

Next-generation probiotics: an outlook into current applications and future developments

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Abstract

The probiotics field, a historically popular yet scientifically debated discipline, is moving beyond a decades-long promotion of ‘first-generation’ food-derived strains towards the development of ‘next-generation probiotics’ (NGP) or ‘precision probiotics’, natural and engineered strains featuring improved human colonization, clinical efficacy and safety profiles. In this Review, we outline the evolution of NGP and means by which their development is designed to tackle challenges of live bacterial therapy related to colonization resistance, in-host evolution, long-term safety and insufficient understanding of therapeutic and off-target mechanisms of activity. We showcase how a variety of emerging strategies enable the identification of NGP strains and define consortia featuring therapeutic potentials in metabolic, immune and oncological diseases. Finally, we discuss how computational and artificial intelligence (AI) advances can reshape NGP development, including AI-based discovery of strains and bioactive compounds; computational-driven design of engineered microorganisms and multi-kingdom consortia; and AI-assisted structural and metabolic network-based modelling predicting personalized NGP function, interactions and therapeutic impacts.

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Review article

Introduction

Probiotics are formally defined as “live microorganisms that when administered in adequate amounts, confer a health benefit on the host”¹. Effective probiotics should combine environmental resilience², competitiveness within the ecological niche, and bioactivity such as an ability to modify immune responses³. Suggested probiotic functional outputs include, among others, production of metabolites that affect host metabolism and health or the fitness of the microbiome, the release of antimicrobial compounds^{4,5}, modulation of bile acid pools⁶, enzymatic detoxification⁷, cytokine-modulated immune regulation³ and pathogen suppression^{1,8,9}.

‘First-generation’ probiotics is an informal term relating to the timeline of development in probiotic approaches and research (Fig. 1). This term refers mostly to the classical food-derived strains chosen

for their preservation properties and/or evidence for enrichment in healthy individuals (for example, *Lactobacillus*¹⁰, *Bifidobacterium*¹⁰ and *Saccharomyces*¹¹ species, among others). In some cases, probiotics are prescribed along with prebiotics (hence termed ‘synbiotics’), specialized plant fibres and complex carbohydrates that are indigestible by the host but act as food for beneficial gut microorganisms^{12–14} (Fig. 1).

Until today, most ‘first-generation’ probiotic formulations are being marketed and regulated as food supplements or dietary ingredients rather than as pharmaceutical drugs. This classification exempted them from the rigorous, multi-phase clinical trials and approval processes required for medical treatments¹⁵. Consequently, probiotics have achieved substantial commercial success, with the global market reaching approximately US\$86 billion in 2025 (ref. 16). Although widely used, their efficacy and safety remain subjects of an intense debate⁹.

Traditional probiotics

Classification



Probiotics — first generation

Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host



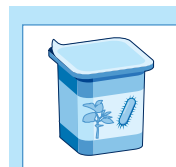
Prebiotics

Plant fibres and complex carbohydrates that are indigestible by the host but act as food for beneficial gut microorganisms

Key features

Chosen for preservation properties and/or evidence for enrichment in healthy individuals, with established safety histories and clinical data

Nutritional intervention exploited to improve host health by enrichment of gut microbiome diversity for preventive measures, or against pathological conditions



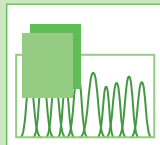
Synbiotics

Classical probiotics strains paired with selective prebiotics as a food source for the probiotic microorganisms

The combined approach promotes survival of the probiotic strain in the gut, thus improving efficiency

Next-generation probiotics

Classification



Probiotics — next generation

Live microorganisms derived directly from the human gut, ensuring better adaptation to the intestinal environment



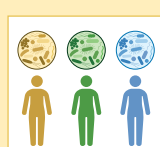
Engineered probiotics

Microorganisms produced by modifying original probiotics through gene or chemical editing

Key features

Identified through advanced comparative analysis of the gut microbiota using next-generation sequencing and bioinformatics tools

Designed to compensate for natural probiotics limitations while providing enhanced therapeutic benefits



Precision probiotics

Host or microbiome-guided selection and engineered strains tailored for individual patients

The most advanced step in probiotic research; a targeted approach motivated by evidence that colonization and response to empiric probiotics are highly personalized

Fig. 1 | Evolution of next-generation probiotics therapy.

The field of probiotics has seen much growth and development over time, from the discovery of the first probiotics strain, through the integration of diet, improving niche compatibility and maximizing probiotic adaptability. With the rise of technological advancements came further optimization of probiotics strains, and the development of engineered next-generation probiotics opened the field for a more precise therapeutic potential. The most recent stage of this evolution is precision probiotics, designed with a bottom-up approach wherein personalized target-based screenings allow tailored probiotic therapies.

A notable exception is the Biocodex *Saccharomyces boulardii* CNCM I-745 strain, which has been recognized as a medicinal product by several European regulatory authorities for the treatment of antibiotic-associated diarrhoea¹⁷.

Contemporaneous with these regulatory nuances and impactful novel findings on the gut microbiome, the spectrum of probiotic strains and their applications have recently broadened, giving rise to next-generation probiotics (NGP)⁸. Unlike first-generation strains, NGP are typically identified through advanced comparative analysis of the gut microbiome, using next-generation sequencing and bioinformatics to isolate bacteria demonstrating specific health benefits. These strains are often derived directly from the human gut, ensuring better adaptation to the intestinal environment and high abundance, thereby improving their therapeutic scope⁸ (Fig. 1). Furthermore, the development of engineered probiotics (achieved through genetic and chemical modification) offers a strategy to address colonization and clinical efficacy limitations¹⁸ (Fig. 1). The most substantial evidence of this shifting landscape is the recent US Food and Drug Administration (FDA) approval of the first live biotherapeutic products (LBPs). This category includes REBYOTA (faecal microbiota, live-jslm)¹⁹ and VOWST (faecal microbiota spores, live-brpk)²⁰, the latter being a defined microbial consortium developed by Seres Therapeutics to prevent the recurrence of *Clostridium difficile* infection^{21,22}. As the first orally administered, FDA-approved microbiome-based therapy, VOWST represents a landmark transition from traditional supplements to rigorously regulated NGP consortia. Other LBP indications are currently in various phases of clinical trials for treating disorders such as antibiotic-resistant infections and complications associated with allogeneic stem cell transplants^{23,24}.

'Precision probiotics' is a term commonly interchangeable with NGP, referring to host or microbiome-guided selection and engineered strains tailored for individual patients^{25,26}. Precision probiotics constitute a targeted approach motivated by evidence that colonization and response to empiric probiotics are highly personalized and represent a recent and advanced step in probiotic research^{25,26} (Fig. 1). Recent advances in artificial intelligence (AI) and machine learning present compelling opportunities to further refine the personalized match between host, condition and NGP strains^{27,28}. Given the frequently overlapping use of the terms NGP and precision probiotics, we refer to both as NGP. In this Review, we explore means by which past probiotics challenges may be met through NGP use; we examine how present and emerging strategies are facilitating the development of NGP in the prevention and treatment of human communicable and non-communicable diseases, and we highlight how future use of AI may transform rational NGP design towards enhanced personalization, efficacy and safety.

Meeting past probiotic challenges

Several reasons account for the lack of generalizability of clinical efficacy of many formulations of first-generation probiotics. One reason is that they constituted a one-size-fits-all approach that often ignored the vast interpersonal variability of the host microbiome²⁹ and disease context. As such, many first-generation probiotic strains failed to stably engraft in the host, as they were unable to overcome the colonization resistance induced by the resident commensal ecosystem³⁰. Additionally, the suggested health benefits of many first-generation probiotics remained debated⁹, and in some cases, such health claims lacked sufficient scientifically proven mechanisms of activity, further contributing to a scepticism of their promoted generalizable effects³¹.

Additional factors that limit our ability to evaluate clinical efficacy include studies not carefully considering differences in the strains examined, the dosages administered, the methods of administration, and the duration of treatment³². Although first-generation probiotics are consumed by millions worldwide and generally regarded as safe for human use, safety concerns have been reported among populations at risk, including cases of fatal systemic probiotics-driven sepsis in immunocompromised hosts^{33,34}, and a probiotics-induced induction of long-term dysbiosis upon co-administration with antibiotics³⁵. Strain selection of NGP that takes colonization capacity, efficacy and safety into account may help to address these challenges. For example, a *Bacteroides fragilis* strain isolated from human stool, reflecting adaptation to the human gut niche and a capacity to support the growth of other commensals, was shown to protect against *C. difficile*-associated disease in preclinical rodent models³⁶. Although evaluated in animals, this finding illustrates how selecting strains with ecological fitness for the human gut may translate into functional benefits³⁶. Additionally, the understanding of complex NGP effects and mechanisms of activity may enable to decode complex, disease-specific and at times opposing clinical effects. For example, the commensal anaerobe NGP *Akkermansia muciniphila* has been shown to modulate gut barrier integrity through its secreted protein Amuc_1409 that increases the dissociation of E-cadherin- β -catenin complex and results in the activation of WNT- β -catenin signalling³⁷ and the reduction of inflammation³⁸. Furthermore, *A. muciniphila* has been shown to confer health effects in cardiometabolic disease³⁹ and amyotrophic lateral sclerosis, potentially through nicotinamide⁴⁰. However, in specific dietary restrictive conditions, excessive mucus degradation by *A. muciniphila* can be detrimental and increase the risk for colitis and infectious diseases⁴¹. Multiple sclerosis is associated with elevated levels of *A. muciniphila*; however, this effect might be compensatory and is controversially discussed⁴². In all, the immense promise of designing NGP for specific functions is dependent on overcoming these fundamental biological obstacles, while reaching an in-depth understanding of mechanisms of colonization, interspecies communications and trans-kingdom downstream effects. In the following sections, we discuss some of these major challenges and explore the emerging strategies and prospects used by the NGP field towards their resolution.

Tackling the colonization challenge

A distinction is commonly made between transient passage, in which ingested probiotics are temporarily detectable during or shortly after supplementation and stable engraftment, which describes the integration and persistence of a strain within the microbial community of the host beyond the supplementation period⁴³. Traditional assessment of probiotic 'colonization' involved an indirect quantification of probiotic faecal accumulation²⁶. A direct and invasive assessment of transient or persistent probiotic colonization along the human gastrointestinal tract (GIT) has been rarely performed and has identified a substantial proportion of humans featuring a marked colonization resistance to exogenous probiotics²⁶. In colonization-resistant probiotics consumers, the exogenous strains accumulated in stool following their oral consumption yet failed to approximate to the intestinal mucosal layer or induce a quantifiable niche-specific change in the host gut transcriptome²⁶. Whereas some argue that transient passage in the absence of any colonization may confer health benefits and would be arguably beneficial when it comes to FDA approvals⁴⁴, most others agree that sustained therapeutic NGP effects necessitates a stable albeit transient engraftment⁴⁵. The GIT is the most common target for probiotic

Box 1 | Challenges of next-generation probiotics colonization across human body niches

Key physicochemical and biological barriers can limit the colonization and efficacy of probiotics in different anatomical sites^{187–192}, as detailed below.

Oral application

The oral cavity exhibits a pH ranging from 5.6 to 7.49, which can transiently decrease to more acidic levels in the morning and following food intake. Saliva serves as a critical host factor, containing digestive enzymes and other antimicrobial components that regulate microbial populations and contribute to the maintenance of oral homeostasis, acting as a first-line defence against colonization of bacteria including probiotics¹⁸⁷. In the stomach, the enzyme pepsin, together with a highly acidic environment with a pH between 1.0 and 3.5 (ref. 188), functions as a major host defence mechanism by inhibiting the survival and growth of most ingested microorganisms, including probiotic bacteria. Within the gastrointestinal tract (GIT), the pH ranges from 6.0 to 7.4 (ref. 188), providing a more favourable environment for microbial colonization. Host factors such as bile acids have a key role in modulating microbial composition and activity. Furthermore, colonization resistance in the GIT is maintained through the action of bacteriocins, microcins, antimicrobial peptides, short-chain fatty acids and nutrient depletion, all of which contribute to limiting the establishment and overgrowth of exogenous microorganisms. In addition, the oxygen gradient needs to be considered when colonizing bacteria.

Suppositories

The vaginal microbiome is sustained within a pH range of 3.8 to 5.0 (ref. 189), primarily maintained by acid-producing *Lactobacillus* species, which generate lactic acid and other antimicrobial metabolites that inhibit the colonization of pathogenic bacteria and fungi.

Topical application

The skin represents another acidic habitat, with a pH between 4.1 and 5.8 (ref. 190). Its physicochemical properties, including bactericidal fatty acids, a dry surface and high salt concentrations, serve as natural host defence factors. Additionally, serine proteases Esp2 and Esp3 contribute to colonization resistance by enhancing antimicrobial activity and preventing excessive microbial proliferation.

Inhalation

Other tissues, including those in the lung, are thought to contain a low biomass microbiome¹⁹¹, yet this notion is debated. Understanding the potential colonization (or lack thereof) of these 'sterile' niches is required in validating or refuting such low biomass colonization and its ramifications on tissue-specific functions. With respect to inhalational administration of probiotics, even though mice have been successfully treated with inhalation of probiotic lactobacilli¹⁹², the safety of live bacterial inhalation in humans is not established. The lung is a sensitive organ, requiring careful consideration of delivery strategies, dosing, bacterial viability and the potential for infection or unwanted immune response.

interventions and provides several hurdles to exogenous bacterial colonization (Box 1). Although encapsulating bacteria can overcome some of the hurdles associated with extreme gastric conditions^{46,47}, bile and digestive enzyme-induced bacterial inactivation and shifting oxygen gradient along the GIT and host specific transit time⁴⁸ remain barriers to probiotic colonization^{2,49}. Genomic analyses of *Clostridium perfringens* strains from preterm infants highlight that closely related strains can differ markedly in virulence, underscoring the importance of strain-level variations in colonization and host interaction⁵⁰. Probiotic strain variations can also result in marked differences in bacterial behaviour^{51,52}, including an impact on the ability to colonize the GIT⁵³. Suggested mechanisms proposed to drive such strain-specific colonization patterns include altered expression of surface proteins⁵⁴ and variations in antibiotic resistance⁵⁵ or fitness⁵⁶.

Beyond these biophysical and probiotic strain-related factors, the endogenous microbiome remains one of the most dominant barriers to exogenous bacterial colonization along the human GIT⁵⁷. *Escherichia coli* Nissle strain, for example, produces bacteriocins, microcins and other competitive molecules that actively inhibit the establishment of other competing strains⁵⁸. Other commensal mechanisms that prevent microbial invasion include the secretion of metabolites such as short-chain fatty acids (SCFAs), acetate, butyrate and propionate, which inhibited colonization of *Citrobacter rodentium* in a mouse infection model⁵⁹; and a variety of innate and adaptive immune mechanisms induced by prokaryotic and eukaryotic commensals, such as secreted anti-microbial peptides (Fig. 2a). Interpersonal variations in the microbiome can lead to differences in the abundance of functional enzymes such as peptidoglycan hydrolases produced by some archaea^{60–62},

metabolites such as secondary bile acids including deoxycholic acid^{63,64} and inter-individual host variability in anti-microbial immune responses. These factors could potentially modulate the responses to colonizing NGP by directly, or indirectly (through induction of host immune responses) shaping how incoming bacteria compete, integrate or fail to incorporate into the local microbial ecosystem⁶⁵. Cross-commensal interactions may further contribute to such colonization resistance. For example, as shown in faecal samples of hospitalized patients and mice with acute leukaemia, *Lactobacillus rhamnosus* and *Ligilactobacillus murinus* promote the growth of butyrate-producing *Clostridiales* species, which in turn deplete nutrient sources available to pathogenic multidrug-resistant Enterobacteriaceae⁶⁶ (Fig. 2a). Similarly, *Lactobacillus gasseri*, but not *Lactobacillus acidophilus*, has been reported to enhance the growth of Muribaculaceae family members, thereby preventing *Clostridioides difficile* colonization in mice⁶⁷ (Fig. 2a). Similar complex interspecies microbial interaction dynamics within a microbial community can influence not only pathogen resistance but also the establishment and persistence of probiotic strains²⁶. Antibiotic treatment can help to free up new niches in facilitating probiotic bacterial colonization, but some evidence suggests that it comes at the cost of probiotic-mediated prevention of the re-colonization of beneficial gut commensals³⁵.

Diagnostically, the functional classification of NGP based on metabolic or ecological traits, rather than on traditional species-level taxonomy, may help to circumvent conserved interspecies defence strategies such as niche occupation⁶⁸, resource competition⁶⁹ or bacteriocin-mediated⁷⁰ exclusion. Furthermore, the prediction of resistance to NGP colonization would benefit from the development

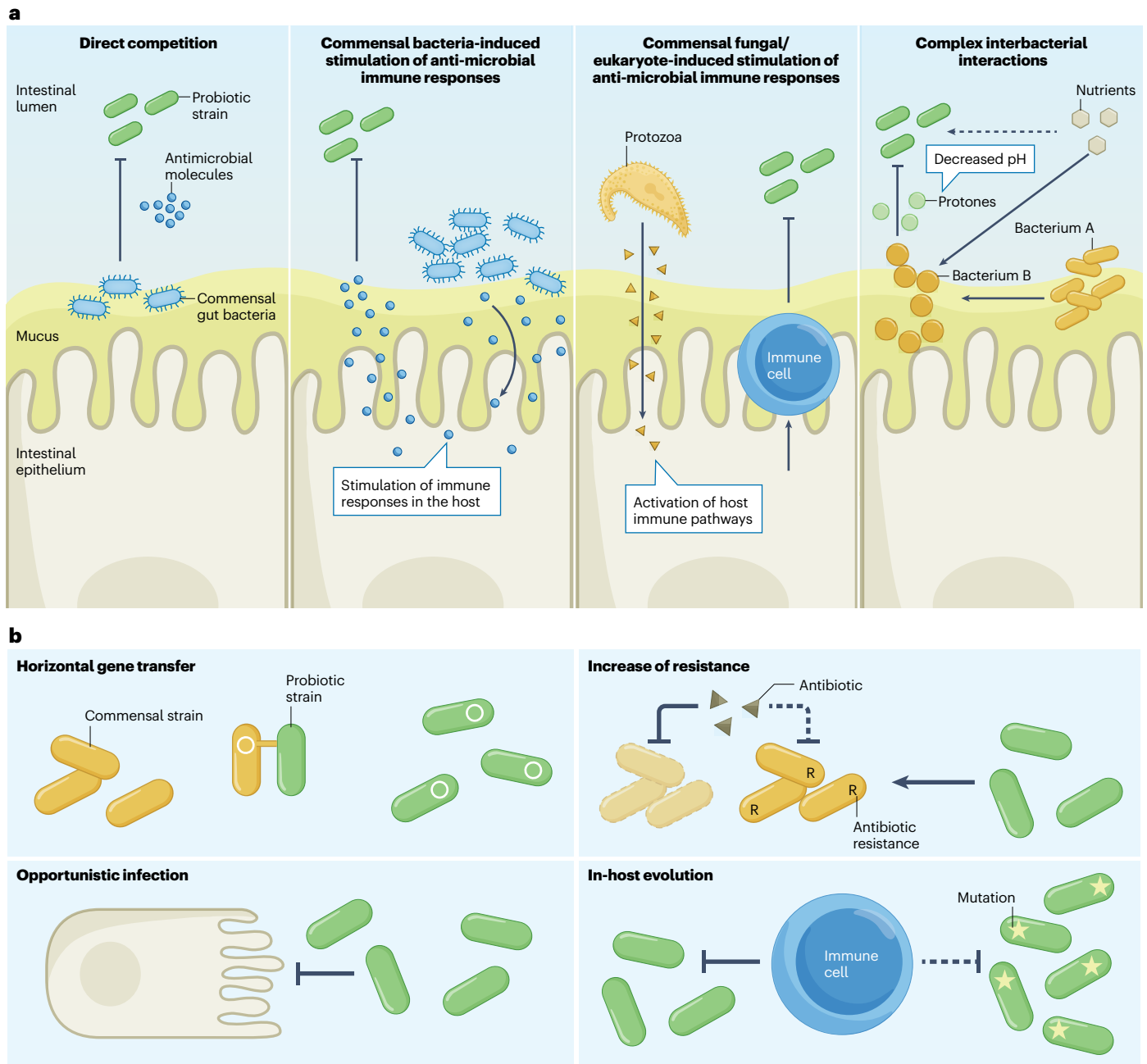


Fig. 2 | Challenges and risks of NGP use. a, Colonization resistance can prevent the establishment of next-generation probiotics (NGP) through different mechanisms: commensal gut bacteria release antimicrobial molecules, which inhibit the growth and colonization of competing microorganisms. Additionally, some commensal bacteria stimulate host immune responses, thereby enhancing the ability of the host to block colonization by exogenous bacteria. Fungi, protozoa and other resident eukaryotic gut microorganisms can also activate host immune pathways that contribute to colonization resistance to probiotics. Furthermore, complex interactions among commensal species have a crucial role in maintaining microbial balance. For example, bacterium A may promote the growth of bacterium B, which in turn acidifies the local environment and competes for nutrients required by invading bacteria. These cooperative and

competitive interactions collectively prevent the establishment of foreign microbial species, including NGP. **b**, Potential risks associated with NGP include the transfer of genetic elements such as plasmids from genetically modified strains to other members of the microbial community, raising concerns about horizontal gene transfer of virulent microbial traits. Moreover, probiotics have been shown to increase the resistome (the collection of antibiotic resistance genes within the commensal microbiome) through mechanisms that are not yet fully understood. In immunocompromised individuals, strains previously considered harmless may act as opportunistic pathogens. Finally, once bacteria colonize the host, they may undergo in-host evolution, adapting to their specific ecological niche, which could lead to unintended or potentially harmful phenotypic changes over time.

of non-invasive ‘companion diagnostic’ methods that assess the existing microbiome composition of an individual⁷¹ and its colonization-resistance mechanisms, thus informing the use of compatible NGP. Untangling the role of secreted metabolites and their antimicrobial function could further help in building such colonization-focused predictive networks. Therapeutically, microbiome-targeted interventions aiming to alter the function of the ecosystem towards a more ‘NGP welcoming’ configuration may include dietary interventions that modulate microbial community composition and metabolic capacity⁷². As such, individualized or standardized dietary regimens before probiotic administration may enhance bacterial survival⁷³ and, thereby, enhance colonization potential. Other approaches may include metabolite supplementation (‘postbiotic therapy’)⁷⁴ and phage-based suppression of commensals that are contributing with resistance to NGP colonization⁷⁵. Testifying to the possible relevance of phages in promoting microbial engraftment, a study has demonstrated that microbiome transplantation against *C. difficile* infection was supported by donor-derived bacteriophages, especially Caudovirales, which highlights how phages can influence colonization outcomes and microbiome remodelling⁷⁶.

Optimization of host–strain specificity, or human trophism, is also optional in that sense. Engineered NGP strains can be rationally optimized to enhance niche-specific fitness traits, such as bile salt resistance⁷⁷, mucin adhesion⁷⁸, nutrient-competition capacity, oxygen-gradient tolerance⁷⁹, and evasion of bacteriocins and microcins, to optimize their colonization. For example, the inherent tolerance of *Lactocaseibacillus paracasei* to bile acids mediated by its expression of cardiolipin synthase and associated cardiolipin production⁷⁷ may be leveraged by introducing these genes into NGP to improve their colonization potential.

Overcoming inter-individual variability in probiotics effects

Even when an NGP strain features a general colonization adaptability to the human GIT, such exogenous strain may induce a clinical response in one individual suffering of a given condition, but it may fail to do so in another individual with a comparable risk²⁶. The reasons for such inter-individual variability to live bacterial therapy responses are diverse and range from host genetic variability⁷⁴, differences in immune responses⁸⁰, varied composition and function of the indigenous microbiome²⁶, and diet⁷³. A diagnostic methodological obstacle in assessing such inter-individual variability is that many studies heavily rely on faecal microbiome profiling, which only captures a limited and spatially averaged snapshot of the GIT ecosystem. This can obscure micro-niche heterogeneity in gastrointestinal probiotic responses⁸¹. A deeper characterization of inter-individual differences in NGP activity may enable the development of common predictive mechanisms and non-invasive biomarkers, ultimately paving the way for targeted, personalized probiotic therapies²⁶. Machine-learning approaches are particularly well suited to integrate personalized multi-omics datasets, from genomics over metabolomics to immune profiling, to discover patterns that link host-microbiome states with colonization and efficacy outcomes. To ensure robustness, these analyses should incorporate diverse datasets from different geographic regions, age groups and patient populations with varied medical backgrounds⁸². Promising findings should be validated using controlled gnotobiotic or conventional mouse models to assess causality and dissect mechanistic pathways. Of note, mouse models present limitations as not all human colonizers will be able to colonize the GIT of mice.

An example of a recent effort to identify effective NGP classes, or ‘probiotypes’, based on their differential impacts on host features, is a study that explored the potential of personalized NGP supplements to counteract physiological, immune and microbial changes associated with ageing-related disease. By analysing the gut microbiome of 297 older adults using metagenomic sequencing, the researchers identified distinct *Lactobacillus* and *Bifidobacterium* species patterns corresponding to differences in demographics, microbial compositions, cognitive performance and neuroimaging findings, possibly enabling future NGP personalization based on host stratification⁸³. Although not an intervention study by itself, the work may provide a framework towards utilization of distinct candidate probiotypes that correlate with cognitive and neurological outcomes. Utilization of these and other disease-related probiotypes merits future validation in experimental models to reach the point wherein such identified candidates can be tested in clinical trials.

Understanding live therapeutic function

A shortcoming of many first-generation probiotic formulations, and a key reason for their frequent inconsistent clinical efficacy, is the lack of deep mechanistic understanding of their proclaimed activity⁸⁴. Popularized probiotics strains were often selected based on historical food use or simple association with health, and their ‘black box’ nature made it difficult to predict or explain their function, or to generalize suggested observations across strains, individuals and diseases. For instance, several studies report that administration of gut-derived probiotic strains such as *L. rhamnosus* or *Bifidobacterium lactis*⁸⁵ correlates with improved outcomes in respiratory conditions, including reduced exacerbations in asthma⁸⁶ and attenuated lung injury in experimental sepsis⁸⁵, yet these observations provide insufficient mechanistic explanations and reproducibility connecting gut exposure to remote lung effects.

In meeting this challenge, NGP research needs to integrate a more refined and granular assessment of causality and mechanism of activity by utilizing a growing number of research tools spanning animal experimentation, organoids, organs-on-chips⁸⁷, and artificial ex vivo gut platforms⁸⁸. Even with these state-of-the-art technologies, identifying NGP functions may constitute a daunting task. The gut ecosystem involves thousands of microbial species, metabolites and host factors, creating an intricate network of cross-kingdom interactions, host–microorganism signalling and personalized metabolic outputs⁸⁹. As such, the mechanisms by which live therapeutics exert their effects on the host and indigenous microbiome may be equally diverse and multi-layered, and include direct host signalling, metabolic activity, competitive exclusion, niche modulation and community-level interactions⁹⁰. Regardless of these challenges, understanding such molecular-level mechanisms of activity and interaction creates therapeutic opportunities by simplifying and generalizing NGP-associated interventions. For example, an NGP-mediated therapeutic effect that is mediated by a single metabolite (for example, SCFA production)⁹¹ opens the door to ‘postbiotics’ or metabolite-based therapies that may supplement or replace the need for live-cell therapeutics. Furthermore, identifying the specific host receptors and signalling pathways involved in an NGP-mediated effect allows for the development of small-molecule host receptor agonists or inhibitors that could mimic or enhance the probiotic effect⁹² (Box 2). For example, the uniquely structured, O-antigen-lacking lipooligosaccharide of *A. muciniphila* predominantly activates Toll-like receptor 2 (TLR2), inducing a strong anti-inflammatory interleukin-10 (IL-10) response and, thereby, explaining its beneficial interaction with the host, making it a potential therapeutic⁹³.

Box 2 | Advancing probiotic efficacy: mechanisms, monitoring and clinical translation

Although probiotics are defined as live microorganisms that confer a health benefit when administered in adequate amounts, their clinical application is often hindered by a lack of standardized dosing and a clear understanding of their site-specific functional engagement^{26,193,194}.

Dose and frequency

The pharmacology of probiotics remains a substantial hurdle in clinical practice^{195,196}. Unlike traditional drugs, probiotic dosage (measured in colony-forming units (CFUs)) is often arbitrary and does not account for the viability loss during shelf-life or the transit through the upper gastrointestinal tract^{193,194,197–200}. To advance the field, probiotic trials should incorporate quantitative survival, persistence and functional activity measurements over time, using strain-specific molecular tracking to define exposure–response relationships rather than relying solely on nominal CFU dosing^{195,198,199,201}. Expert opinion suggests that instead of static dosing, future trials should utilize dose-ranging studies to determine the minimum viable count needed to activate a specific functional pathway¹⁹⁶ (for example, reaching a threshold of short-chain fatty acid (SCFA) production) rather than simply aiming for faecal recovery^{202,203}. Frequency of consumption is equally critical as most strains do not permanently engraft and require continuous administration to maintain a functional presence^{26,45,199,204–206}.

Mechanisms of action

Probiotics influence host health through a variety of strain-specific and context-dependent pathways^{26,45,196,207}. Microbiome-facing mechanisms include the competitive exclusion of pathogens for nutrients and adhesion sites, alongside the production of antimicrobial bacteriocins^{208–210}. Host-facing mechanisms involve the strengthening of the epithelial barrier via the regulation of

tight-junction proteins and the modulation of immune responses through pattern-recognition receptors^{211–213}. Furthermore, metabolic signalling such as bile acid biotransformation (via FXR and TGR5) and the production of metabolites such as SCFAs allows these organisms to exert systemic effects on metabolic and mucosal homeostasis^{214–217}.

Clinical biomarkers of efficacy

Determining probiotic efficacy could be improved by moving beyond stool culturomics towards mechanism-informed biomarkers^{26,204,218}. Evidence of passage and viability can be confirmed via targeted quantitative PCR or metagenomic strain tracking in stool^{199,201,219}. For barrier-related claims, the lactulose–mannitol permeability test or blood markers such as intestinal fatty acid-binding protein (I-FABP) provide objective measures of mucosal integrity^{220,221}. Immunomodulatory success can be monitored via faecal calprotectin (for intestinal inflammation) or systemic cytokine profiles (IL-10 or TNF)^{222–224}. Metabolic engagement is best assessed through targeted metabolomics of stool and serum to measure shifts in SCFAs or secondary bile acid pools^{218,225}.

Dietary substrates and engraftment

Diet is a major determinant of probiotic function and niche establishment^{26,195,207}. Dietary fibres are known to act as ‘selective substrates’ (prebiotics), opening ecological niches for newly introduced strains^{226,227}. Without these substrates, even high-dose probiotics may fail to engraft or produce the necessary metabolites required for a health benefit^{26,45}. Furthermore, engraftment is highly individualized, often depending on the baseline diversity of the resident microbiome and the availability of specific carbohydrate-utilization pathways. Mounting research suggests a role for synbiotic approaches (combining strains with their preferred substrates) to improve clinical predictability^{26,45,228}.

With these mechanistic opportunities notwithstanding, disentangling complex mechanisms of activity to predict therapeutic success constitutes a formidable challenge that is not easily overcome. Harnessing advanced computational and AI tools to assist in such mechanistic quests is discussed in the section ‘Computational and AI-driven advances in next-generation probiotics design’.

Navigating safety and off-target effects

As noted above, in the past decades, probiotics have been consumed by millions of individuals and are generally regarded as safe. However, it is also worth mentioning that effective interventions usually are accompanied by adverse and off-target effects^{94,95}. Although these do not necessarily imply that such intervention may not be used under the right clinical context, they must be rigorously studied and reported to minimize consumer risk. Indeed, adverse effects exerted by earlier probiotic formulations remained underreported^{9,96}. Some probiotic studies have been noted to feature enhanced efficacy and reduced adverse effect profiles compared with other probiotics assessments^{35,97,98}. The expected enhanced therapeutic potential exerted by precision and engineered NGP also inherently means that their consumption may be associated with a wider repertoire of off-target and adverse effects⁹⁹. As such, approval of NGP use

necessitates a rigorous assessment of safety and unintended off-target consequences.

Of particular concern in this regard is the risk encountered by administration of live bacteria to immunocompromised hosts^{94,95}. In some cases, a careful and rational NGP administration to immunocompromised hosts may offer therapeutic potentials. For example, a precisely selected six-species NGP consortium can mitigate graft-versus-host disease (GVHD) in immunocompromised mice more effectively than standard probiotics⁹¹. Adaptation of such NGP use into immunocompromised human populations, as well as other populations at risk including children and older individuals, will necessitate a careful and prospective assessment and clinical stratification of risk versus benefit.

A major challenge regarding administration of live NGP relates to a risk of in-host evolution, often neglected in clinical safety assessments^{100–104} (Fig. 2b). A landmark study has demonstrated that even a well-characterized probiotic strain, *Lactobacillus reuteri*, rapidly accumulates mutations in regulatory genes during mono-colonization in mice¹⁰⁵. This evolution altered the microbial cell wall structure and gene expression profile, leading to enhanced immune evasion¹⁰⁵. This finding underscores that a probiotic strain introduced into a host is not a static entity but a dynamic organism subject to evolutionary pressures, which could alter its function and safety profile over

time. Similarly, the administration of probiotics can increase the risks for antibiotic resistance. Although first-generation probiotics have been shown to decrease the amount of antibiotic resistance genes in some, but not all antibiotic-naive humans¹⁰⁶, antibiotic-treated participants co-administered with probiotics feature an increase in the microbiome 'resistome', mainly associated with an expansion of bacteria carrying vancomycin resistance genes¹⁰⁶ (Fig. 2b). However, such genes might not be expressed by all bacterial species, and further assays examining the actual resistance of these bacteria are required for verification¹⁰⁷.

Genetic modification of probiotics introduces additional safety challenges, including the potential for horizontal gene transfer of engineered constructs to resident microorganisms or pathobionts and the release of modified organisms into the environment⁹⁹. A novel approach called Rapid and Integrated Bacterial Evolution Analysis can evaluate the pathogenic development of a hypermutable *Klebsiella pneumoniae* strain within 1 month¹⁰⁸. By experimentally increasing mutation rates and applying defined selective pressures, this framework rapidly identifies genetic and phenotypic changes associated with virulence. When applied to candidate probiotic strains, such evolution models could function as a preclinical safety stress test to evaluate the evolutionary stability of the strains and their potential to acquire pathogenic traits before clinical use, particularly in vulnerable patient populations. Furthermore, human-to-germ-free mouse microbiome transfers could help to unravel potential risks exerted by certain NGP¹⁰⁹ to the mammalian host and its microbiome. Integrating kill switches, genetic circuits that trigger cell death when engineered organisms leave controlled conditions, or synthetic auxotrophies that make organisms dependent on artificial nutrients, may prevent NGP from thriving in the human habitat beyond a predetermined period, thereby

avoiding their persistent colonization, if not required anymore¹¹⁰, and undesirable long-term effects. These promising strategies¹¹¹ merit further clinical testing in the human setting (Fig. 2b).

Beyond the achievement of better know-how as to the adverse and off-target effects of NGP, and construction of useful means of minimizing such effects, a paradigm shift in the NGP regulatory oversight is essential. The most viable prospect for ensuring NGP safety is to move these advanced therapeutics out of the 'supplement' category and into the 'drug' category, as is the case for any other live-cell therapy¹¹². Classifying precision and engineered probiotics as living biotherapeutics would mandate the rigorous, multi-phase clinical trial process required for FDA (or equivalent) approval, ensuring their safety and efficacy are proven before market access.

Current experimental concepts driving the development of next-generation probiotics

In the rapidly expanding field of NGP, multiple efforts are already underway to meet the above challenges with an expanding toolbox, assessing candidate NGP strains as preventive and treatment measures in a variety of diseases. In this section, we exemplify key emerging concepts that lead these efforts towards the construction of effective, safe and clinically useful NGP.

Synthetic consortia

An emerging concept of NGP is that, in some cases, the utilization of combinations of strains may confer colonization, displace pathogens and obtain efficacy advantages (Fig. 3). One study has created a large, defined human-derived microbial consortium (119 strains) and, through repeated augmentation based on faecal-challenge engraftment, established a reproducible and highly resilient community

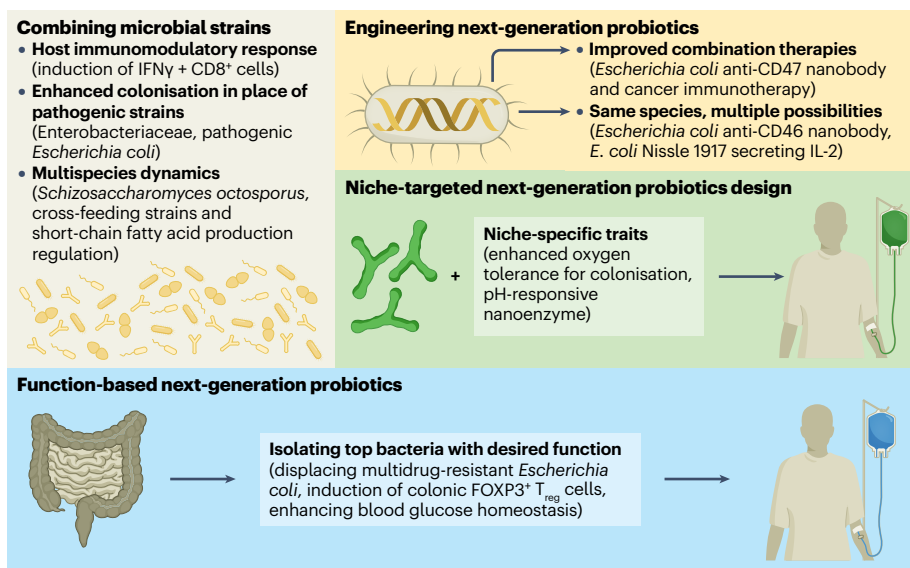


Fig. 3 | Emerging strategies in the development of NGP. The figure presents a schematic overview of several innovative strategies for the development of next-generation probiotics (NGP). One approach involves the use of defined multi-strain and multi-kingdom consortia for improved modulation of host immune responses, enhanced colonization and better multispecies interactions that can promote cross-feeding and regulation of microbial metabolites such as short-chain fatty acids. Another strategy is based on a function-first discovery approach, in which bacterial strains are selected according to specific desirable

traits (for example, displacement of multidrug-resistant microorganisms, enhancement of blood glucose homeostasis, or induction of targeted immune responses). Additional approaches include molecular engineering for targeted therapeutic delivery, as well as niche-focused design to enhance colonization and site-specific activity. Together, these complementary pathways are presented as routes towards safe, mechanistically rational and clinically effective NGP, linking high-level concepts to translational goals and experimental workflows in the field. T_{reg} cell, regulatory T cell.

showing enhanced colonization resistance against a pathogenic *E. coli* strain, alongside the ability to recapitulate host immune and metabolite phenotypes¹¹³. These results provide direct evidence that carefully assembled multi-strain consortia can achieve superior engraftment and functional efficacy compared with unoptimized mixes¹¹³. When designing multi-strain consortia, it is important to consider the cost-effective considerations of such a product, as a large variety of different strains can be quite costly and complicated to manufacture for a shelf-product. Another key example is a study in which an 18-strain consortium derived from human stool was isolated and selected for its ability to suppress multidrug-resistant gut pathobionts from the Enterobacteriaceae family (in particular, *Klebsiella* and *Escherichia* spp.), which are often resistant to conventional antibiotic treatment¹¹⁴. This approach also recognized that antibiotic susceptibility varies substantially between species, strains and antibiotic classes. The consortium colonized the metabolic niche through the regulation of gluconate availability and competition for this carbon source, with an efficiency that a single probiotic strain or an unrefined mix could not achieve. As a result, pathogen-driven intestinal inflammation in *Klebsiella*-driven and *Escherichia*-driven induced colitis and Crohn's mouse models was reduced, demonstrating functional decolonization and ecological control by multi-strain mixes¹¹⁴.

Human-derived, defined bacterial consortia can reproducibly elicit protective immune responses. For example, an 11-strain commensal consortium isolated from the stool of healthy humans robustly induced intestinal interferon- γ -producing CD8⁺ T cells and enhanced antitumour immunity in mice, demonstrating that carefully selected multi-strain formulations can produce specific, therapeutically relevant host responses not achievable by single strains¹¹⁵.

In some cases, such NGP combinations may span across kingdoms. For example, a study has analysed the taxonomic abundance and function of commensal gut bacteria and fungi across nine cohorts to associate responses to immune checkpoint inhibitor (ICI) therapy in pan-cancer models with bacterial and fungal dynamics¹¹⁶. In combining bacterial and fungal data into a 'multi-kingdom' model, the authors found strong candidate predictors of treatment response and prognosis in gastrointestinal cancers, melanoma, renal cell carcinoma and non-small-cell lung cancer, and thus improved prediction accuracy and correlation with better survival outcomes. The study has highlighted a specific synergistic interaction between the yeast *Schizosaccharomyces octosporus* and key butyrate-producing bacteria, most notably *Faecalibacterium prausnitzii*. This interaction was found to be a stronger predictor of ICI efficacy than either kingdom alone, suggesting that fungal-derived metabolites or structural components may provide a preferential environment for specific bacterial taxa driving systemic antitumour immunity. Distinct microbial metabolic modules (for example, starch and sucrose metabolism and butanoate metabolism) were more abundant in responders than non-responders, supporting a model in which coordinated fungal–bacterial cross-feeding around complex polysaccharides and SCFA production is associated with positive ICI responses¹¹⁶. The finding that key fungal–bacterial interactions may be predictive of ICI response broadens the entire conceptual framework for NGP to the possibility of harnessing mixed-kingdom consortia as adjuvant treatments in such clinical indications. However, the associations and predictions made in this¹¹⁶, and other studies, require demonstration of causality and elucidation of trans-kingdom mechanisms of colonization, activity and safety. In particular, safety considerations become increasingly important as consortia complexity increases, as the inclusion of a greater number of strains may enhance unintended inter-microbial interactions, altered ecological behaviour, horizontal gene transfer, or host-related adverse

effects (Fig. 2a,b). In such studies^{113–116}, detailed explorations of in vivo mutualistic and antagonistic effects exerted by the consortium partners on each other and on the indigenous microbiome will constitute exciting avenues of future research.

Function-based discovery of next-generation probiotics

Commensal strain function, rather than ontology, is emerging as a leading parameter guiding the construction of NGP^{8,25}. This approach is based on 'function-first' selection and mechanism-driven design (Fig. 3). An early example of this strategy was a study identifying its marked reduction in the mucosa-associated microbiome of patients with Crohn's disease during relapse¹¹⁷. The mechanism of action described in the study involved the production of butyrate and the secretion of the microbial anti-inflammatory molecule (MAM), inhibiting the NF- κ B pathway and inducing the production of anti-inflammatory cytokines such as IL-10. These properties established it as a leading candidate for treating inflammatory bowel diseases, through the restoration of immune homeostasis and protection of the gut epithelial lining¹¹⁷. The therapeutic scope of *F. prausnitzii* has recently expanded into oncology, wherein clinical data reveals that its baseline levels predict patient responses to ICIs¹¹⁸. Experimental evidence from another study demonstrates that specific strains, such as EXL01, can enhance the efficacy of anti-PDL1 therapy by promoting T cell activation and restoring the gut microbiome after antibiotic treatment. This work (although still in preclinical stages) highlights the transition of NGP from gut-centric supplements to sophisticated live biotherapeutics capable of modulating systemic immune responses in cancer patients¹¹⁸. An elegant example for an NGP with a function-first application is provided in a study that screened 430 human commensal strains for the functional ability to displace multidrug-resistant *E. coli*. The candidates were selected based on measured function (decolonization activity), and the study then examined the mechanism (carbohydrate and nutrient competition) driving competition between the candidate probiotics and the pathogenic strain, to enable rational and optimal selection¹¹⁹.

A function-first approach can involve NGP consortia and host phenotypic alterations. The discovery of *A. muciniphila* as a cornerstone of the NGP domain was driven by human clinical observations, wherein its abundance was found to be significantly higher in lean individuals than in those with obesity¹²⁰. Mechanistically, this bacterium reinforces the intestinal barrier and improves metabolic homeostasis through the action of the outer membrane protein Amuc_1100, which interacts with TLR2 to reduce gut permeability and systemic inflammation. Clinically, human trials have demonstrated its therapeutic potential, showing that oral supplementation is safe and effectively improves insulin sensitivity and reduces cholesterol in volunteers with overweight and obesity¹²⁰. A study has isolated a defined consortium of human-derived, spore-forming Clostridia species that potently induce murine colonic FOXP3⁺ regulatory T cells (specialized immune cells in the gut lining) that are crucial for maintaining immune tolerance to harmless gut microorganisms, preventing excessive inflammation, and promoting tissue repair¹²¹. Another study has demonstrated that adhesion of specific microorganisms to intestinal epithelial cells constitutes a key signal for induction of intestinal T helper 17 (T_H17) cells, creating a 'border patrol' against pathogenic microorganisms¹²². Both studies provide a clear demonstration of a function-driven strategy for NGP discovery, highlighting how particular species and their definitive trait determine host immune polarization.

Function-first approaches can be utilized to enhance metabolic health, for example, glucose control. Indeed, the gut microbiome has been suggested to modulate host glucose regulation^{123–126} and, thus, potentially reduce diabetes complications such as retinopathy¹²⁷, neuropathy^{128,129}, nephropathy¹³⁰ and cardiovascular diseases^{131,132}. In an attempt to develop NGP that improve glucose regulation, 12 murine bacterial strains were isolated based on their glucose consumption rate, with three of the strains (*L. rhamnosus*, *L. reuteri* and *Lactobacillus salivarius*) exhibiting the highest glucose utilization and, therefore, selected to formulate an NGP cocktail. In a mouse model of high-fat diet, this NGP preparation induced a significant reduction in blood glucose levels, body weight and body fat percentage¹³³. Similarly in the cancer context, a function-first approach was applied to assemble a six-species NGP cocktail from faecal samples of patients with human stem cell transplant, selecting strains based on their in vitro SCFA production⁹¹. This NGP formulation significantly increased survival rates and intestinal SCFA concentrations in GVHD-inflicted mice compared with standard probiotics, which highlights their potentially superior therapeutic efficacy in preventing or treating this devastating complication of allogeneic bone marrow transplantation⁹¹. The study underscores the marked strain-to-strain variability in SCFA output within common gut taxa (for example, *Bifidobacterium longum*, *Clostridium bolteae* and *Blautia* spp.) and shows that rational selection of high-producing, cross-feeding strains can augment luminal butyrate and other SCFAs in vivo⁹¹. This work, therefore, represents a key conceptual advance in ‘reverse translation’ of strains directly obtained from the target human population, using defined consortia to enhance a microbiome-derived protective function that is often quantitatively reduced or dysregulated in the context of disease (SCFA-mediated epithelial and immune regulation) and moving from a protective clinical observation directly to a defined therapeutic¹³⁴. However, although this NGP cocktail demonstrates promising efficacy in murine models, the absence of mechanistic validation and engraftment data in humans limits conclusions about their translational potential, particularly given that SCFA are produced by a broad range of commensal gut bacteria and are, therefore, not a unique functional trait of the selected strains¹³⁴. Another example involved an NGP combination of three bifidobacteria (*Bifidobacterium adolescentis*, *B. longum* and *Bifidobacterium bifidum*) that negatively correlated with *Fusobacterium nucleatum*, a known colorectal cancer-associated microorganism^{135–137}. In vitro, each of the three *Bifidobacterium* strains significantly inhibited *F. nucleatum* growth (24–65%), and the combination achieved approximately 70% suppression. In a small human pilot study (40 patients on probiotics, 32 controls), a 4-week administration of this NGP consortium increased bifidobacteria in the stool and significantly lowered *F. nucleatum* and other colorectal cancer-associated markers¹³⁷.

Engineering next-generation probiotics

In many cases, understanding the desired functions of NGP is insufficient to identify suitable microbial strains that both colonize effectively and carry the molecular machinery needed to fulfil the desired disease-modifying function⁹. In this context, molecular engineering may provide an attractive means in optimizing NGP function (Fig. 3). A major focus is engineering bacteria to act as selective in-body drug delivery vehicles, thereby potentially uncovering precise and effective therapeutics that exceed those of traditional molecular approaches¹⁸. For example, an engineered non-pathogenic *E. coli* strain containing a synchronized-lysis circuit was able to selectively lyse within the tumour microenvironment of lymphoma, melanoma and breast cancer

models, releasing an encoded anti-CD47 nanobody (an anti-phagocytic receptor that is overexpressed in numerous human cancer types)¹³⁸. The engineered strain produced robust tumour regression, induced dendritic cell-dependent phagocytosis and systemic antitumour immunity, and improved survival in the murine tumour models. In addition, the study has shown that a combination with systemic checkpoint inhibition (specifically anti-PDL1 therapy) further enhanced efficacy, triggering a systemic antitumour response¹³⁸. Another example of an engineering approach involves the generation of NGP capable of secreting immune modulatory cytokines. Inflammatory bowel disease (IBD) is an auto-inflammatory disorder marked by a compromised intestinal barrier and mucosal epithelium. IBD-associated microbiome is characterized by a significant reduction in taxonomic alpha-diversity, a marked depletion of butyrate-producing Firmicutes (such as *F. prausnitzii*), and a corresponding bloom of pro-inflammatory proteobacteria¹³⁹. This state of dysbiosis is not a static marker of disease but a dynamic shift often driven by the oxidative stress of the inflamed gut environment¹⁴⁰, leading to an uncontrolled damage of the GIT tissue. The complex dynamics of the GIT in IBD, the inherently poor intestinal colonization capacity of the IBD-inflicted gut¹⁴¹, and the need for a potent immunomodulatory function of an effective probiotic make NGP supplementation especially challenging in this disease^{141,142}. A strain of *E. coli* Nissle 1917 (ECN) was engineered to secrete IL-2, an immunomodulatory factor that promotes in-tissue differentiation of anti-inflammatory regulatory T cells, and coated with Eudragit L100-55 (an anionic, pH-dependent copolymer used in pharmaceuticals to dissolve specifically in the upper small intestine) to improve intestinal bioavailability¹⁸. IL-2 secretion was induced by oral arabinose to provide a gut-specific release of the cytokine. The engineered NGP featured a substantial therapeutic efficacy in an acute dextran sodium sulphate mouse model of IBD, evidenced by improved body weight recovery, mucosal damage healing, reduced inflammatory cell infiltration, and gut microbiome modulation¹⁸. This approach constitutes an elegant example of the potential use of NGP as a programmable drug delivery vehicle, offering a substantial advantage over systemic drug administration. The synthetic biology avenue is further combined with a physical delivery technology (the cell coating) to overcome fundamental hurdles related to bioavailability¹⁸.

Design of niche-targeted next-generation probiotics

The growing realization that commensals and NGP feature unique metabolic fitness traits and host-specific biophysical requirements represents a compelling opportunity to harness such know-how towards the design of niche-targeted NGP (Fig. 3 and Box 1). As a proof-of-concept of such approach, a probiotic strain (*B. longum* BL999) was coupled with a pH-responsive peroxidase-like nanozyme (B-FeAu) that functions as both a ‘nano-promoter’ and a ‘nano-effector’ in a colorectal cancer model in which the system only self-activates within the tumour microenvironment¹⁴³. Mild reactive oxygen species initially stimulate NGP metabolism and acid production, which further triggers strong production of reactive oxygen species for cancer cell elimination. The engineered NGP (termed BL999@B-FeAu) showed a potent antitumour efficacy in subcutaneous, orthotopic and colitis-associated colorectal cancer models, with a minimal toxicity and a high tumour specificity¹⁴³. This strategy of an engineered ‘switchable’ NGP platform offers a precision approach for live microorganism-mediated tumour therapy, although at this point, results are limited to preclinical mouse models, and merit further clinical translation. Another example of harnessing niche-adapted commensals is the adaptive evolution of the strictly

anaerobic human symbiont *F. prausnitzii* to increase oxygen tolerance, thereby improving its robustness during manufacture and delivery as an NGP, together with emerging data that strain-dependent oxidative stress responses and controlled oxygen gradients can support its persistence and function at the oxygen-graded mucosal interface⁷⁹.

In all, the studies exemplified in this section demonstrate the progress being achieved in the field of NGP, in leveraging innovative new concepts towards the development of bioactive experimental strains that feature predefined colonization capacity and host-modulatory disease-specific and site-specific functions. Many additional development efforts are underway in utilizing these and other novel concepts towards the development of NGP approaches as means of preventing and treating a variety of human diseases. Some of these efforts, and associated challenges they aim to meet, are depicted in Table 1.

Computational and AI-driven advances in next-generation probiotics design

Despite the above exciting developments, the advent of NGP is still hindered by an immense biological complexity of both NGP strains, the human host and its microbiome^{9,26,45}. Disentangling these multiple variables, cross-interactions¹⁴⁴ and mechanisms of activity to predict an NGP therapeutic success is a challenge that is often beyond traditional statistics¹⁴⁵. AI, deep learning, machine learning and advanced statistical modelling are emerging as essential tools in managing this complexity by enabling personalized NGP design through the integration of diverse 'big data' sets¹⁴⁶. These tools can potentially assist in prioritizing therapeutic substrates, strains and compounds, thereby enabling predictive power even in data-scarce settings.

However, as models rely on these various modalities of new data, the quality of this data is a major requirement¹⁴⁷. Although there is not one single comprehensive computational pipeline (or AI tool) dedicated to NGP design, integrating existing or emerging AI-based tools may be useful to optimize NGP research and development efforts. However, such AI utilization in NGP development is challenged by common pitfalls of AI systems, including issues related to transparency, intentionality, risk and participatory oversight¹⁴⁷, and data quality. Many of these pitfalls can affect each other (for example, poor data governance can undermine transparency and increase risk).

Predictions of molecular interactions

Models trained on extensive public multi-omics datasets may identify promising microbially derived proteins and metabolite biomarkers. Recent advances in AI are uncovering mechanistic insights, while dramatically reducing the necessary laboratory work to bridge the translational gap¹⁴⁸. Tools such as AlphaFold 3, Boltz-2 (refs. 149,150), RapiDock¹⁵¹ and others utilizing generative deep learning architectures have revolutionized protein structure and interaction modelling. Given the vast landscape of bacterial strains, metabolites, substrates and clinical variables, AI and machine learning may help to screen NGP-derived metabolites for bioactivity^{152–154} and predict ligand–receptor interactions. A critical distinction worth mentioning is that many of these tools are optimized to predict the structural configuration of a ligand–receptor interaction, or, in other words, the specific geometry of the binding site, which is computationally distinct from quantifying binding affinity, or the strength of the interaction. Although high-throughput docking can successfully identify potential binding partners, accurately predicting the thermodynamic affinity often requires separate, more computationally intensive methods^{155,156}. Researchers are already looking to expand the repertoire of these tools with varying degrees of success. For example,

BoltzGen¹⁵⁷ was developed to design host-specific ligands based on an input protein. Rather than iterate through a list of ligands searching for a host, this type of generative AI could begin with a host target and generate a list of ligands that might act on it with varying avidity.

These tools may also allow computational researchers to explore potential ligand–receptor interactions and generate hypotheses about how NGP-derived compounds might affect host biology. However, because protein folding is a computational task performed before binding predictions, errors in both steps can compound. Furthermore, if there are strong imbalances in the training datasets, these models may not truly learn biological significance but rather memorize a subset of patterns¹⁵⁸.

Assuming data quality can be ensured, these systems can be used to evaluate thousands of microorganism–compound combinations and rank the most promising options for experimental follow-up, similar to how data-driven models have predicted candidate strain engraftment from baseline community composition¹⁵⁹. Of note, protein folding and binding tools, although powerful, are not perfect. Machine learning or AI models can run the risk of overfitting its training data, which means that they have learned dataset-specific noise and correlations rather than the generalizable underpinnings of a system, leading to poor predictive performance on new, unseen data¹⁵⁸. Furthermore, the current generation of these tools does not consider conformational changes or agonist and antagonist interactions with host receptors¹⁵⁸.

Metabolic modelling

Although AI excels at identifying patterns across high-dimensional multi-omics datasets, it often lacks the mechanistic constraints necessary to ensure biological plausibility. Microbial community-scale metabolic modelling (MCM) bridges this gap by providing a mathematical framework for microbial interactions. Many AI and machine learning predictions are based on correlations and are heavily dependent on the quality of their training data, making them more likely to identify spurious associations that lack true biological mechanism or causal relevance. MCM enables the design of microbial consortia to generate targeted levels of metabolites and other bioactive compounds tailored to individualized needs without requiring massive training datasets^{160–162}. A novel resource advancing this mechanistic approach is AGORA2, a database of 7,302 high-quality, strain-resolved genome-scale metabolic reconstructions for human-associated microorganisms¹⁶¹. This compendium models the functional output of microbial communities using constraint-based analysis rather than just using presence or absence of specific microorganisms. AGORA2 was curated to incorporate metabolic pathways for 98 commonly prescribed drugs, enabling personalized predictions of microbial drug biotransformation¹⁶¹. The accuracy of this resource was demonstrated in a cohort of 616 individuals, wherein models predicted that the potential of the gut microbiome to metabolize drugs varied significantly and correlated with clinical factors such as age, sex, body mass index and clinical phenotype¹⁶¹. This type of mechanistic modelling provides granular detail that, when paired with AI-driven pattern recognition, could enable the rational design of interventions taking individual host–microorganism interactions into account.

A demonstration of AI-based predictability of NGP metabolic capacity was provided in a study that utilized MCMs and machine learning to select for butyrate-producing consortia¹⁶³. The researchers built genome-scale metabolic models for 19 gut isolates, simulated butyrate output for more than 450,000 synthetic consortia, and then trained regressors on network-derived features to predict butyrate production¹⁶³. This achieved strong correlations when further tested

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Table 1 | Examples of applications of NGP across non-communicable diseases, and metabolic, immune, ageing, gastrointestinal and oncologic indications

Category	Pathology	Microorganism or strategy	Mechanism	Stage	Challenges	Ref.
Combining microbial strains	Colonization resistance and pathogen decolonization	Large defined human-derived consortia (multi-strain mixes)	Augmented engraftment and metabolic niche competition → resist pathogen colonization and recapitulate host phenotypes	Preclinical: in vivo faecal- challenge and augmentation models	Design complexity, reproducible engraftment, ecological interactions	113
	Pathogen-driven intestinal inflammation (colitis and Crohn's disease models)	Human stool- derived 18-strain consortium	Metabolic niche colonization (gluconate competition) → suppress Enterobacteriaceae and relieve inflammation	Preclinical: mouse colitis and Crohn's disease models	Translation to humans; ecological stability	114
	Colorectal cancer, melanoma	Defined 11-strain human commensal consortium	Induces intestinal IFN γ +CD8 $^+$ T cells → enhanced antitumour immunity	Preclinical: in vivo tumour models	Specificity of immune activation; safety	115
	Gastrointestinal cancers, melanoma, non-small-cell lung cancer, and renal cell carcinoma	Multi-kingdom (bacteria and fungi) consortia and signatures	Inter-kingdom interactions (for example, <i>Schizosaccharomyces octosporus</i> +SCFA producers) predict ICI response	Clinical: multi-cohort association studies	Causality, stability and mixed-kingdom therapeutic design	116
Function-based NGP discovery	Crohn's disease and IBD	<i>Faecalibacterium prausnitzii</i>	Significant reduction in disease states; potential anti-inflammatory role	Clinical: observational	Cultivation of anaerobic strains; variability across cohorts	117
	Non-small cell lung cancer, melanoma	<i>F. prausnitzii</i>	Live biotherapeutic potential in cancer patients to enhance therapy	Preclinical: in vivo tumour models, in vitro human cell models, clinical data reanalysis	Standardization of LBP manufacturing; dosage optimization	118
	Obesity and metabolic health	<i>Akkermansia muciniphila</i>	Detection in lean individuals versus absence in individuals with obesity; metabolic regulation	Clinical: observational	Large-scale validation; safety in vulnerable populations	120
	Decolonization of MDR <i>Escherichia coli</i>	Function-first screening of 430 human commensals for decolonization activity	Carbohydrate and nutrient competition drives displacement of MDR <i>E. coli</i>	Preclinical: in vitro screening and mechanistic dissection	Reproducibility, mechanistic optimization for translation	119
	Immune tolerance, regulatory T cell induction	Defined spore-forming Clostridia consortium	Induction of colonic FOXP3 $^+$ regulatory T cells → immune tolerance	Preclinical: in vivo mouse studies	Translating immune polarization to humans	121
	T $_H$ 17 induction and mucosal defence	Adherent specific microbes to intestinal epithelial cells	Microorganism adhesion to intestinal epithelial cells triggers T $_H$ 17 cell induction	Preclinical: mechanistic mouse studies	Balancing protective versus pathogenic T $_H$ 17 responses	122
	Metabolic disease (glucose regulation)	Precision-designed NGP cocktail selected by glucose consumption	High glucose-utilizing strains reduce host blood glucose and metabolic parameters	Preclinical: in vivo (high-fat diet mice)	Personalization to host microbiome; human translation	133
	GVHD	Six-species NGP from HSCT patient isolates selected for SCFA production	Restore SCFA secretion → improved intestinal homeostasis and survival	Preclinical: in vitro SCFA assays and in vivo mouse model of GVHD	Patient microbiome heterogeneity; modelling human GVHD	91
Colorectal cancer	Combination of <i>Bifidobacterium adolescentis</i> , <i>Bifidobacterium longum</i> , <i>Bifidobacterium bifidum</i>	Competitive suppression of <i>Fusobacterium nucleatum</i> → reduced colorectal cancer-associated markers	Preclinical: in vitro and small human pilot study (4 weeks)	Small pilot study; needs larger RCTs and clinical end points	137	
Engineering NGP	IBD	Engineered <i>E. coli</i> Nissle 1917 (IL-2 and coating)	In situ IL-2 delivery → regulatory T cell induction and local immunomodulation	Preclinical: in vivo (DSS-induce mouse model of colitis)	Activation control, safety, regulatory hurdles	18
	Lymphoma, melanoma and breast cancer	Engineered <i>E. coli</i> (anti-CD47 nanobody)	Synchronized lysis in TME → release of nanobody → enhanced immunity	Preclinical: in vivo tumour models	Specificity of immune activation; safety	138

Table 1 (Continued) | Examples of applications of NGP across non-communicable diseases, and metabolic, immune, ageing, gastrointestinal and oncologic indications

Category	Pathology	Microorganism or strategy	Mechanism	Stage	Challenges	Ref.
Niche-targeted NGP design	Colorectal cancer	Engineered <i>B. longum</i> BL999+nanozyme	TME-activated ROS via nanozyme and probiotic metabolism → cancer cell killing	Preclinical: in vitro and mouse models	ROS safety, off-target effects	143
	Gut colonization capacity	Evolved and engineered <i>F. prausnitzii</i>	Enhanced oxygen tolerance → improved colonization and viability in the gastrointestinal tract	Preclinical: evolutionary engineering	Maintaining function with adapted traits	79

DSS, dextran sulphate sodium; GVHD, graft-versus-host disease; HSCT, haematopoietic stem cell transplantation; IBD, inflammatory bowel disease; ICI, immune checkpoint inhibitor; LBP, live biotherapeutic product; MDR, multidrug-resistant; NGP, next-generation probiotics; RCT, randomized controlled trial; ROS, reactive oxygen species; SCFA, short-chain fatty acid; T_H17 cell, T helper 17 cell; TME, tumour microenvironment.

on experimentally measured consortia. In developing a future NGP, a computational pipeline could scale from a small, curated strain panel to thousands of candidate genomes by automatically reconstructing metabolic networks, encoding cross-feeding probabilities and pathway capacity as features, and using machine learning models to score which single strains or consortia have the highest tendency to deliver a target metabolite profile in vivo. Beyond bacteria, fungal commensals are increasingly being reconsidered for their immunomodulatory and metabolic roles¹⁶⁴. However, the combinatorial space of mixed-kingdom probiotics remains largely unexplored. Deep learning tools, particularly graph-based neural networks and trans-kingdom embedding models¹⁶⁶, may help to predict stable bacterial–fungal consortia and anticipate unintended interactions that might disrupt host homeostasis¹⁶⁷.

Repurposing next-generation probiotics

Network-based computational screening offers a powerful method for identifying new therapeutic avenues from existing research. A study has used link prediction methods on a bipartite graph constructed from the ProBio database and ICD-10 disease codes (International Classification of Diseases 10th Revision code, which is used to code and classify medical diagnoses) to forecast novel probiotics–disease interactions¹⁶⁸. The network mapped known interactions between probiotic strains and ICD-10 disease codes. By analysing this web, the model identified high-probability ‘missing’ connections wherein probiotics with overlapping treatment profiles probably share unrecorded therapeutic effects¹⁶⁸. This network model predicted new prophylactic and therapeutic roles for known probiotics with high accuracy, assessed via a ‘holdout’ dataset of known interactions (which the model had not been exposed to)¹⁶⁸. Specifically, the study has identified potential applications for *Lactobacillus jensenii* in managing Crohn’s disease and ulcerative colitis, and for *L. acidophilus* in treating urologic and female genital illnesses, such as urinary tract infections and vaginitis¹⁶⁸. This in silico approach effectively narrows the search space, identifying promising candidates for further validation. Although no new in vivo studies were conducted, the authors performed a literature-based validation of the top 20 predictions, finding that 30% of these computationally identified links were corroborated by existing empirical data from independent research¹⁶⁸.

This result suggests that graph network models facilitate strain repurposing by simultaneously predicting novel therapeutic applications and simulating how community interactions will modulate the strain function. Predictive filtering allows for the rational selection of strains or consortia, which can considerably reduce the cost and

time of preclinical and clinical trials for specific health conditions. However, this assumes that the input data is of high quality and that the modelling approach is applied correctly. Although the previous example did not incorporate trans-kingdom data, this method could be useful when controlling for non-bacterial constituents of consortia. Graph network models excel here over simple correlation-based approaches because they can map the indirect relationships and dependencies within a community. This can then be expanded to include the mycobiome, modelling how key fungal species (such as *Candida albicans*)¹⁶⁹ might act as ‘modulators’ of predicted bacterial functions, paving the way for mechanistically designed, mixed-kingdom probiotic consortia.

Personalizing the use of next-generation probiotics

AI, machine learning and other computational advancements can be broadly applied to personalize NGP to the individual¹⁶⁰, including aspects that are not necessarily related to engineering specific bacteria or metabolic outputs. Features specific to each individual can be used as inputs for such personalization and include a myriad of host and microbiome factors¹⁷⁰. For example, different body sites have different exposures to oxygen, pH, immune surveillance and nutrient availability, all of which differentially shape the resident microbial niche^{171,172} and the associated responses to NGP in different body sites, disease contexts and individuals. Despite the current scarcity of data accounting for such person-specific features (such as host protein expression, levels of antimicrobial peptides produced, and clinical features), future models trained on skin, oral, vaginal and gut microbiome datasets carry the potential to identify commensal strains with niche-specific fitness, adhesion capabilities and competitive exclusion potential that may enable harnessing different NGP to predefined personalized and clinical contexts. A recent study has used MCMs to create personalized ‘invasion assays’ that predicted an individual’s risk of pathogen colonization¹⁶⁰. This model moves beyond simple composition to simulate the resident microbial niche, identifying specific metabolic vulnerabilities (such as succinate or ornithine dependency) that allow *C. difficile* to gain a foothold. The framework was then used to predict the efficacy of an NGP cocktail through calculation of the capacity of different NGP combinations to outcompete pathogens for specific resources. In all, this approach maximizes the therapeutic potential of an NGP by ensuring that the live bacterial treatment and pathogen are in direct, mechanistic competition for the same nutritional niche. Similar computational approaches can be developed to optimize NGP treatments in a variety of microbiome-contributed non-communicable diseases¹⁷³.

Optimizing the delivery of next-generation probiotics

Practical constraints, such as culturing strict anaerobes, maintaining viability through the gastric passage, or masking unpleasant taste, could be addressed using computational modelling, assuming data on the relationship between bacterial strains and host conditions (such as pH, transit time and time until effectiveness) can be assessed. AI can assist in designing micro-encapsulation matrices, predicting shelf-stable formulations, and optimizing delivery routes (for example, buccal films, enteric-coated capsules or fermented food vehicles). For instance, active machine learning can address the challenge of formulation by predicting how hundreds of different excipients – the inactive ingredients used in capsules and matrices – will affect NGP viability. An example of this is a study that presented a strategy to assess excipient–probiotic interactions, attempting to reduce a common downstream formulation bottleneck¹⁷⁴. The researchers started with a small dataset of just six excipient–probiotic interactions. They trained a machine learning model to serve as a default, and each prediction was associated with an uncertainty score (indicating how confident the model is in its prediction)¹⁷⁴. A low certainty score would imply that a model has trouble classifying an observation owing to lack of data or observations. When the model predicted a higher uncertainty for a specific excipient–microorganism combination, the researchers prioritized this for a wet-laboratory follow-up. On the basis of the true label in the experiment, the model was trained with this updated data¹⁷⁴. By testing just three such formulations, the final accuracy of the model was substantially improved, paving the path to efficiently designing stable formulations¹⁷⁴. This subset of machine learning can be particularly valuable for many wet-laboratory optimization tasks because it is designed to perform well in low-throughput experimental settings wherein labelled data are limited.

Large language models

Beyond conventional machine learning pipelines, large language models (LLMs) are rapidly emerging as powerful tools with the potential to reshape NGP discovery by translating fragmented biomedical literature into clear knowledge. An example of LLM application in biomedical research demonstrated how pretrained LLMs combined with named-entity recognition and relation-extraction pipelines were used to automatically mine over 30,000 PubMed abstracts and generate domain-specific knowledge graphs linking foods, chemicals and diseases¹⁷⁵. The system managed to extract thousands of previously unstructured relations, such as food–disease ‘cause and treat’ links and food–chemical ‘contains’ associations, with a mean precision of roughly 70%. Applied to NGP development, an analogous LLM-driven pipeline could systematically mine existing microbiome literature to construct strain–metabolite–host interaction graphs, identify underexplored immunomodulatory or metabolic functionalities, and highlight strain–disease hypotheses that currently remain buried¹⁷⁵.

LLMs further offer the potential to enhance NGP research by acting as adaptive research copilots across experimental design, data interpretation and clinical translation. If integrated with metabolic networks or multi-omics data, LLM agents can summarize multiple sources, generate ranked candidate hypotheses, propose experimental validation strategies, and assist with biomarker interpretation. In principle, these tools could bridge the knowledge gap between genome-derived predictions and phenotypic screening by recommending specific strain panels for testing based on literature-derived functional motifs (for example, bile salt hydrolase activity, SCFA biosynthesis and epithelial adhesion). LLM systems may eventually offer natural-language

interfaces for physicians prescribing personalized probiotic regimens while retaining linkage to formal evidence graphs and decision rules.

Researchers have additionally examined the part LLMs can play in the enhancement of clinical trial design by reviewing and analysing existing protocols¹⁷⁶. In evaluating 15 phase I–III protocols, researchers found that ChatGPT-4o achieved 95% accuracy in extracting study design attributes and successfully identified key regulatory guidelines for statistical analysis plans and pharmacokinetics–pharmacodynamics¹⁷⁶. Although the study highlights the potential for LLMs to streamline these traditionally labour-intensive reviews, the authors emphasize a ‘human-in-the-loop’ approach to safeguard against subtle contextual errors in complex trial designs. Because an LLM can be fed a number of outcomes that a trial needs to track, it may assist in summarizing protocol requirements and highlighting key design parameters, potentially helping investigators to evaluate appropriate sampling windows or longitudinal study designs.

Notwithstanding this potential, the deployment of LLMs in NGP research is also challenged by substantial limitations. Chief among these are hallucinations^{177,178}, the generation of fluent but factually incorrect or ungrounded outputs, and the quality of training data¹⁷⁹. Systematic evaluation of state-of-the-art models revealed that hallucinations arise from both prompt-related ambiguity and intrinsic model behaviour, even under controlled prompting strategies and evaluation benchmarks such as TruthfulQA and HallucinationEval (now known as HalluClean)^{180–182}. Although structured prompting techniques such as chain-of-thought reasoning reduce hallucination rates, they are not consistent fixes. However, this is a rapidly growing field with improving capabilities, and we estimate that LLMs of the future (or equivalent technology) will suffer from these problems in fewer instances than they do today. For current generation models, some researchers have suggested roles for retrieval-augmented generation to reduce these issues^{183,184}, but there is no consensus on the matter. It remains probable that expert-driven verification is crucial at present before novel biological claims are treated as actionable hypotheses. Equally important are the infrastructural inequities associated with large-scale model training and inference costs¹⁸⁵: enterprise LLM providers charge by the token, requiring expenditure accessible only to well-funded academic centres, potentially restricting participation from less affluent research environments. Together, LLMs should be viewed not as autonomous discovery engines but as strong hypothesis generators. Responsible adoption requires safeguards against hallucination, transparent retrieval-based grounding, and broad investment in accessible AI infrastructure to ensure equitable participation in this emerging frontier¹⁸⁶.

In all, the AI approaches depicted above sketch out independent niches of potential for an AI-enabled workflow in which mechanistic modelling and supervised learning will narrow the NGP strain and consortium search space, while active learning will focus limited wet-laboratory capacity on the highest-value experiments across both biology and formulation (Fig. 4). Predictive screening tools such as these can be used to guide candidate selection for further *in vitro* work and could be complemented by protein interaction tools such as AlphaFold 3. Additionally, these AI-based approaches could also assess the probability of small molecules and proteins derived from NGP to feature bioactive host effects. Although each tool occupies its own niche, appropriate AI tool integration could accelerate the overall development process. Ultimately, AI can be used to speed up the research life cycle, integrating multimodal inputs to design NGP while accounting for host anatomy and real-world constraints. However, as it

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cannot be effectively used without a clear scientific direction, proper application will most probably continue to rely on the curation of high-quality clinical data and rigorous validation.

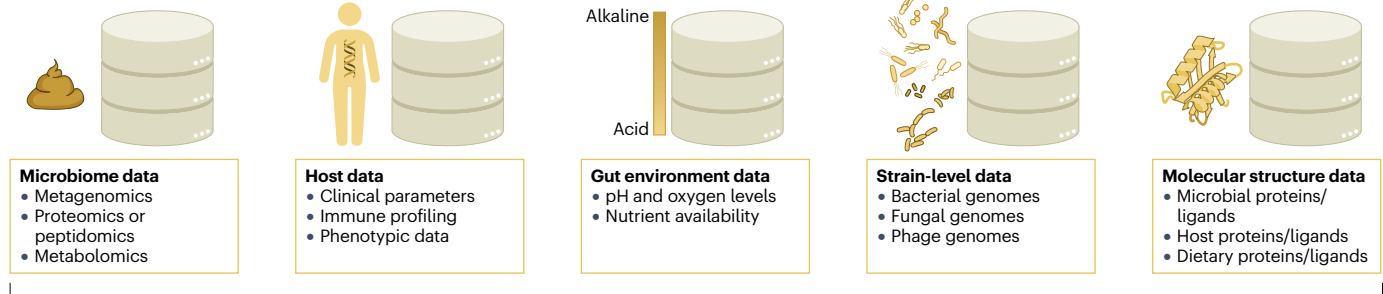
Conclusion

This Review has charted the journey of NGP development and application from empirically selected strains to the brink of rationally designed

Step 1: Data integration

Collection and curation of wide variety of data modalities (examples underneath are not exhaustive)

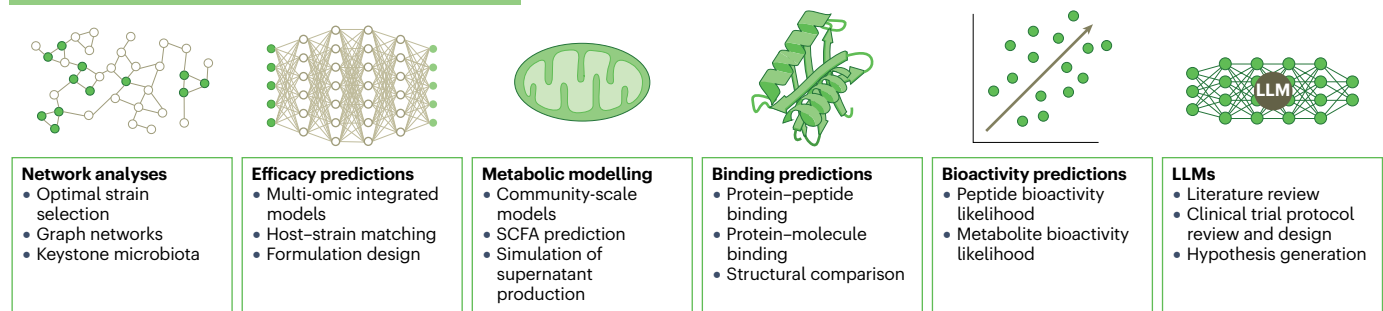
Greater numbers of observations and wider types of data collection help the predictive accuracy of models in step 2



Step 2: Modelling and prediction

Incorporation of multi-omic data to predict optimal consortia, desired microbial products and interactions with host

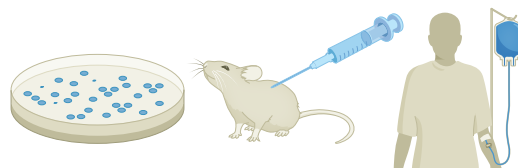
Each method or tool can be used independently or in conjunction to take various data modalities and help to prioritize candidates for step 3



Step 3: Lab work and optimization

Testing and verification of prioritized candidates in a wet lab setting with collection of additional data

A final list of generated high-quality candidates can be tested and used for modelling refinement in steps 1 and 2. Failures of the modelling stage can help to inform computational researchers where tools need improvement



In vitro and in vivo validation

- Experimental confirmation
- Assessment of adverse effects
- Data collection for additional testing



Additional observation data

- Highlights modelling failures
- Refinement of data modelling
- Feedback loop for computational teams

Fig. 4 | A conceptual framework for the iterative design and validation of next-generation probiotics. Data integration (step 1) involves collecting and curating diverse, high-dimensional datasets. These data types can include microbiome data (for example, metagenomics, proteomics, metabolomics), host data (for example, immune profiles, clinical parameters), gut environment data, and specific genomic or molecular structure data for bacteria, phages and their products. In the next phase (step 2), computational models are used to analyse the complex, multi-omics datasets from step 1. This *in silico* phase aims to generate testable hypotheses and prioritize candidates for intervention. It encompasses a wide range of tools and methods, such as network analyses to identify keystone taxa;

metabolic modelling to predict community function (for example, production of short-chain fatty acids (SCFAs)); large language models (LLMs) to summarize the literature for hypothesis generation; and structural and binding prediction tools (such as AlphaFold 3 and BoltzGen) to assess molecular interactions and bioactivity. The most promising candidates predicted in step 2 are advanced to experimental validation (step 3) by *in vitro* and *in vivo* testing (in cell cultures and animal models) to confirm efficacy, assess mechanisms and ensure safety. Overall, the framework is iterative, as the additional observation data generated during validation serve as a critical feedback loop, refining the models in step 2 and enriching the initial data collection in step 1 for subsequent rounds of design.

Glossary

Engineered probiotics

Genetically modified microorganisms designed to perform specific functions (for example, nutrient competition and optimal colonization).

First-generation probiotics

Traditional live microorganisms including *Lactobacillus* or *Bifidobacterium* species found in yogurt and fermented food that provide general health benefits when consumed in adequate amounts.

Live biotherapeutic products (LBPs)

A regulatory term for biological products that contain live organisms (such as bacteria) and are used to prevent, treat or cure a disease.

Next-generation probiotics (NGP)

Live microorganisms identified through advanced comparative analysis of the gut microbiome, using next-generation sequencing and bioinformatics to isolate bacteria with defined health benefits.

Postbiotics

Inanimate microorganisms and/or their components (for example, metabolites) that confer a health benefit on the host.

living therapeutics. The leap to ‘next-generation probiotics’ has the potential to be fundamentally enhanced by recent advances in AI and computational methods, which is shifting the paradigm from descriptive discovery to predictive and generative design. The emerging NGP field, and associated experimental and computational tools that it utilizes, increasingly allow researchers to screen for function, model community-scale metabolic interactions, and rationally engineer microorganisms for specific tasks. However, our survey also highlights that computational power does not bypass biological reality. The formidable, personalized nature of colonization resistance remains the primary obstacle to efficacy, whereas the potential for in-host evolution and horizontal gene transfer represents a critical, underexplored safety challenge. The experimental examples, although promising, underscore that the field is still in its nascent stages, with few interventions yet meeting the true standard of ‘precision’ or ‘personalized’.

Looking ahead, the integration of multi-kingdom (for example, bacterial–fungal) interaction data and host multi-omics into these AI frameworks will be essential and exciting. The future of the field lies not only in predicting which strain works but also in iteratively designing a strain or consortium with a predefined therapeutic function, tailored to the specific microbial and immune landscape of an individual, and targeted to predetermined disease niche while minimizing off-target

Prebiotics

Non-digestible food components (plant fibres) that selectively feed and stimulate the growth of beneficial bacteria.

Precision probiotics

Targeted microbial strains selected based on the unique microbiome profile or a specific health condition of an individual.

Synbiotics

A synergistic combination of both probiotics and prebiotics designed to improve the survival and activity of the live microbial supplement.

Synthetic consortia

Rationally designed microbial communities composed of two or more well-characterized, often genetically distinct strains developed to leverage metabolic cross-feeding, niche specialization and division of labour to perform complex biological functions more effectively than a single strain.

effects. Achieving this vision will demand rigorous clinical validation, the curation of high-quality, multimodal datasets, and the development of robust safety controls to ensure the safe and reversible translation of these powerful new therapeutics. Additionally, one major hurdle in probiotic research is bringing probiotics from bench to bedside, which requires standardized definitions for optimal dose and frequency of administration, an aspect that further complicates clinical translation. Advancing the field will require dose–response trials, testing the frequency of consumption, and using standardized dietary regimes when consuming probiotics.

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Competing interests

E.E. is an adviser to Purposebio and Zoe on topics unrelated to this work. The remaining authors declare no competing interests.

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