shown to prevent development of obesity, with bacterial pasteurisation increasing stability and efficacy of the species. Initial proof-of-

concept studies have taken place in humans and shown that both live and pasteurised *A. muciniphila* is safe to use in humans and improves several metabolic parameters [12]. Live *A. muciniphila* is already on the market in a multispecies **synbiotic** preparation, containing inulin, *Bifidobacterium longum* subsp. *infantis* and other anaerobic bacteria (*Clostridium beijerinckii, Clostridium butyricum,* and *Anaerobutyricum hallii)* and was shown to improve glucose levels in type 2 diabetics [13].

The gut microbiome will not be the only source of new candidate probiotic strains (Figure 1). Niches of strong interest for discovery of new species, and as targets for intervention, include the female urogenital tract, oral cavity, nasopharyngeal tract, and skin [14–16]. Species or genera associated with health in these regions are being investigated as potential interventions to restore microbial populations and therefore physiological homeostasis in disease states. Examples include the skin commensal isolate *Staphylococcus hominis* for eczema and atopic dermatitis [17], and *Lactobacillus crispatus* for vaginal dysbiosis [18]. **Fermented foods** are the most common natural source of potentially probiotic strains of LAB, and consumption has been associated with significant health benefits, including reduced risk of type 2 diabetes and cardiovascular diseases [19] as well as a putatively beneficial metabolomic profile [20]. These foods are most likely the major source of LAB in the human gut microbiome [21] and show potential for future probiotic development. Fermented and unfermented food sources of future probiotics may include fruits, vegetables, grains/cereals, dairy, meat and fish products, and honey, as well as environmental sources such as soil [22].

In addition to the core heartlands of gut and immune health, emerging target conditions for probiotic therapy include subfertility [23], liver disease [24], mood disorders [25], cognition [26], oral health [27], asthma [28], metabolic disease [29], hypercholesterolaemia [30], and obesity [31].

Significant emphasis will be placed on investigating the safety of novel species and genera considered for the development of new probiotic products [8,9]. Many commonly exploited and currently available probiotic strains benefit from a **generally recognized as safe** (**GRAS**) status in the USA or belong to species with **qualified presumption of safety** (**QPS**) status with the European Food Safety Authority (EFSA), yet this is not yet the case for other candidate novel probiotic species that have no history of use. Submission through GRAS, QPS, and novel food frameworks may enable a path to commercialisation, and for pharmaceutical applications, novel regulatory frameworks are emerging, for example, the **live biotherapeutic products** category being defined by the Food and Drug Administration (FDA)[1] and the European Directorate for the Quality of Medicines [32]. A complete characterisation of strains from these new species will likely be required [33], comprising retrospective analysis of possible human disease linked with the taxa considered, full genome sequence, antibiotic resistance genes, toxin genes, transferrable genetic elements, virulence factors, proven safety in animal models, pharmacokinetics, pharmacodynamics, and Phase I–III trials. Many live biotherapeutic products with appropriate clinical evidence will fall within the current scientific definition of probiotics [5] (Figure 2), albeit attracting specific regulatory attention.

The discovery of defined therapeutic **microbial consortia** with network interactions and synergistic effects [34] will augment the development of single-strain organisms in the future and remain in the scope of the current probiotic definition, if well characterised [5] (Figure 2). Adjacent to probiotics, **postbiotics**[1] – microbial fragments and metabolites [35] – have been shown to share many, though not all, mechanisms of their live probiotic counterparts. Some new promising gut isolates will also most likely be commercialised under the postbiotic category, such as the

[1] An updated expert consensus panel definition, convened by ISAPP, is currently in press.

Glossary

Bioinformatics: computational analyses of biological data.
Candidate prebiotic oligosaccharides: oligomers that may fulfil the current criteria for prebiotic but lack (*in vivo*) confirmation currently.
Crossfeeding: whereby one or more metabolic products from microorganism(s) can serve as growth substrates for others.
Faecal microbial transplantation: the transfer (through a processed mixture of liquid stool) of healthy bacteria from a donor into the intestines of the patient (recipient).
Fatty acids: carboxylic acids, with aliphatic chains, that can be either saturated or unsaturated.
Fermentable fibres: dietary fibres broken down by microbial growth in the gut.
Fermented foods: foods and beverages that have involved microbial growth and activities.
Generally recognised as safe (GRAS): a notification to the FDA stating that a substance is generally recognised, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use.
Genetically modified organisms: organisms whose genetic material has been altered using genetic engineering techniques.
Gut-simulation models: *in vitro* systems that mimic the human or animal gut.
Live biotherapeutic products: biological products that contains live organisms and are applicable to the prevention, treatment, or cure of a disease.
Metabolome: the combined metabolic outputs of the host and its microbial content.
Metagenomic: the study of the collective genome of microorganisms from an environmental sample.
Microbial consortia: a mixture of microbial species with symbiotic interactions. It can include well-defined consortia with fully characterised members or undefined mixtures.
Microbiome signature: a characteristic pattern of microorganisms.
Next-generation probiotics: probiotics from genera with no history of use as probiotics, and which are likely to be delivered under drug regulatory frameworks.

abovementioned pasteurised *A. muciniphila* or bioactive proteins from this species that have shown beneficial effects [36].

Prebiotics – Novel Substances, Sources, and Applications

At the instigation of the prebiotic concept for gut microbiota management [37], and for many years since, the primary premise of prebiotics has been selectively fermented carbohydrates acting in the colon and modulating levels of resident lactobacilli and bifidobacteria said to elicit health effects. In recent years, **omics** techniques have improved mechanistic *in vitro* and *in vivo* research, as well as human clinical trials, to determine a fuller extent of prebiotic impacts. Current targets for prebiotics have now expanded, beyond LAB, to a wider range of microbial responders [6]. Similar to probiotics, these include candidate health-promoting genera such as *Roseburia* spp., *Eubacterium* spp., *Akkermansia* spp., *Christensenella* spp., *Propionibacterium* spp., and *Faecalibacterium* spp. Prebiotics may be used to stimulate the growth of these and other bacterial groups directly or indirectly through **crossfeeding** interactions. One of the key health-promoting benefits of such genera is their production of short-chain **fatty acids** (SCFAs) that regulate a range of gut and ex-gut functions, including gut epithelial and mucus barrier function, immunity, inflammation, glucose and lipid metabolism, energy expenditure, and satiety [10].

Currently, a narrow range of confirmed prebiotic substances exists, with galactans and fructans (e.g., inulin) dominating the market. The desire to stimulate a wider group of commensal organisms has allowed the development of novel candidate prebiotic compounds (Figure 2). These will likely include carbohydrate-based substances derived from plants – the source of traditional prebiotics such as inulin – but may also include those that mimic animal-derived substrates (oligosaccharides present in milk; O-linked glycans present in mucins), yeast-based substances, and many **non-carbohydrate substances** including polyphenolics, fatty acids, herbs, and other micronutrients. Over 8000 known **polyphenols** exist in plants, vegetables, and fruits, and many reach the colon intact, to be utilised by resident microorganisms [38]. Some polyphenols have been shown to have prebiotic potential, such as cranberry-rich extracts stimulating *A. muciniphila* [39], or to provide antimicrobial action against pathogens [40].

In the future, prebiotics will likely be isolated from novel sources (Figure 1) as focus on sustainability, cost, and scale emerges [3]. The 1.3 billion tons of food waste generated annually in the food chain[ii] represents a rich and sustainable source of natural bioactive ingredients. Many side streams from fruit, vegetable, and grain processing contain potential prebiotics, such as pectin from orange peel [41] and arabinoxylans from distillery and brewing waste [42]. Future prebiotic compounds may also be chemically or structurally modified by the application of sonication, high pressure, acid, enzyme and oxidation treatments, in order to modify functionality. Further, unique combinations of prebiotics in optimised mixtures may provide the ability to create new profiles of benefits [43].

There is also growing interest in the use of prebiotics to affect other microbiomes within the host, such as the female urogenital tract, oral cavity, and skin. As an example, prebiotic glucomannan hydrolysates have been shown to modulate the skin microbiome and reduce acne when administrated topically [44]. There is also interest in more targeted prebiotic delivery within the distal colon for treatment or prevention of colorectal cancer and ulcerative colitis. A mixture of different chain length prebiotics, or specific delivery technologies, may allow delivery of intact prebiotics towards the distal colon and selective stimulation of carbohydrate-metabolising genera therein, reducing local proteolysis and the concomitant production of undesirable metabolites. Such modulation of the colonic microbial **metabolome** to a healthier profile is likely to become a key target for prebiotics, beyond simple microbial growth promotion [45]. The ability of prebiotics to

Non-carbohydrate substrates: microbial growth factors that are independent of saccharolytic growth.
Omics: comprehensive analysis of complete genetic or molecular profiles of organisms, including genomics, transcriptomics, proteomics, or metabolomics.
Polyphenols: naturally occurring plant compounds containing phenol groups.
Postbiotics: [i]bacterial fragments with or without bioactive products of microbial growth that are of benefit for the host.
Prebiotic: a substrate that is selectively utilised by host microorganisms conferring a health benefit.
Probiotics: live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.
Qualified presumption of safety (QPS): a status granted to genera, species, or subspecies of microorganisms by the EFSA after an application is received and an assessment of available evidence on characterisation, safety, and intended use is conducted.
Synbiotics: a mixture, comprising live microorganisms and substrate(s) selectively utilised by host microorganisms, that confers a health benefit on the host.
Vitamins: organic molecules that are essential micronutrients to support an organism's metabolism.

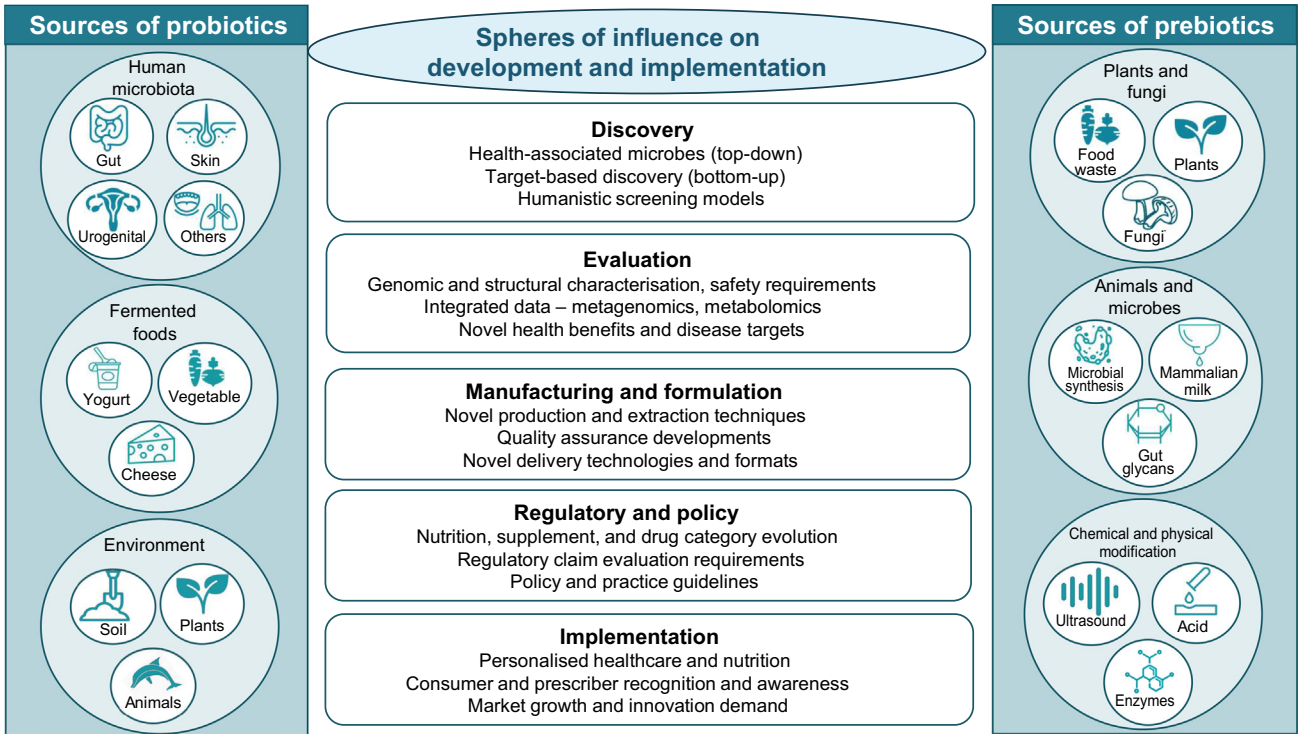

Figure 1. Influences on the Future of Prebiotics and Probiotics. The figure shows current and emerging influences on probiotics and prebiotics, including novel sources, new discovery and evaluation techniques, manufacturing and formulation advances, regulatory and policy changes, and influences on implementation in nutrition and healthcare. It is important to note that developments in one area influence growth in others – for example, the discovery of novel probiotic genera necessitates evolution in manufacturing techniques and regulatory frameworks.

control detrimental bacterial growth via pathogen exclusion and virulence attenuation is an area of interest and may extend beyond bacteria into viral pathogens [46]. Prebiotics, such as human milk oligosaccharides (HMOs), may also act as decoy receptors that prevent the attachment of pathogenic microorganisms, or via immunomodulatory interactions with host gut epithelial or immune cells [47].

As of December 2020, there were 245 registered clinical trials (ClinicalTrials.gov) which have completed evaluation of prebiotics (alone or in combination with probiotics) on ageing, autism, bariatric surgery, colic, colon cancer, atopic dermatitis, constipation, diarrhoea, infant growth, irritable bowel syndrome (IBS), obesity, and other conditions. The number of studies and investigational targets are suggestive of significant investment in the development of prebiotics as bioactive ingredients or supplements for a range of potential applications.

Stretching the Boundaries of Prebiotics

Polyphenols and other intestinal microbiome-modulating carbohydrates (including resistant starch, polydextrose, xylo-oligosaccharides, pectin, and HMOs) are considered prebiotic candidates but have not yet met the ISAPP consensus definition [6,48]. Also, overlapping and adjacent to the current boundaries of prebiotics (Figure 2) are **fermentable fibres** and other substrates such as **vitamins**, minerals, and fatty acids [6]. While all require further determination through *in vivo* studies, such compounds are emerging to be utilised by the indigenous microbiome and may have the potential to impart beneficial health effects upon the host.

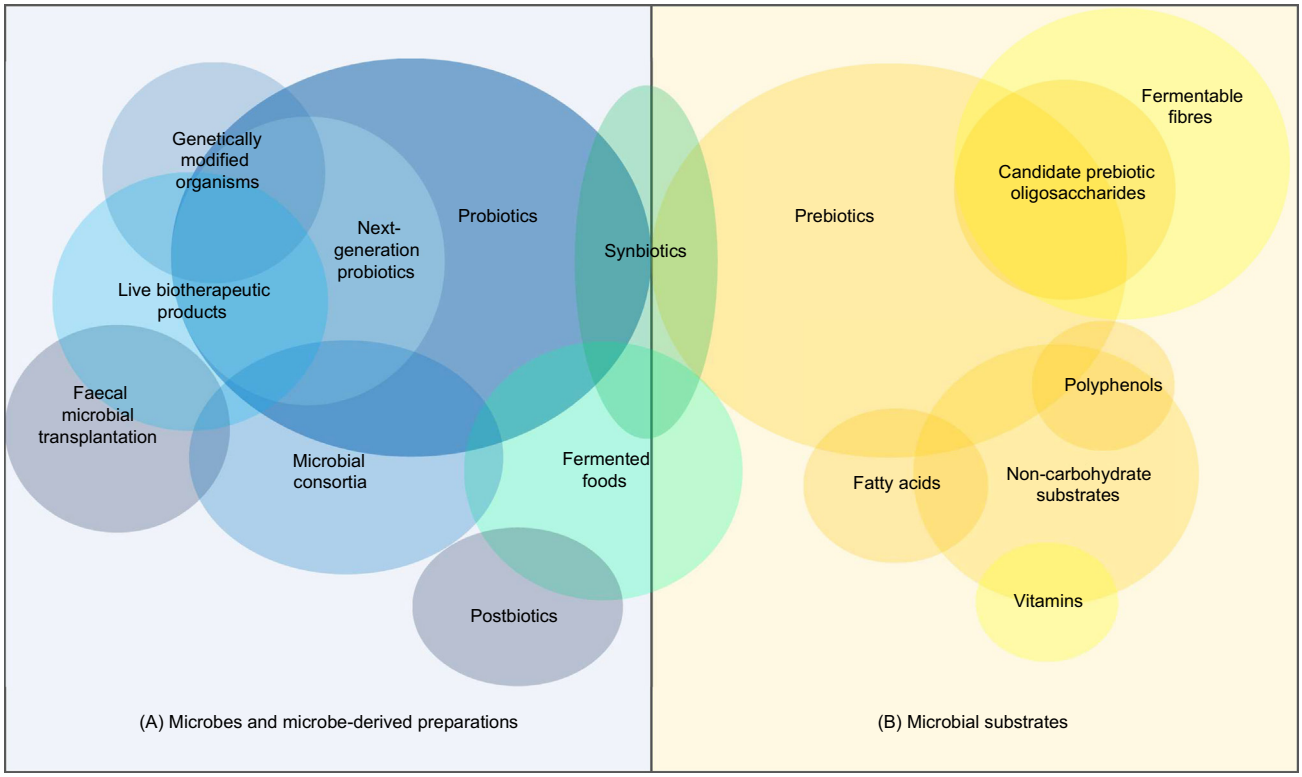

Figure 2. Probiotics, Prebiotics, and Adjacent Fields. Many areas of new discovery intersect and are adjacent to the currently defined fields of probiotics and prebiotics. The figure depicts a conceptual map of these established and emerging concepts, with (A) microbes and microbe-derived preparations represented in shades of blue; (B) microbial substrates represented in shades of yellow; and preparations containing both represented in green. Current and potential relationships between fields are depicted by the overlap of shapes (note: the size and shape representing each field is for visual purposes only). Refer to the Glossary for definitions of each field.

During the most recent decade, several papers have proposed alternative definitions of prebiotics, with broader scope in order to better integrate emerging microbiome-modulating compounds [49–51]. In particular, the requirement for 'selective utilisation' by a limited number of species or genera has been questioned (despite an overall consensus in the literature to the contrary). As our capacity has grown for detailed resolution and deeper understanding of the members and interactions within the microbiome, one assertion is that differentiating in a binary manner between growth effects on beneficial and nonbeneficial growth targets has become increasingly difficult. Proponents also note that some of the health benefits of currently recognised prebiotics may be ascribed to non-species-specific ecological or functional modulation of the microbiome, such as increases in SCFA production or broad compositional modulation [51]. It is important to recognise that blurring the boundaries between prebiotics and fermentable fibres in such a way would be in conflict with the premise upon which prebiotics were built, that is, as a method for selectively enhancing the growth of specific microorganisms with associated health benefits.

Other nonfermentable modulators of the microbiome also lie adjacent to the prebiotic scope (Figure 2) and will play a role in the future of microbiome modulation. While vitamins are normally absorbed in the small intestine, their administration in large amounts or in colon-targeted formulations can exert modulation of the colonic microbiome, as has been demonstrated with both riboflavin and niacin [52,53]. Furthermore, genomic studies have suggested that B vitamin

exchange may be a component of normal symbiotic relationships among gut microbial species [54], and several lines of evidence suggest a potential corrective role for colonic vitamin administration in some disease states [55].

Synbiotics and Complex Mixtures

Combining the effects of fermentable substrates and live microorganisms are blends known as synbiotics. Synbiotics may be complementary or synergistic in nature. Complementary synbiotics are comprised of a combination of an accepted prebiotic and probiotic, as their mechanisms of action can be independent of each other, and both the prebiotic and the probiotic must have their own demonstrated health benefits. Alternatively, synergistic synbiotics contain a fermentable substrate for the coadministered live microbe, where the substrate and the microbe may or may not be able to elicit a health benefit independently of the other. In this case the individual components do not necessarily need to be confirmed probiotics or prebiotics (Figure 2); however, they must have a demonstrated health benefit in combination [56]. Similar to the probiotic and prebiotic fields, the future of synbiotics will be influenced by the development of novel strains and substrates, informed by and targeted to vacant microbiome niches in individuals and subgroups, with potential applications in both gastrointestinal and ex-gut sites.

Other possible mixtures, which sit outside of the synbiotic definition, will likely be developed in the future, as food industry trends around gut health show no sign of slowing. Such mixtures may include combinations of live or killed microorganisms, coupled with potential microbiome-modulating compounds such as fermentable substrates, vitamins, and minerals, phytochemicals, and other plant-based materials. Fermented foods can be considered to be naturally occurring microbiome-targeted mixtures (Figure 2), delivering microbes and microbial substrates together, with a range of bioactive fermentation metabolites [19]. The increasing popularity of such foods will likely drive everyday consumer recognition of prebiotics, probiotics, and synbiotics moving forward.

Personalised Probiotics and Prebiotics

The search for individual and group **microbiome signatures** to predict disease incidence, progression, and response to treatment is a key area of microbiome research, enabled by efficient and powerful processing of large data sets [57]. Unique taxonomic profiles and specific genera and species have been associated with health and disease status [58] as well as host biomarkers, dietary and lifestyle characteristics [59] in large cross-sectional studies. Based on these data, there is significant interest in targeted strategies to modulate microbial composition within hosts on a personalised or population subgroup level. Probiotics and prebiotics present as promising candidate interventions with the potential to 'redirect' these signatures towards health [7,57,60], utilising multiple potential modes of action (Table 1).

One approach is to replace under-represented microbes in vacant taxonomic niches; however, LAB show only limited potential for direct microbial 'replenishment' strategies as they represent only a small proportion of the adult gut microbiome. While probiotics have demonstrated little impact or disruption to microbiome composition in healthy states [61], some lactobacilli and *Saccharomyces* species have demonstrated partial restoration of commensal microbiome groups in various manifestations and models of dysbiosis, including antibiotic administration, alcohol-induced disruption, and in children with cystic fibrosis [62–64]. Substantially modifying the adult microbiome in the future through replenishment strategies may be a possibility through the development of novel microbiome-derived probiotics (see earlier), with the majority of current candidates identified through top-down approaches as health-associated microbes [7]. However, it is also important to note that probiotics can exert independent health benefits outside

Table 1. Precision Application of Probiotics and Prebiotics – Potential Strategies

Target	Target component	Precision application strategy
Host	Health state	Target unique host health states • Targeted treatment of symptoms and disease states utilising specific clinical trialled probiotic strain/s or prebiotic compound/s
	Genotype, phenotype, environment	Tailor for unique host characteristics • Match suitability of specific probiotics and prebiotics for particular diet, lifestyle, demographic, genetic factors
Microbiome	Composition	Fill vacant compositional niches ('missing' microbes) • Reintroduce health-associated bacteria with microbiome-derived probiotics • Promote growth of under-represented species through targeted prebiotics or crossfeeding interactions from probiotics
		Reduce over-represented microbes • Inhibit growth of detrimental microbes by creating unfavourable environments, e.g., production of antimicrobial compounds
	Function	Fill vacant functional niches ('missing' functions) • Augment beneficial metabolite levels through provision of relevant prebiotic substrates and/or probiotics with specific metabolic capacity
		Reduce deleterious microbial metabolites • Inhibit the production, or support the catabolism, of detrimental compounds

of colonisation or microbiome modulation, and that probiotic ability to modulate the microbiome should not be considered a prerequisite for its utility.

Prebiotics may also assist to correct compositional imbalance through promoting the growth of under-represented species. While conventional prebiotics are primarily known for their bifidogenic effects, crossfeeding interactions have shown the potential of inulin to modify some other limited intestinal microbiome groups, including *Faecalibacterium* spp. and *Anaerostipes* spp. [65]. In the future, novel and emerging prebiotic compounds may be able to be used in targeted ways to manipulate the microbiome and its metabolic outputs. Many of the structural characteristics of prebiotics are known to influence which microbes can utilise the substrate, including monosaccharide structure, degree of polymerisation, branching, linkages, and addition of functional groups or other modifications [43]. Clinical intervention studies utilising multiple sources of either type II [66] or type IV [67] resistant starch, each with distinctive structural properties, have demonstrated modulation of specific taxa and SCFAs unique to each compound. To aggregate data from disparate studies, Lam and Cheung [43] proposed the creation of a multidimensional prebiotic structure–microbiome matrix, sequentially testing and collating data from prebiotic interventions and mapping the resulting microbiome impact from each structural variation. Such information could be teamed with machine learning to predict structural characteristics of a prebiotic required for the modulation of specific microbiome profiles, and lead to custom prebiotic and synbiotic production based on these characteristics [43]. Data collection and predictive modelling could also capture microbial metabolic interactions [57], layering in the potentially complex ecosystem effects of designer prebiotic administration and mixtures thereof.

Taxonomic microbiome characterisation is increasingly being combined with **metagenomic** or metabolomic data to understand what functions microbes might be performing. Integrated data sets may assist to identify loss of microbiome functions, or vacant 'functional niches', important to host health and providing further potential for precision medicine intervention [68]. A recent longitudinal study [69], employing metagenomic and metabolomic analyses in samples from IBS

patients and healthy controls, found differences in the levels of certain faecal metabolites and relative abundance of taxa between the IBS constipation and diarrhoea subtypes and healthy controls, as well as targets for potential probiotic intervention being proposed. For example, elevated levels of primary bile acids could be addressed by the introduction of bile acid-metabolising microbial consortia; suboptimal hypoxanthine production could be augmented through local microbial production or inhibition of enzymatic breakdown; and SCFA and tryptamine production could be increased for motility benefits in constipation [69]. Prebiotics and synbiotics may also assist to address over- or under-represented metabolic microbiome pathways in the future. Direct application of postbiotics to replenish missing compounds [60] is also a promising area of future investigation.

Other opportunities for precision application include targeting therapies based on the prediction of responders and nonresponders. It has been shown that specific prebiotics may be more able to confer a health benefit when they are given to an individual with appropriate baseline commensal microbes to utilise the substrate. Microbial composition at baseline has been demonstrated to predict microbiome response, fermentation rates, and butyrate production resulting from resistant starch [66,70] and hydrolysed guar gum [71] supplementation. Interindividual intestinal microbiome differences have also been linked to differential clinical response to prebiotics with regard to stool consistency [71] in healthy adults and hepatic lipid metabolism in hepatic steatosis patients [72]. This provides the possibility for more optimal matching of prebiotics to individual microbiome characteristics. Metabotypes will also likely play a key role in personalised prebiotic innovation into the future. The ability of individuals to metabolise polyphenols into smaller bioavailable metabolites through gut microbiota is one example. Specific bioactive molecules may only be produced from polyphenols in the presence of specific gut microbiota species [73], and production of these metabolites may serve as a useful surrogate marker of the ability of a subject to benefit from a given polyphenol.

Nonresponse is also an issue for probiotics [74], which may be overcome in the future with precision application. For probiotics, the baseline microbiome provides a degree of colonisation resistance that can impact the duration of residence as well as the penetration into the mucosal microbiome, and potentially impact biological activity [7]. Several studies have demonstrated variability in probiotic colonisation between individuals [75,76], either persistently or transiently during supplementation; however, whether there is a resulting effect on clinical outcomes is currently unknown. Zmora and colleagues [76] identified multiple host immune factors (as well as microbiome composition) as determinants of mucosal colonisation of administered probiotics. Transcriptomics from mucosal biopsies revealed caecal host immune activity against Gram-positive bacteria in colonisation-resistant individuals, inviting speculation of a personalised predisposition to the creation of an inhospitable environment to probiotics.

Vacant functional niches identified by metabolomic stool features may also be predictive of probiotic response. In children with gastroenteritis, a meaningful reduction in diarrhoea and lower intestinal inflammation in response to *Limosilactobacillus reuteri* DSM 17938 intervention was predicted by lower baseline levels of faecal metabolites, including lactate [77]. Authors suggested that a vacant metabolic niche may have existed in the responders that was amenable to the activity of lactobacilli. In healthy individuals administered *Lacticaseibacillus paracasei* DG, baseline levels of butyrate were found to predict the directional butyrate response to therapy [78]. These results provide the potential for faecal metabolomics to be incorporated into future baseline measures to observe and predict individual response to probiotic therapy.

Future research could support the realisation of possibilities in this field in a number of ways. Enhanced characterisation of the biological response to probiotics and prebiotics in clinical trials

would enable deeper understanding of these interventions and their potential for precision application. Calls have been made for increased use of integrated, multi-omic approaches to characterise probiotic and prebiotic effects, including metagenomic, metatranscriptomic, and metabolomic technologies [79,80]. Recognition of the relevance of effects within the mucosal microbiome will likely predict inclusion of more invasive sampling in future trials as well as the development of noninvasive techniques for sampling different regions of the gut [60]. Such deeper characterisation would be coupled with analysis and reporting of clinical and biomarker response at subgroup and individual levels. Improved reporting of responders and nonresponders in intervention trials would enable aggregation of individual or small-subgroup data into meta-analyses to better develop predictive modelling of microbial and host responses to prebiotics [79] and probiotics. Further, the inclusion of multiple prebiotics or probiotics in head-to-head comparative studies [7] will enable the detection of unique directional effects through direct comparison, and facilitate identification of optimal interventions for groups, subgroups, or individuals.

Hypotheses developed from these avenues of research could be tested prospectively in future clinical trials, with cohort selection or group stratification based on microbiome and other host factors, such as diet or genetics. Subject selection may be possible on baseline factors, or in the case of identifying metabotypes, a run-in period may be utilised to identify the response of a specific microbial species and/or the production of specific metabolites after a short period of intervention. The exclusion of a large portion of nonresponders in the screening phase of clinical trials would increase predicted effect sizes and effectively test personalisation hypotheses.

As effective biomarkers of probiotic and prebiotic response are identified, implementation into practice may warrant the conduct of extensive testing at the individual level [7], a potentially costly and burdensome activity. While microbiome testing services for the general public are commercially available, accessibility constraints such as cost and service delivery exist, and use in nutrition and healthcare is not yet widespread [81]. Furthermore, extensive validation and refinement of putative host or microbiome biomarkers will be required for clinical implementation. Ease of use through the integration of data sets into digitalised decision-making tools [7] will facilitate consumer nutrition and healthcare implementation alike, and effective dissemination through education and practice guidelines will influence readiness and acceptance from healthcare professionals to adopt a personalised approach in their practices [82].

Global Healthcare Challenges

Probiotics and prebiotics may also play a role in the future in addressing current and emerging healthcare challenges, including those caused by microbes.

The rise of antimicrobial-resistant pathogens is a World Health Organisation priority, and the use of antibiotics as growth promoters and for infection prophylaxis in animal husbandry has led to large-scale antimicrobial resistance [83]. While bans on their use as feed additives now exist in many Western nations[iii,iv], in middle-income countries antimicrobial use [84] and resistance [83] in livestock continues to increase. In the future, probiotics and prebiotics may be increasingly used as alternative growth-promoting and health-enhancing feed additives, due to their modulating effects on animal immunity, gut microbiota, feed intake, and productivity [85,86]. Certain probiotic strains have shown results in the decolonisation of antimicrobial-resistant pathogens from the human gut [87,88], and both probiotics and prebiotics may provide protective effects against multidrug-resistant infections via effects within the microbiome, epithelial barrier, and immune system [89], suggesting potential for a future role in reducing the burden of antimicrobial resistance in hospitals and healthcare. Furthermore, probiotics and their antimicrobial by-products are being investigated as novel future alternatives to antibiotics [90,91].

Over the last decade, the challenges of influenza and coronaviruses, including H1N1 (swine flu), severe acute respiratory syndrome coronavirus [SARS-CoV (SARS)], and SARS-CoV-2 [coronavirus disease 2019 (COVID-19)], have created massive healthcare, societal, and economic burdens. In the period between the emergence of a new mutant virus and the development and testing of an effective vaccine, safe and low-cost prophylactic agents with nonspecific immune, anti-inflammatory, and antiviral effects could provide an avenue for intervention. Probiotics and prebiotics have been suggested as candidate components of preventative and acute care strategies for COVID-19 infection [92–94]. Known for their ability to regulate multiple aspects of the immune response [95], a body of evidence has previously demonstrated the effectiveness of specific probiotics in preventing upper respiratory tract viral infections [96] and reducing the risk of ventilator-associated pneumonia [92]. One report on a small group of hospitalised COVID-19 patients found reduced morbidity and mortality with the addition of a multistrain probiotic formulation to standard care [97]. While widespread clinical data do not yet support their use for prevention or treatment of serious viral infections, this represents a potential future area of investigation for probiotic and prebiotic research to investigate their safety and effectiveness as adjunctive therapies.

New Discovery and Research Methodologies

Significant developments in technologies and associated methodologies have enabled many advances in the prebiotic and probiotic field in recent years and will continue to play a key role in the future. As the breadth of technologies and **bioinformatic** techniques expands, increased emphasis is being placed on the reproducibility of microbiome results and removal of bias introduced by protocols. Interpretative variations can enter at many stages, including collection and processing of samples, gene sequencing, database use, and data analyses, any of which may cloud biologically relevant signatures including interindividual variations [98]. Furthermore, sample size and statistical power will become increasingly important when conducting microbiome studies [99].

Microbiome-derived leads have generated many current candidate probiotic genera of interest. However, target-based approaches are also being widely utilised for the development of probiotics, where libraries of bacteria are screened *in silico*, *in vitro*, or *ex vivo* for mechanistic activity on host- or microbiome-related pathways of interest [7]. Furthermore, there are increasing regulatory and scientific demands to support clinically proven beneficial activities of prebiotics and probiotics with detailed mechanistic insights. These challenges are beginning to be met through the development, improvement, and implementation of existing and new discovery and research methodologies. An expanding group of 'omics' technologies, responsible for many recent advances, are now moving from stand-alone data-generating vehicles to fully integrated, systems biology-oriented 'meta'-technologies [100]. Future and ongoing efforts will no doubt improve current bioinformatics tools for data interrogation, integration and processing, with holistic predictions being facilitated through machine learning and artificial intelligence [101–103].

The cost of molecular methodologies has been decreasing, being powered by microfluidics and nanofluidics, which generate an astonishing amount of data. The miniaturisation of enzymatic reactions has also allowed for absolute quantification of molecules in droplet quantitative PCR reactions, including high-throughput qPCR and digital PCR [104], which rely on robotics to dispense volumes in the range of 10 nanolitres. Such technologies have permitted, for example, the absolute quantification of 12 *Bifidobacterium* species in response to prebiotic galactooligosaccharides (GOS), utilising volumes as low as 3 µl of total DNA [105]. Likewise, improvements in sequencing quality beyond traditional short-read next-generation sequencing, as well as longer-read generating platforms, including single-molecule real-time (SMRT) sequencing,

which has been developed based on the nonclonal amplification and sequencing of single DNA molecules [106], will also expand our ability to better characterise microorganisms to species and even strain levels.

Likewise, novel high-throughput cultivation approaches, such as single-cell encapsulation in droplets of a monodisperse microfluidic double water-in-oil-in-water emulsion (EMD) [107,108], the ichip [104], and employment of multiple culture conditions associated with long incubation periods, have permitted the identification of new microbial species [109]. Such novel isolates, for example antibiotic-producing bacteria with potential beneficial effects [107,110], can now be rapidly and comprehensively characterised through next-generation sequencing and liquid chromatography–mass spectrometry analysis. Recently developed instruments like the Prospector System (GALT, CA, USA), which allow the simultaneous cultivation and screening of isolates based on specific phenotypes, the ability to use substrates of interest like prebiotics, or the generation of metabolites, will rapidly advance the collection, characterisation, and application of novel strains.

Advances in bioinformatic platforms and parallel-computing workflows have enhanced our ability to convert the terabytes of sequence data into useable data in a fraction of the time, allowing for generation of probiotic databases and large-scale analyses of probiotic strains [111,112]. Similarly, movement of some software and wrapper programs into more user-friendly graphical user interfaces, rather than only being available through cumbersome command lines, has also helped to remove analysis barriers for probiotic and prebiotic effects.

Nonetheless, it should be borne in mind that many such technologies deliver *in vitro* information on potential prebiotics and probiotics that may not represent *in vivo* behaviour. A lack of translation of *in vitro* mechanisms to clinical effects can occur in probiotic and prebiotic development. Moreover, while *in vitro* fermentation models are currently used in development to simulate impacts of prebiotics and probiotics on the microbiome, such models do not replicate the host physiological interactions. Current efforts to improve these translation challenges include the development of humanistic models for screening, including humanised microbiome animal models, organoids from human biopsy samples, coculture experiments of epithelia and microbes, 'organ on a chip' models, and 'in human' discovery models. Such models will increasingly be used not only for discovery and prediction purposes but also to elucidate the mechanisms of action of previously demonstrated clinical effects. None will replace the necessity of *in vivo* assessments, which remain definitive tests for probiotic and prebiotic efficacy.

Quality Assurance Developments

There is demand for incorporation of temperature- and moisture-stable probiotics and prebiotics into novel foods and supplements, in increasing levels and complex combinations. This trend has placed a burden on the evolution of quality assurance methodologies to ensure stability, shelf life, batch consistency, and functional integrity of such bioactive ingredients [113–115]. Over the years, numerous studies have called into question the quality and purity of commercial probiotic products [116], and calls have been made for standardised assessment and certification tools to improve trust from end users and other stakeholders [2]. The same should be applied to prebiotics.

Appropriate identification of a probiotic strain indicates to which genus, species, and subspecies or strain it belongs [117], according to valid (and for lactobacilli, recently updated [118]) nomenclature. Conventionally, phenotypic methods of probiotic identification are used in commercial quality control laboratories, which provide low resolution, often to the genus level only [119].

The availability of whole-genome sequencing (WGS) and next-generation sequencing (NGS) have changed studies of microbial communities and are routinely used in food quality and safety practices [120]. However, these techniques are not currently widely adopted in quality control laboratories, despite their potential to enable accurate identification of probiotics in multistrain mixes, as well as the presence of potential contaminants [121,122]. Increased utilisation will depend on time-to-results reduction, cost effectiveness, and curation of databases[v] [123].

Microbial viability is classically measured through culturability, that is, colony-forming units (CFUs); however, this method has significant limitations, including an inability to enumerate organisms in a viable but nonculturable state [124]. Laboratories currently seek faster, cheaper, less labour-intensive, and more reliable methods, with applicability across various matrices and emerging new genera. Viability measurement by flow cytometry is available for certain species of LAB, and the combination with polyclonal antibodies shows promise for enumerating multistrain mixes [125]. Flow cytometry can also be applied to assess damage from oxidative stress [126]. Other emerging alternatives to plating methods include chip-based digital PCR, droplet digital PCR, or electrochemical magnetic bead-based immunosensor techniques [127–129]. Importantly, as the majority of probiotic efficacy trials report doses in CFUs, a rationale for equivalence of new quantification results to classical colony-count techniques will be needed for use in quality assurance of commercial products. Furthermore, widespread industry adoption of these approaches will require scaling to the routine laboratory, standardisation, and methodological validation [130]. Application of these new methods to test products during human intervention trials will also help to ensure implementation.

Improvements in the stability of live cells in products throughout shelf life across different product formats will continue to be a future focus. Understanding strain phenomics and fluxomics with big data and machine learning may also help mastering product efficacy and stability in various applications [131]. Furthermore, beyond simple probiotic viability measures, the integrity of extracellular structures, enzymatic activities, or other effector molecules may help to assess probiotic functionality in the final application [132], provided such candidate markers are able to demonstrate a robust role in probiotic efficacy [132–134].

For prebiotics, it is well established that they may be degraded by food-processing methods, including pH changes and heat [135,136], and therefore widespread adoption of standardised quality assurance methods for prebiotic products is an important step in the future of effective, reliable, and diverse products. Prebiotic quality assurance can take the form of chemical and structural analyses, which determine dose and maintenance of molecular integrity; or functional assays, to measure retention of activity (i.e., microbial metabolism of the prebiotic and the associated health benefit in final products). Relatively simple functional assays enable the quantification of prebiotic effects on bacterial growth [137] and have been successfully used to determine the effect of food-processing techniques on prebiotic properties [138]. These culture media-based assays which use single organisms, coupled with culture enumeration of the target microbes, may be superseded in routine use by more complex models in the future. **Gut-simulation models** capture the complex interaction of digestive processes and mixed microbial fermentation and are often combined with DNA-based methods to ensure comprehensive determination of microbial response [139]. Such techniques can be applied to prebiotic food products, overcoming the need for purification steps required for chemical analysis techniques.

Direct prebiotic compound analysis can take the form of chromatographic and electrophoretic methods for quantitative information, while spectroscopic techniques provide more detailed structural characterisation [140]. Gas chromatography and high-performance liquid chromatography

are frequently used for prebiotic determination [140]; however, their application in commercial food matrices is complex and might not always be accessible for routine quality control. To address this, a method of high-performance anion-exchange chromatography with pulsed amperometric detection has been coupled with characterisation by size-exclusion chromatography with multiangle light scattering and refractive index detection. This method provides a sensitive and reliable approach for accurate quantification of prebiotic compounds in complex dietary matrices without the need for fractionation [141]. Such methods may become increasingly important in the future as prebiotics become incorporated into an ever-expanding variety of foods, beverages, and other consumer products. Microstructural morphological examination via environmental scanning electron microscopy and detailed structural analysis via spectroscopic techniques can provide detailed information on new candidate prebiotics [142]; however, they are unlikely to be used in quality assurance laboratories on a routine basis. For novel polymeric prebiotics with highly complex structures, an approach similar to the use of trypsin for the study of complex proteins has been proposed, whereby structure-specific glycosidic hydrolases can be used to cleave oligomers more amendable to mass spectrometry approaches [43].

Implementation into Policy and Practice

While a convincing body of evidence [143,144] exists on the effectiveness of certain probiotics and prebiotics for a broad range of health applications, their consistent implementation into nutrition and healthcare remains limited. Sentiments from both healthcare practitioners and consumers commonly contain a degree of scepticism about the potential usefulness of probiotics and their level of clinical evidence [145].

The ability to communicate about probiotics and prebiotics to key stakeholders is heavily influenced by the regulatory environment. Probiotics and prebiotics hold different regulatory status across countries and regions, further differing in considerations for use in product formulations, health claims, labelling, or other end-user communication [146]. While many countries have successfully implemented specific regulatory frameworks and authorised evidence-based probiotic and prebiotic claims, in other jurisdictions approved claims are scarce and current communication about health benefits in the market is therefore limited. The European framework in particular (Box 2) is an important influence on the future of industry-driven probiotic and prebiotic research, given its stringent requirements and significant market size. The scientific and technical requirements for bringing products to market and for claims are therefore very diverse around the world, just considering food and dietary supplements. Stakeholders operating under distinct jurisdictions need to comply with these divergent frameworks, sometimes with different levels of scientific requirements for the same concepts [146]. Several evidence-based categorisations and straightforward criteria might be considered for probiotics [5,117]. Existing and new initiatives for further international harmonisation should be encouraged[vi]. Working towards convergent frameworks, and at the same time adapting these to upcoming scientific and technological discoveries will be one of the major implementation challenges for future prebiotics and probiotics. It is likely that a reconciliation between scientific recognition and regulatory acceptance is the way forward and further harmonisation of regulatory approaches will improve the uptake of prebiotic and probiotic products into mainstream nutrition and health care.

The translation of scientific findings into changes to healthcare practice has repeatedly been shown to be a lengthy and inconsistent process [147]. Strategies aimed at microbiome modulation for health maintenance and disease prevention are currently notably absent from most health care systems, although professional guidelines have made some recommendations [148]. While the growing field of implementation science aims to address common issues across many healthcare fields [149], a novel approach being carried out in the UK is direct political influence

Box 2. Challenges and Opportunities in a European Framework

The EU-wide framework for nutritional and health claims is a challenging case for probiotics and prebiotics in foods and food supplements. In this market, probiotic and prebiotic effects have been largely rejected by assessment panels for health claims, with notable exceptions [155,156], despite being based upon otherwise peer-reviewed scientific evidence, exploiting up-to-date technologies and being, in many cases, mechanistically driven [155–157].

The EFSA operates an ingredient-specific pre-market approval process for any health claim, with a rigorous evaluation process. Regulations specify rules for the authorisation of nutritional and health claims in Member States, clarified by the Commission in specific guidance. Interested companies must follow a specific application process. To prepare dossiers, applicants can rely on extensive guidance, revised according to experience in a process open to public consultation. The EFSA is responsible for evaluating the scientific basis of applications; it then publishes evaluations as Scientific Opinions in the open-access *EFSA Journal*[vii]. Finally, claims are authorised according to the final decision by the Commission and Member States, including actual claim wording and conditions of use. A public EU register, with over 2300 entries as of 2020, is available, including nonauthorised claims. Member States are responsible for implementing the regulations and the authorised claims.

As highlighted in specific reviews, few applications for prebiotics and probiotics have been successful, lacking either sufficient characterisation, a beneficial effect for human health, establishment of a cause-and-effect relationship, or biological plausibility [146,155–158]. This limited positive track record creates a high uncertainty and unpredictability, especially for future applicants in the always-evolving field of human health and nutrition. The EFSA follows technical and scientific developments and reviews its procedures and methodologies regularly, as illustrated by guidance on weight of evidence [159], biological relevance [160], on whole-genome sequencing data[viii], or the uptake of *Lactobacillus* genus taxonomy changes in QPS [161]. However, for future applications to be successful, further evolution in scientific standards will be necessary to accommodate future scientific and technological developments such as those described in this review, and especially cope with long-standing, wide-ranging trends such as systems biology and omics methods, personalised nutrition, or the role of the gut microbiome in human health.

via the translation of key microbiome science into emotive and relevant benefits for policy makers. In the All-Party Parliamentary Group (APPG) on the Human Gut Microbiome, a group of political campaigners and microbiome scientists aim to educate and convince key decision-makers (including politicians) to incorporate the role of the gut microbiome into policy. In the complex field of prebiotic and probiotic science, a fact-based 'storytelling' or narrative approach 'decodes' the science, making it accessible, relevant, comprehensible, and increases the chances of engagement and action [150,151]. Realising the full potential of probiotics and prebiotics in healthcare will require increased recognition at all levels, from consumers, prescribers, and governments, and subsequent integration into policy, practice, and lifestyle.

Concluding Remarks and Future Perspectives

The wealth of research into microbiome-targeted nutrition and therapeutics has expanded the fields of probiotics and prebiotics as well as many related interventions. Both within and outside of the current definitions, new probiotics and prebiotics will emerge, challenging scientific as well as regulatory definitions. Many substances will be derived from novel sources that meet economic and environmental needs to target a growing range of compositional and functional niches within the microbiome. Industry trends and consumer preferences will continue to drive demand for integration of probiotic, prebiotic, and other bioactive substances into a plethora of formats, supported by advancements in delivery technologies and quality assurance. While the gut will likely remain as the heartland of these therapies, clinically proven applications will continue to expand in the respiratory system, immune system, urogenital tract, skin, nervous system, oral cavity, cardiometabolic system, and weight-management field. Emerging healthcare challenges will drive research into new areas of global health importance, and a growing body of evidence for key applications will guide increased implementation in healthcare policy and practice.

Accelerating advances in biotechnology and bioinformatics show no sign of slowing and will provide detailed mechanistic insights into the action of prebiotics and probiotics as well as leads to identify new candidate organisms and substrates. Discovery and validation techniques will

Outstanding Questions

Can we facilitate the reintroduction of 'missing' desirable microbial species into vacant niches in a microbiome?

Will novel probiotic strains prove to be safe and achieve relevant health outcomes?

Will the most promising probiotics of the future be derived from human, animal, or plant hosts, foods or other sources?

Can we achieve better translation of candidate microbes and substrates to proven probiotics and prebiotics with health benefits, through the development of *in situ* and in-human discovery models?

How do we move beyond binary methods of microbial classification from 'health-associated/beneficial' or 'disease-associated/detrimental', to more sophisticated understanding of ecosystem interactions, functionality, and therapeutic targets?

What are the host-related or microbiome-related factors that determine individual response or nonresponse to probiotics and prebiotics?

How can we continue to elucidate a deeper mechanistic understanding of probiotic and prebiotic mechanisms of actions?

Will the putative effects of prebiotics on pathogen exclusion and virulence attenuation provide a useful pathogen-control strategy for the microbiome?

Will synbiotics provide significantly enhanced health benefits compared with individual probiotic and prebiotic application, due to cooperative interactions in the intestine?

Will the advent of live biotherapeutic products and genetically modified organisms alter the public perception and utilisation of probiotics?

What other metabolic pathways in microbes (such as vitamin utilisation in the colonic microbiome) can be exploited for microbiome modulation?

Which structural or functional features of prebiotics and probiotics will be

continue to undergo refinement, increasing reliability and reproducibility of study findings. This will further enable the comparability of data sets and larger aggregate insights from multiple research streams. These insights, as well as continued investment into large intervention and population-based studies, will uncover new ways to improve dietary relevance and clinical efficacy as well as target these interventions and tailor them to individuals' biology and microbiome. Such a vision is our predicted future of probiotics and prebiotics (see also Outstanding Questions).

most informative for verification in quality assurance assays for food and supplement products?

How can effective messages about probiotic and prebiotic effects be translated to consumers, health agencies, and key opinion leaders?

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Declaration of Interests

M.C., V.B., R.G., D.G., S.M., D.O., M.I.P., and R.E.S. are involved in research and development projects as employees of companies that produce, sell, and distribute food and/or healthcare products, including probiotics and prebiotics (M.C., Metagenics; V.B., General Mills; R.G., GSK Consumer Healthcare; D.G., Symrise Nutrition; S.M., Reckitt Benckiser; D.O., Danone Nutricia Research; M.I.P., Winclove Probiotics; R.E.S., DSM Nutritional Products).

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M.A.A., A.B., K.H., K.S.S., D.S., J.V., and G.G. have no conflicts of interest to declare.

Resources

[i]www.fda.gov/files/vaccines,%20blood%20&%20biologics/published/Early-Clinical-Trials-With-Live-Biotherapeutic-Products–Chemistry–Manufacturing–and-Control-Information–Guidance-for-Industry.pdf

[ii]www.fao.org/food-loss-and-food-waste/en

[iii]https://ec.europa.eu/commission/presscorner/detail/en/IP_05_1687

[iv]www.accessscience.com/content/u-s-bans-antibiotics-use-for-enhancing-growth-in-livestock/BR0125171

[v]www.genome.gov/about-genomics/fact-sheets/DNA-Sequencing-Costs-Data

[vi]www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-720-39%252FCRDs%252FCRD03.pdf

[vii]https://efsa.onlinelibrary.wiley.com/journal/18314732

[viii]www.efsa.europa.eu/en/consultations/call/public-consultation-efsa-statement-requirements-whole-genome

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