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# The pros, cons, and many unknowns of probiotics

Jotham Suez[1,7], Niv Zmora[1,2,3,7], Eran Segal[4,5*] and Eran Elinav[1,6*]

**Consumption of over-the-counter probiotics for promotion of health and well-being has increased worldwide in recent years. However, although probiotic use has been greatly popularized among the general public, there are conflicting clinical results for many probiotic strains and formulations. Emerging insights from microbiome research enable an assessment of gut colonization by probiotics, strain-level activity, interactions with the indigenous microbiome, safety and impacts on the host, and allow the association of probiotics with physiological effects and potentially useful medical indications. In this Perspective, we highlight key advances, challenges and limitations in striving toward an unbiased interpretation of the large amount of data regarding over-the-counter probiotics, and propose avenues to improve the quality of evidence, transparency, public awareness and regulation of their use.**

The concept of oral consumption of microorganisms as a means of inducing health benefits has intrigued humans for centuries. The term 'probiotics' first appeared in this context in 1974 and has conceptually evolved to its current common definition as live microorganisms that confer a health benefit when consumed in adequate amounts, suggested by the Food and Agriculture Organization/World Health Organization in 2002 (ref. [1]). Nowadays, probiotics constitute a constantly growing multi-billion-dollar industry[2] and are one of the most commonly consumed food supplements worldwide[3]. Foods such as yogurt, cheese, ice cream, snacks and nutrition bars, breakfast cereals and infant formulas are supplemented with probiotics, as are cosmetic products. Probiotics are also commercialized as lyophilized pills[4]. Probiotic consumption is widely supported by physicians[5], specifically gastroenterologists[6].

The popularity of probiotics notwithstanding, data from decades of research on the efficacy of probiotics in the treatment and prevention of disease often point toward opposing conclusions and remain conflicting, debated and confusing in many cases. Moreover, the major medical regulatory authorities, such as the European Food Safety Authority[7] and the US Food and Drug Administration[8], have yet to approve any probiotic formulation as a therapeutic modality. As a result, marketing of probiotics as dietary supplements is often driven by properties such as safety, viability in the gastrointestinal (GI) tract and lack of impact on the taste of food, rather than by unequivocal health-promoting effects[9]. This confusing state merits better evidence-based proof of the impacts that probiotics have on humans and their adverse effects[10].

In this Perspective, we will highlight and discuss some of the major prospects and limitations of the current approach to probiotic research, present challenges in the interpretation of available data and suggest possible strategies to clarify these issues and transform investigation of probiotics into a more reproducible and universally accepted measurement-based approach. In our work, the reviewed over-the-counter microbial interventions will be termed probiotics regardless of their benefit and efficacy or lack thereof. Of note, the aim of this Perspective is not to review investigational, non-commercially-available 'next-generation' microbial therapy approaches that are being proposed as interventions for various medical indications. These are discussed elsewhere[11]. We will highlight notable examples to discuss the following: the 'knowns' and challenges with respect to the strength of evidence and clinical interpretation of studies assessing the health benefits of probiotics; the suggested probiotic mechanisms of action, relating to the debate of whether these will require gut colonization; interactions of probiotic strains with the gut microbiome; safety; and future directions.

## Clinical efficacy

The effects of probiotics on humans have been extensively studied both by scientists and the food and drug industry for decades. This has led to multiple suggested prophylactic and therapeutic health indications and claims, such as prevention or treatment of acute, antibiotic-associated and *Clostridium difficile*–associated diarrhea; amelioration of inflammatory bowel disease and irritable bowel syndrome (IBS); and reduction of risk for neonatal late-onset sepsis and necrotizing enterocolitis. Other claims include, among many others, eradication of *Helicobacter pylori*, reduction in incidence and severity of respiratory infections, alleviation of depression, prevention or treatment of atopic dermatitis and reduction of cardiovascular risk factors associated with the cardiometabolic syndrome[10]. Regretfully, despite the fact that some clinical trials related to the above health claims are of high methodological quality and validity[12–16], for most of the above indications, there are also studies of similarly high methodological quality featuring negative or opposing results, collectively leading to conflicting, ambiguous and debatable overall conclusions.

The current confusing situation may stem from a number of issues, including the fact that many readouts from probiotic trials are based on empirical clinical data that vary in collection methodology, clinical endpoints and analytical rigor. Many reports use qualitative, self-reported parameters of 'well-being', such as emotional or social function[17,18]. Others provide quantification of markers that do not necessarily have clinical significance, for example clinically insignificant reduction of the inflammatory marker C-reactive protein (CRP) in healthy individuals[19], or elevation of glucose-stimulated glucagon-like peptide 1 (GLP-1) in glucose-tolerant individuals[20]. Likewise, there is great variability in the systems analyzed in these trials, including extrapolations from cell cultures, in vitro studies,

[1]Immunology Department, Weizmann Institute of Science, Rehovot, Israel. [2]Digestive Center, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel. [3]Internal Medicine Department, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel. [4]Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel. [5]Department of Computer Science and Applied Mathematics, Weizmann Institute of Science, Rehovot, Israel. [6]Cancer-Microbiome Research Division, DKFZ, Heidelberg, Germany. [7]These authors contributed equally: Jotham Suez, Niv Zmora. *e-mail: eran.segal@weizmann.ac.il; eran.elinav@weizmann.ac.il

animal models and human studies that may be observational or randomized, placebo-controlled trials. At times, even among high-quality, placebo-controlled studies, different trials uncover conflicting putative benefits of probiotics[21,22].

Another contributor to the variability in probiotics research is the disparity of studied strains. The dominant microorganisms used in the probiotics industry even nowadays belong to the *Lactobacillus* and *Bifidobacterium* genera, as well as *Lactococcus* spp., *Streptococcus thermophilus*, *E. coli* Nissle 1917 and the yeast *Saccharomyces boulardii*[23]. While some health-associated mechanisms of action are common in multiple probiotic genera and species (for example, the production of bile salt hydrolases)[24], other traits may be species- or even strain-specific, or may require interaction between different strains to produce an effect.

To counteract the above methodological and analytical limitations and to overcome underpowered findings, researchers and clinicians frequently integrate results from multiple studies in the form of systematic reviews and meta-analyses. The use of such tools may be highly useful in revealing general trends; however, it may also be susceptible to biases that can be introduced in each analytical step[25], such as the inclusion of outlier studies that dominate the collective results and obscure actual effects, or the lack thereof. In particular, meta-analyses concerning probiotics tend, at times, to group studies testing various unrelated supplemented microorganisms under the same umbrella, thereby risking over- or misinterpretation of results[26,27]. Consequently, even meta-analyses addressing similar topics may conflict with one another[28,29]. Thus, in our view, meta-analyses can complement, but not replace, high-quality, large-scale, multicenter, randomized controlled clinical trials.

Moreover, unlike animal models, humans are highly heterogeneous in terms of diet, age range, genetic background and gut microbiome configuration, and may therefore respond differently to the same intervention. Indeed, several probiotics studies have indicated the importance of precision because of differential outcomes that depend on factors related to the host and their microbiome or diet (Fig. 1). Specifically, as further discussed in the following sections, the degree of gut colonization by probiotics considerably varies between individuals, which may drive the differential effects of probiotics on their hosts and/or their gut microbiomes.

Finally, many of the probiotics studies are linked, funded, initiated and endorsed by commercial entities of the probiotic industry or professional lobbying groups that are heavily associated with and funded by the same industry[30]. While this reality by itself does not necessarily compromise the validity of such studies, there is a need and interest in independent corroboration of efficacy claims through nonaffiliated research by scientific and medical entities. Examples of some of the indications in which probiotics are most commonly associated with a beneficial outcome are described below.

**Acute gastroenteritis.** Probiotics have been suggested to be effective prevention against or therapeutics for various pediatric and adult etiologies that manifest as acute diarrhea. Several meta-analyses and systematic reviews have indicated that some preparations[31], especially those containing *S. boulardii*[32], *Lactobacillus rhamnosus* GG (LGG)[33] and other strains within the *Lactobacillus* genus[34], may ameliorate acute diarrhea in children and shorten its duration by approximately 1 day. Likewise, probiotics have been shown to be effective in the prevention and treatment of acute diarrhea in adults, and it has been suggested that various preparations, in particular those involving *S. boulardii* and *L. rhamnosus*, improve antibiotic-associated diarrhea both in healthy children[35] and adults[36,37], and in hospitalized patients[38].

In contrast, other studies and meta-analyses have shown contradictory results with respect to the effectiveness of probiotics in diarrhea prevention in children[39], adults[21] and the elderly[37,40]. Notably, the results of two recent high-quality, large-scale, multicenter, randomized placebo-controlled trials assessing treatment with *L. rhamnosus* (LGG or R0011), with or without *Lactobacillus helveticus* R0052, in over 1,800 children who presented with acute gastroenteritis to the emergency department demonstrated no clinical benefits[41,42]. An earlier meta-analysis in over 4,000 children showed that the quality of evidence with regard to this indication was low to very low[43], leading to the omission of probiotics from one set of clinical management guidelines[44], whereas another study still advocates for the use of LGG and *S. boulardii* while stating that the evidence upon which these recommendations are based is of low quality[45]. Notwithstanding this dispute, many parents 'self-treat' their children when they contract gastroenteritis with 'functional foods' containing probiotics[46].

***Clostridium difficile*–associated diarrhea.** *C. difficile* thrives in the gut when microbiome-conferred colonization resistance is compromised, such as upon antibiotic treatment in hospitalized patients. The result is a disease that can range in severity from mild diarrhea to a life-threatening condition termed pseudomembranous colitis. Several meta-analyses have shown a cumulative beneficial outcome of orally administered probiotics: prevention of *C. difficile* infection or its associated morbidity[47], especially when administered close to antibiotic exposure[48]. A follow-up 2017 meta-analysis of 8,672 cases (comprising different probiotic strains, ages, doses and timings of administration) further uncovered moderate beneficial evidence for prevention of *C. difficile*–associated diarrhea (CDAD) in patients treated with antibiotics, but indicated that there was a considerable heterogeneity between trials and used a post hoc analysis that suggested no significant effect of probiotics on CDAD prevention in trials with human subjects at low and moderate baseline CDAD risk[49]. Another meta-analysis concluded that, of the various probiotic strains, only *S. boulardii* was effective against *C. difficile*[50], though a different meta-analysis relating specifically to *S. boulardii* found that it reduced CDAD risk in children, but not in adults[51], with a low quality of evidence noted[52].

Upon further examination of the individual studies forming the basis of these meta-analyses, we discovered that *C. difficile* incidence during the trial period was nonexistent (8 trials, Supplementary Table 1) or low in the majority of the trials in both the placebo and the treatment groups, and the vast majority of trials included in the meta-analyses (34 trials, Supplementary Table 1) did not demonstrate that probiotics of different strains had a significant effect on CDAD or *C. difficile* infection. While this may be related to insufficient power of these studies for demonstration of an effect in the context of the low incidence of *C. difficile*, two randomly controlled trials (RCTs) featuring populations with a high incidence of *C. difficile*, including the largest trial of probiotics for this indication to date, did not find a difference between the treatment and placebo groups[40,53]. Thus, the preventive effects of probiotics against CDAD are mostly supported by a minority of studies that demonstrate a significant effect[16,38,54–57], of which two are non-peer-reviewed conference abstracts[58,59]. While *C. difficile* incidence in the placebo groups was very high in most studies that uncovered a beneficial effect[16,38,54,55,57], other studies, in which CDAD was uncommon, yielded a lower level of evidence with respect to the efficacy of probiotics in prevention of CDAD[30,60]. Together, variable baseline risk of CDAD among cohorts and the fact that the majority of meta-analyses aggregated studies that tested a variety of probiotic strains, both fungal and bacterial[61], may potentially explain the differences in outcomes between studies.

**Irritable bowel syndrome and digestive complains.** IBS is a common and clinically variable disorder of unclear etiology. Trials assessing interventions to alleviate IBS are often limited by the fact that this condition is defined by subjective criteria. As such, it is of paramount importance to ensure that IBS symptom alleviation

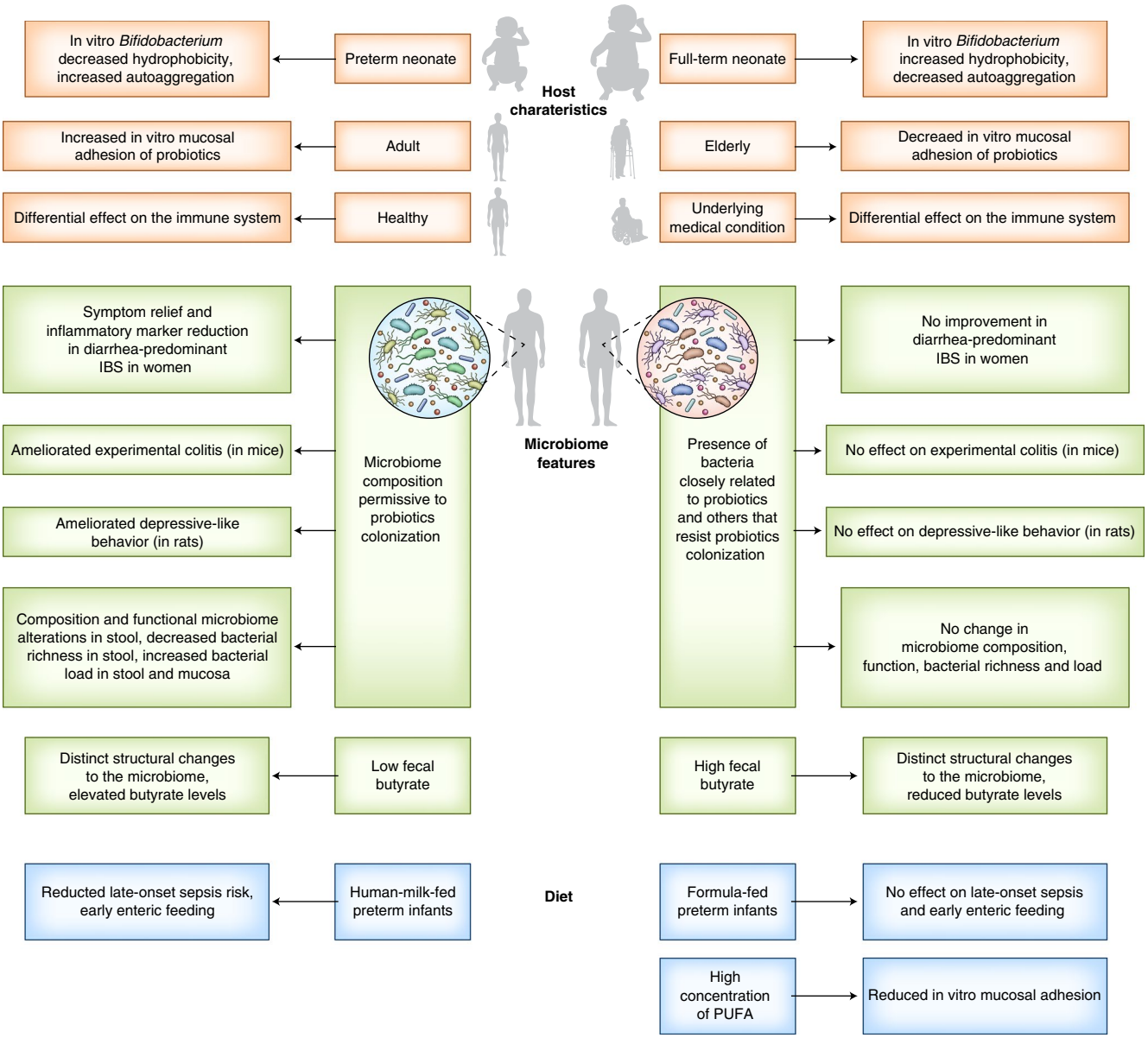


**Fig. 1 | Precision aspects of probiotics.** Distinct initial conditions in the host and their microbiome and varying environmental exposures can result in differing outcomes in different individuals who are supplemented with the same probiotic preparation. In vitro properties of probiotic bacteria, such as adhesion, hydrophobicity and autoaggregation, may vary depending on the host they were isolated from[196,199]. Underlying medical conditions, such as atopic dermatitis[200] or milk hypersensitivity[201], are known to modify the effects that probiotics exert on host immune cells. Features of the indigenous microbiome can also account for different impacts of probiotics on the host, as microbiomes that allow colonization of probiotic bacteria are associated with ameliorated clinical responses in women with IBS[202] and murine models of colitis[203] and depression[204]. These permissive microbiomes are also more prone to compositional and functional alterations in response to probiotics, and the gut epithelium of their hosts exhibits enrichment in distinct pathways compared to that of hosts with resistant microbiomes[89]. Presupplementation butyrate levels are also associated with a differential effect of probiotics on the microbiome and butyrate production or metabolism[205]. Diet may also affect properties of probiotics, as dietary polyunsaturated fatty acids (PUFA) modulates probiotics adhesion in vitro. Similarly, diet may affect clinical outcome, as preterm infants fed with human milk have shown a reduced risk of late-onset sepsis and a shorter time to achieve full enteral feeding, while this is not the case for formula-fed infants[71].

by probiotics is not equal or inferior to that of a placebo effect[62]. One recent meta-analysis has suggested that probiotics may be efficacious in treating symptoms of IBS[63], although it should be noted that none of the single-strain preparations was proven effective for alleviation of abdominal pain or for treatment of bloating, flatulence and bowel urgency. Even within probiotic combinations, some were found to be effective in reducing symptom persistence and abdominal pain scores, while others were not, emphasizing the importance of informed strain selection on disease outcome. Correspondingly, a systematic review of 9 systematic reviews and 35 RCTs did not find evidence for efficacy of various probiotic strains in treatment of IBS symptoms[64].

**Neonatal sepsis.** A promising indication for the efficacy of probiotics is the prevention of neonatal late-onset sepsis and/or necrotizing enterocolitis (NEC), a gastrointestinal disease that typically affects premature newborns[65,66]. Studies in animal models and human cell cultures suggest that the protective mechanism against NEC may

involve antipathogen mucosal protection coupled with induction of maturation of innate immunity and intestinal epithelial cells by some probiotic strains (such as LGG), which prompt an attenuated inflammatory response[67,68]. Furthermore, a recent large-scale RCT strengthened the findings of the aforementioned studies by showing that breastfed infants in rural India ($n = 4,556$ infants) who received a combination of an oral preparation of *L. plantarum* PP 11-217 and prebiotic fructooligosaccharide were protected from neonatal sepsis and death[12]. Nonetheless, in a trial with 1,310 very preterm English infants, *Bifidiobacterium breve* BBG-001 that was enterally fed with formula to infants had no significant effect on prevention of NEC or sepsis[69]. A 2014 Cochrane review (including over 5,000 infants) that did not include these two studies concluded that enteral probiotics containing either *Lactobacillus* alone or in combination with *Bifidobacterium* reduce the incidence of NEC and mortality, but not nosocomial sepsis, in preterm infants[70]. Another systematic review and meta-analysis concluded that probiotics were effective for prevention of late-onset sepsis in preterm infants only when they were given as mixtures (compared to single strains), and only when infants were exclusively human-milk-fed (compared to exclusive formula or mixed feeding)[71]. Two meta-analyses reported no statistically significant effect of probiotics on prevention of NEC[72] or sepsis[73] in infants with extremely low birth weights. Thus, even in this promising indication, precision is warranted, both with regard to the treatment (for example, strain composition, dose, mode of administration and inclusion of prebiotics) and the patient (for example, baseline risk pertaining to birth weight and environmental exposure to microorganisms, and diet). Importantly, the long-term consequences of probiotics on the development of the indigenous gut microbiome and their effect on gut immune, metabolic and anatomical development[74] warrant further studies.

**Acute respiratory infection.** Several systematic reviews and meta-analyses have suggested that probiotics may be effective in reducing the severity, duration and incidence of the common cold, respiratory infections and influenza-like symptoms in children, adults, the elderly and even athletes[75,76]. However, in these meta-analyses, the quality of evidence was stated as being low to very low, and the heterogeneity between studies regarding treatment effect was deemed significant. A meta-analysis encompassing both children and adult studies proposed that probiotics might reduce the severity and duration of respiratory tract infections, but not their incidence[77]. These discrepancies may stem, at times, from reliance on subjective or indirect measures to assess infection, such as self-reporting[78–81], or inference of the duration of disease from the duration of antibiotics treatment or days of absence from work or daycare[75,82]. Discrepancies may also result from unadjusted results when treatment groups are different at baseline (for example, in age and number of preceding infections[82]), subsampling with no clear clinical or biological justification[83,84], unexplained exclusion of trials from meta-analyses[75] and attribution of an effect to treatment despite a counterintuitive dose–response relationship[84]. On a causal level, there is a great need for a data-driven explanation of the mechanisms by which gastrointestinal-localized probiotics would impact a disease involving a remote organ.

## Gut colonization

An unresolved issue associated with the mechanisms of action of probiotics relates to the capacity of the administered microorganisms to stably or even transiently colonize the host gastrointestinal mucosal surface, and whether their colonization is necessary to exert beneficial impacts on the host. The proximity of probiotic strains to the intestinal epithelial layer may be mechanistically crucial to enable host–microbe interactions, such as contact-dependent immune modulation[85,86], metabolite secretion in effective concentrations[87] and mucus layer modification[88]. This decades-long debate is comprised of two inherently distinct colonization-related questions, discussed below.

**Colonization of the gut mucosa during supplementation.** Do probiotics colonize the gut mucosa during consumption? Surprisingly, this critically important topic has not been directly explored in a comprehensive manner in humans until recently. Most claims regarding probiotics colonization have been extrapolated from the assessment of the abundance of probiotics species in stool without direct examination of whether this actually reflects their colonization capacity, or the passage of microbes across the GI tract and their excretion into stool[89]. Like stool assessment, probiotics adherence to human GI cells in vitro[90,91] may be a poor indicator of in vivo colonization due to a myriad of host and microbiome factors that are absent in the in vitro setting.

Direct quantification of mucosal probiotics colonization was determined by endoscopies in a handful of trials, with some studies in humans[92–95] and pigs[96,97]. Some of these studies suggest that probiotic bacteria can be isolated from various GI organs during or even after supplementation, while others show a highly limited and variable colonization pattern, observed in only a minority of tested individuals[98–101]. It is noteworthy that the assessment of the presence of probiotic bacteria by culturing or 16S rDNA techniques in these studies considerably limits distinguishability of the administered probiotic strain and endogenous commensals that are closely related to the probiotic and are of the same species and/or genus (see Box 1). A species- and strain-sensitive metagenomic assessment of human participants evaluated by colonoscopy and gastroscopy before and after consumption of 11 probiotic strains belonging to the 4 most widely used probiotic genera (or placebo)[89] identified an expansion of the mucosa-associated probiotics in 60% of the supplemented individuals and a near-total colonization resistance in the other 40%, even when measured by ultra-sensitive qPCR. The degree of mucosal association was unrelated to the bloom of probiotic strains in stool and could be predicted by a combination of baseline host and microbiome factors, highlighting the potential future prospect of tailoring probiotics to individuals. Interestingly, transplantation of the fecal microbiome from 'resistant' or 'permissive' human individuals into germ-free (GF) mice recapitulated donor susceptibility to probiotics colonization, indicating the existence of a dominant microbiome-mediated colonization-resistance mechanism[89].

Postulated non-colonization-dependent probiotics effects on the host, such as impacts on food digestion, merit evidence-based experimental proof. With this respect, in the above study[89] probiotic strains in 'resistant' individuals were not detected even in the gut lumen during active consumption[102], suggesting that temporarily and/or persistently colonizing mucosa-associated probiotics may serve as an important reservoir for luminal bacteria.

**Post-supplementation persistence in the gut mucosa.** Do probiotics persistently colonize the gut mucosa, even after cessation of consumption? Even in permissive individuals, it remains unclear whether probiotics colonization is maintained after supplementation ceases. In rats fed a fermented milk product (FMP) containing five probiotic strains, all strains were shed in stool during the period of feeding, but only a subset of rats continued to shed one of the five probiotics strains (*L. lactis* CNCM I-1631) at 2 days following supplementation. Transferring the distinct microbiomes of permissive or resistant rats to germ-free rats replicated the colonization permissiveness of the donors[103].

In humans who receive probiotic supplements, detectable shedding of probiotics in stool samples that diminishes following cessation has been described for *Bifidobacterium infantis* 35624 (ref. [104]), *Bifidobacterium animalis lactis* Bb-12 (ref. [105]), *Lactobacillus acidophilus* R52 (ref. [106]), *Lactobacillus casei* DN-114 001 (ref. [107]), *Lactobacillus johnsonii* La1 (ref. [101,108]), *Lactobacillus plantarum*

**Box 1 | Microbiome analysis strategies in probiotics research**

Advances in the field of microbiome research now enable a finer resolution when studying the interaction between probiotics and the resident microbial community while addressing previous methodological limitations and biases to potentially resolve contrasting reports. A major contributor to this confusion is the lenient definition of 'microbiome alterations'. The majority of reports assessing probiotics-induced microbiota modulation utilize 16S rDNA relative abundance (RA) in stool samples. As supplemented probiotic bacteria are excreted in stool, increase in their RA concomitantly leads to a spurious reduction in the relative, but not absolute, abundance of other community members[206], sometimes misleadingly interpreted as microbiota modification[207]. Similarly, introduction of heat-killed bacteria contributes their genetic material to the sample and consequently affects relative abundances[208]. Thus, an increase in the RA of the administered probiotic strain should not be interpreted as a bona fide effect on the microbiome[209]. Another important limitation is the inability of 16S rDNA–based analysis to distinguish between the supplemented probiotic strain and closely related, endogenous members of the same species, leading to an increase in the abundance of the supplemented strain to be interpreted as restoration of the endogenous one[210]. Utilization of culture-based methods or species-specific probes can overcome this caveat by describing probiotics-associated changes in their absolute abundances[211] while accounting for the viability of the supplemented strains[212], but cannot describe global shifts in the microbiome configuration compared to the presupplementation configuration or that after treatment with placebo (beta diversity) or alterations in species richness (alpha diversity). While shotgun metagenomic sequencing may also result in conflicting reports[213,214], it offers the advantage of strain-level resolution and characterization of potential effects of probiotics on microbiome function. Interestingly, several studies have reported probiotics-related effects on the microbiome function in terms of genes, pathways, or microbial metabolites despite no apparent effect on global composition, although these functional microbiome alterations may be the product of genes contributed by the supplemented probiotic strain, rather than modulation of the microbial community[115,215,216]. An additional limitation concerns the definition of the sought-out 'healthy microbiome' that probiotics presumably contribute to. Even when assessing the studies that do suggest probiotics-associated microbiome modulation, no consensus signature of such impacts can be reached, and reports of microbiome changes induced by probiotics are conflicting in many instances. This is the case with, for example, *Clostridium perfringens*[208,211,217] or *Escherichia*[212,217,218], and in various clinical contexts[174]. For example, a probiotics-associated fecal bloom of butyrate-producing bacteria (belonging mainly to Clostridiales) and a reduction in *Bilophila wadsworthia* and *Parabacteroides distasonis* was noted in individuals with IBS ($n = 28$)[213], and mirrored (for *B. wadsworthia*) in a separate cohort of individuals ($n = 107$) in a subset of responders, who experienced alleviation of symptoms following the intervention[202]; however, this was not reproduced by a third RCT ($n = 55$)[219]. Importantly, even in cases in which probiotics administration was associated with changes in the microbiome, these changes could stem from disease modulation rather than directly from exposure to probiotics. To the best of our knowledge, no study to date has demonstrated a direct causal role for probiotics-related microbiome modulation in improvement of a disease phenotype.

299v[109], *Lactobacillus reuteri* DSM17938 (ref. [110,111]), *Lactobacillus rhamnosus* (LGG, R11, 19070-2)[100,106,111] and *Lactobacillus salivarius* CECT5713 (ref. [112]), among others[113]. However, in most studies, follow-up periods were limited to 1–2 weeks after cessation of consumption. Patterns emerging from longer follow-ups suggest both strain- and person-specific persistence variability. Two months following supplementation cessation, *L. rhamnosus* was detected in only one of ten individuals[114], whereas one-third of the *B. longum* AH1206 consumers continued to shed the probiotic species in their stool up to 6 months after discontinuation[115]. Subject- and strain-specific postcessation shedding were also noted in humans supplemented with the aforementioned five-strain FMP, in which only *L. lactis* CNCM I-1631 was shed in stool samples 5 weeks following cessation and only by a subset of individuals that differed in their presupplementation microbiome composition from the noncarriers[103].

## Mechanism of activity

Researchers have postulated that beneficial effects of probiotics occur through diverse mechanisms, including induction of immunomodulation, protection against physiological stress, suppression of pathogens, microbiome modulation and improvement of the barrier function of the gut epithelium (Fig. 2). These mechanistic probiotics studies often suffer from several major limitations, including heavy reliance on utilization of cell-culture systems that do not account for physiological cues that dictate microbe–microbe and microbe–host interactions within the complex GI mucosa microenvironment, and are thus often not replicated in in vivo trials. Other limitations of these studies stem from the poor colonization capacity of exogenous 'human-compatible' probiotics in the murine GI mucosa, compared to that noted in humans[89,116]. Host discordance may be functionally significant, as administration of human commensals to mice can result in a markedly distinct effect on the immune system[117,118] or the host metabolome[119] compared to that of mice harboring a murine microbiome. Importantly, some probiotic traits may be uniformly present between different members of the species or even the genus, for example both *Bifidobacterium* spp. and *Lactobacillus* spp. produce the enzyme β-galactosidase, which may compensate in lactase insufficiency[120,121], while other traits may be species-[122] or even strain-specific[123], or require interaction between probiotic strains[124], as will be further discussed. Several major mechanisms have been suggested to be involved in the effector functions of probiotics, as discussed below.

**Immunomodulation.** Many studies have suggested that there are effects of probiotics on expression of immune-related genes, inflammatory pathway activity and immune marker levels, including modulation of intestinal epithelial cell NF-κB, mitogen-activated protein kinase (MAPK), Akt (also known as phosphoinositide 3-kinase, PI3K)), peroxisome proliferator-activated receptor-γ, CRP, interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-α, IL-1β and interferon γ (IFN-γ), through multiple mechanisms that are mostly contact-dependent (reviewed in ref. [125]). Interestingly, in some studies, live and dead bacteria had a differential effect on gene expression, suggesting that both cell surface and actively secreted molecules may affect the intestinal transcriptome[126]. Additional examples of suggested immune impacts of probiotics on the host include *Lactobacillus*-mediated toll-like receptor 2 (TLR2)-dependent stimulation of TNF-α secretion through lipoteichoic acid (LTA)[127], *B. longum*–mediated contact-dependent IL-10 secretion[128], sortase-dependent pili in *Bifidobacterium* evoking a TNF-α response[90], cell surface exopolysaccharide (sEPS) in *B. longum* 36524 modulating proinflammatory cytokines and T-helper cell 17 ($T_H17$) responses in the gut and the lung[129] and immunostimulatory cell surface appendages, termed SpaCBA, in LGG that mediate (in vitro) both binding to human intestinal mucus and TLR2-dependant modulation of TNF-α, IL-6, IL-10 and IL-12 (ref. [130]).

Additional examples of suggested in vivo mechanisms include induction by LGG of the generation of reactive oxygen species and consequent inhibition of TNF-α-induced intestinal NF-κB activation through SpaC-mediated adhesion to the intestinal

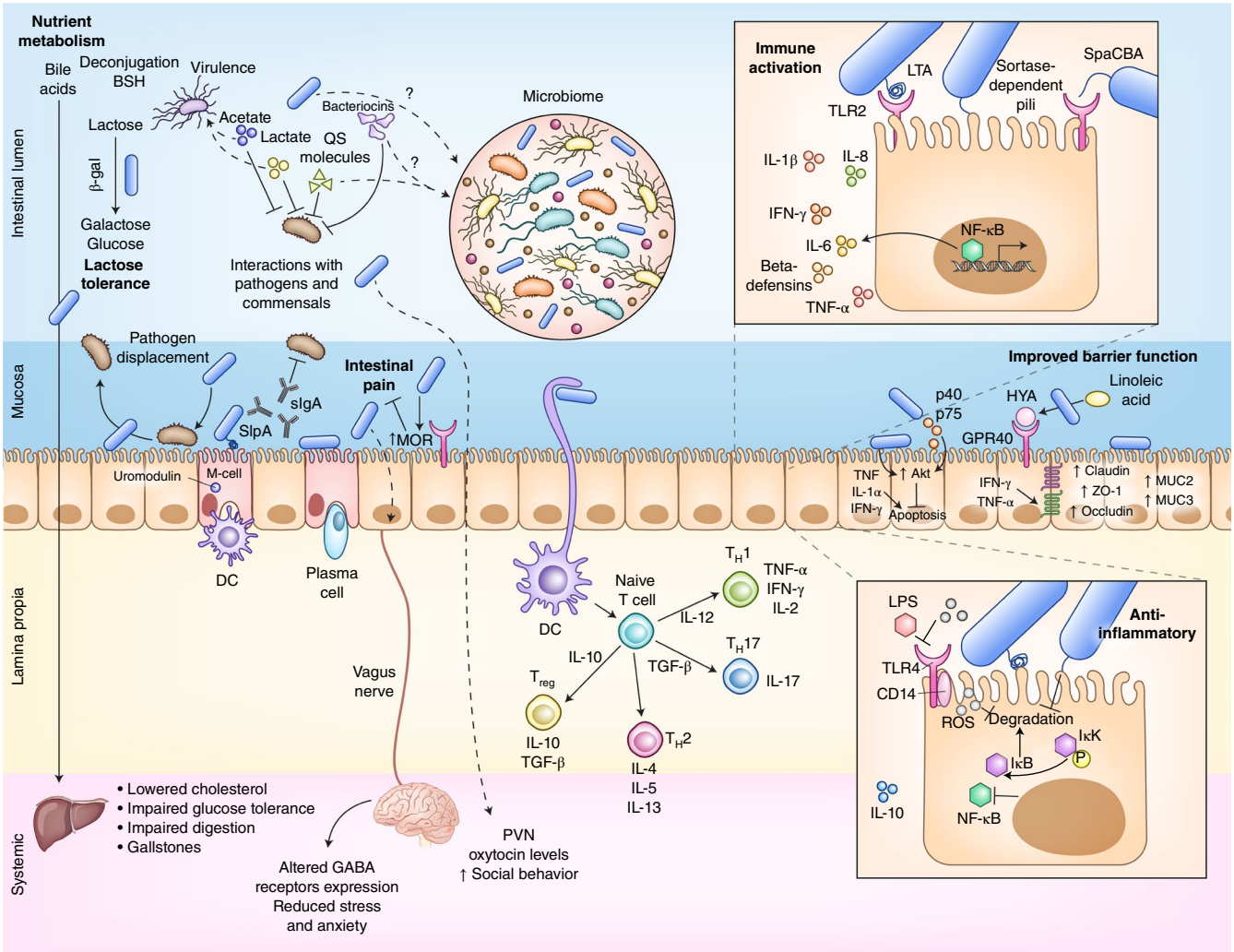


**Fig. 2 | Mechanistic interactions between probiotics and the host and its microbiome.** Probiotics may have several effects on the host, including metabolism of nutrients to improve digestion (e.g., lactose) or produce systemic effects (e.g., bile salts); direct and indirect pathogen antagonism (but potentially also promotion of virulence); improved barrier function, alteration of the microbiome; change of signaling to the nervous system; and immunomodulation. These may be contact-dependent and/or mediated by surface molecules (such as lipoteichoic acid (LTA), sEPS, SpaCBA and sortase-dependent pili), or by secreted molecules (such as short-chain fatty acids, bacteriocins, p40 and p75). Dashed lines represent putative mechanisms. BSH, bile salt hydrolase; β-gal, β-galactosidase; SlpA, S-layer protein A; sIgA, secreted immunoglobulin A; DC, dendritic cell; MOR, mu-opioid receptor; PVN, paraventricular nucleus of the hypothalamus; TGF-β, transforming growth factor beta; TLR, toll-like receptor; LPS, lipopolysaccharide; ROS, reactive oxygen species; $T_H$, T-helper cell; $T_{reg}$, regulatory T cell.

epithelium[131]; peptidoglycan from *L. salivarius* Ls33, but not *L. acidophilus* NCFM, protecting mice from chemically induced colitis in a nucleotide-binding oligomerization domain-containing protein 2 (NOD2)–IL-10-dependent manner[122]; *L. acidophilus* L-92 binding to microfold cells (M cells), resulting in immune modulation by its surface layer protein A (SlpA)[132]; *B. infantis* 35624 inducing TLR2-depended T regulatory cells in humans[133]; and *B. animalis lactis* Bb-12 inducing IgA secretion[134,135]. Collectively, most of the above examples point to a requirement of physical contact or proximity between host cells and probiotics for potential induction of both pro- and anti-inflammatory responses, highlighting the importance of the context in which the probiotics are administered. The clinical outcome of such changes observed in colonized individuals, whether beneficial or not, merits further human studies.

**Protection against pathogens.** Probiotics have been suggested to inhibit pathogen colonization via attachment to epithelial cells and physical blocking of the pathogen's ability to adhere. This has been shown in culture[136] and indirectly in mice for *Salmonella* and *L. acidophilus* LAP5 or *Lactobacillus fermentum* LF33 (ref. [137]). *L. acidophilus* A4 can also antagonize adhesion of *E. coli* O157:H7 to intestinal epithelial cells through upregulation of mucin-2 (MUC2), IL-8, IL-1β and TNF-α (ref. [138]). Several *Bifidobacterium* spp. have been shown to produce acetate in vivo, consequently inhibiting Shiga toxin–producing *E. coli* O157:H7 through acidity-related mechanisms[139]. Several lactic acid bacteria can produce bacteriocins, which are compounds that exhibit antimicrobial activity[140]. For example, production of Abp118 bacteriocin by *L. salivarius* UCC118 protects mice from infection with *L. monocytogenes*[141]. Other mechanisms may involve the disruption of quorum sensing (QS). For instance, *L. acidophilus* La-5 inhibited autoinducer-2 (AI-2) and reduced the expression of some virulence factors of *E. coli* O157:H7 in vitro[142]; *L. acidophilus* GP1B inhibited AI-2 activity of *C. difficile* in vitro, and its administration to mice with *C. difficile* infection improved their survival[143]; and *L. reuteri* RC-14 produced mediators to interfere with *Staphylococcus aureus* QS and thus

repressed its virulence, including the expression of toxic shock syndrome toxin-1 (ref. [144]). Importantly, production of QS molecules and response to QS signals are traits shared between pathogens and commensals[145]; thus, the complexity of QS signals and abundance of responders in vivo may differ from that of in vitro experiments[146], and QS manipulation in vivo can even result in inhibition of commensal bacteria[147].

**Improved barrier function.** Several underlying mechanisms have been suggested for stabilization of gut barrier function by probiotics, and these are reviewed elsewhere[148]. They include upregulation of tight-junction (TJ) proteins (claudin-1, occludin and ZO-1) and improved transepithelial electrical resistance, promotion of mucus secretion (by upregulation of MUC2, MUC3 and MUC1 in colonic epithelial cells) and elevation of butyrate levels, as well as microbiome modulation. These effects may be mediated by locally secreted metabolites. For example, *L. plantarum* produces hydroxy-cis-12-octadecenoic acid (HYA), which has been demonstrated to suppress TJ permeability and the downregulation of occludin, ZO-1 and claudin-1 induced by IFN-γ and TNF-α in culture by regulating TNF receptor 2 expression via the G-protein-coupled receptor (GPR)-40–mitogen-activated protein kinase (MEK)–extracellular-signal-regulated kinase (ERK) pathway[149]. In mice, HYA decreased skin TNF-α and increased claudin-1 in a model of atopic dermatitis[150] and ameliorated pathogen-induced gingival epithelial barrier disruption in a GPR40-dependent manner[151]. Two secreted proteins purified from LGG (termed p40 and p75) have been suggested to promote intestinal epithelial homeostasis by inhibiting cytokine-induced epithelial cell apoptosis[152]. Other effects may require direct mucosal adherence, as demonstrated for MUC3 expression induced by *Lactobacillus* strains in HT29 cells[153], as well as MUC2 and *L. casei* GG in Caco-2 cells[88]. The requirement for adhesion may explain why supplementation with the common commercial VSL#3 probiotic mixture in vivo results in conflicting findings regarding its ability to increase mucin secretion[154,155]. Importantly, when attempting to validate these findings in clinical trials, researchers found inconclusive results, with probiotics-associated improvement observed in some trials[156–158], but not in others[159–162], for multiple underlying conditions. Whether these discrepancies represent the result of variable probiotics colonization not appreciated by early studies remains to be established.

**Additional suggested mechanisms of probiotic action.** One of the prerequisites for commercial probiotics includes resistance to bile salt–mediated growth inhibition. For example, *Lactobacillus* and *Bifidobacterium* spp. resist bile by producing bile salt hydrolases (BSH), which deconjugate glycine or taurine from the steroid core[24]. BSH activity has been associated with systemic beneficial metabolic effects, including reduction in mouse weight gain and levels of plasma cholesterol and liver triglycerides[163], as well as lowering of cholesterol in humans[164]. Nonetheless, deconjugation of bile acids may lead to impaired digestion of dietary lipids and the formation of gallstones[24], as well as impaired glucose tolerance[165].

It has also been suggested that probiotics affect signaling to the enteric and central nervous systems and confer anxiolytic, antidepressant and nociceptive effects to the host[166]. Mice fed with *L. rhamnosus* JB-1 experience specific regional changes in expression of mRNA for γ-aminobutyric acid (GABA)-A and GABA-B receptors in the brain, associated with attenuation of the corticosterone response to stress and anxiety-related behavior, which was not observed in vagotomized animals[167]. Nonetheless, the same strain failed to modulate stress and cognitive performance in humans[168]. In mice, a maternal high-fat diet results in gut dysbiosis of both the dam and the offspring, which has a causal role (as demonstrated by transplantation of the dysbiotic bacteria profile to germ-free mice) in impaired social behavior of the offspring. Treatment with *L. reuteri* ATCC PTA 6475, but not *L. johnsonii* ATCC 33200, restored oxytocin levels in the paraventricular nuclei that were reduced by maternal HFD, and improved social behavior[169]. *L. reuteri* DSM 17938 may also present an antinociceptive effect in rats in a transient receptor potential vanilloid 1 (TRPV1)-dependent manner[170]. *L. acidophilus* NCFM induced expression of μ-opioid and cannabinoid receptors in intestinal epithelial cells and had an analgesic effect in rats[171].

Importantly, clear effects of probiotics in animal models do not necessarily translate to an effect in humans, as was recently demonstrated in a meta-analysis concerning the effect of probiotics on anxiety[172]. Thus, with the potential for probiotics to beneficially influence the gut–brain axis notwithstanding, key molecular players are still unknown and will be critical for proper translation of findings in animal models to human-relevant therapies.

**Box 2 | Quantifying the effect of probiotics on the gastrointestinal microbiome in situ**

While stool samples may not accurately represent the GI mucosa–adherent microbiome[220], only a handful of studies have characterized the effect of probiotics on the intestinal microbiome in situ. A culture-based study of *L. plantarum* 299v–supplemented individuals ($n = 29$) demonstrated an enrichment of *Clostridia* in fecal samples, but not in the rectal or ascending colon mucosa[99]. Likewise, no significant alterations at the lower GI luminal or mucosal microbiome were noted in probiotic-supplemented humans, compared either to their own baseline or to placebo-administered individuals[89]. In rats, VSL#3 exacerbated the reduction in luminal species diversity associated with the induction of chemically induced colitis, but had no effect on the mucosa-associated microbiome[221]. In contrast, in a mouse model of colitis-associated colorectal cancer (azoxymethane-treated *Il10*$^{-/-}$ mice), VSL#3 supplementation resulted in mucosal expansion of Proteobacteria and reduction in Verrucomicrobiaceae, Porphyromondaceae and *Clostridium*, changes that were associated with enhanced tumorigenesis[222]. Conflicting results regarding probiotics-related microbiome modulation were also observed in patients with pouchitis[156,223], although the reported alterations may be merely stemming from the introduction of the VSL#3 bacteria into the niche[223].

## Interactions with the host indigenous microbiome

While the impact of probiotics on the host may not necessarily relate to their interactions with the indigenous microbiome, their use is often associated with claims related to beneficial modulation of the microbiota and normalization of a perturbed microbiota, either as favorable outcomes on their own or as a mechanism by which probiotics protect the host against disease[1]. Nonetheless, the extent, if any, by which probiotics modulate the host intestinal microbiota in healthy individuals remains highly debated. This is highlighted by a 2015 systematic review that reported a lack of evidence for an effect of probiotics on the microbiota in six of the seven studies analyzed[173], as well as by an earlier systematic review analyzing different trials using probiotics, of which only 21% resulted in microbiome alterations[174]. Importantly, presumed effects on the host microbiome may stem from analytical biases (Box 1), and there is a paucity of trials characterizing the effect of probiotics on the gastrointestinal microbiome in situ (Box 2).

One important determinant that may affect the ability of probiotics to modulate the microbiome is the endogenous microbial milieu in the gut before exposure to probiotics, which may differ between individuals. Antibiotics significantly perturb the microbiome[175], thus relieving colonization resistance to probiotics[116],

**Table 1 | Caveats in the probiotics field and proposed strategies to overcome them**

| Limitation | Current state | What can be done |
|---|---|---|
| **Conception** | Probiotics often regarded as a homogenous entity | Strain-level resolution of clinical and mechanistic studies |
| | | Avoid bundling of strains in analyses |
| **Spectrum** | Strain selection confined to few genera | Novel candidate microorganisms with suggested health benefits from recent microbiome research |
| **Research approach** | Trial and error–based | Mechanism-based |
| **Research methodology** | Sample size inadequate at times | Sample size based on power analysis |
| | Endpoints indirect, irrelevant and/or poorly or subjectively defined | Highly valid and reliable endpoints |
| | | Account for placebo effect |
| | Adverse events under-reported | Report adverse events and side effects |
| **Sampled material** | Effect evaluated remotely from target site (stool) | Effect evaluated in situ through endoscopic sampling |
| **Reliance on models** | In vitro models lack probiotics–microbiome and probiotics–host mucosal interactions | Human trials as the mainstay of probiotic research; in vivo and In vitro experimentation used to validate human trials and further explore mechanisms of action |
| | In vivo models may not be compatible with human probiotics | |
| **Stratification and personalization** | One-size-fits-all therapy | Precision therapy based on host and microbiome characteristics, as well as diet |
| **Safety** | Insufficient reporting of safety outcomes, especially in the long term | Long-term safety, especially for critically ill and immune-compromised individuals, as an obligatory quality-control measure |
| **Motivation** | Driven by commercial interests | Driven by medical interests |
| | Regulated as dietary supplements, so proof of efficacy not mandatory | Regulated as drugs, so proof of efficacy under scrutiny by medical authorities |

but also to pathogens[176]. In this context, probiotics are postulated to serve as placeholders in the cleared niche, preventing pathogen colonization and antibiotic-associated diarrhea[35], or as a means of correcting antibiotic-associated dysbiosis[1], but evidence to support an ability of probiotics to facilitate reconstitution of the gut microbiome following perturbation with antibiotics is often based on bacterial cultures or specific fluorescence in situ hybridization or qPCR probes, which represent only a minimal fraction of the perturbed microbiome, and, even using this methodology, the restoration reported may be partial[177,178] or minimal[179] and is highly debated[174].

Overall, the majority of studies do not support a role for probiotics in compositional or functional microbiome modulation other than transient presence of the probiotic strains themselves during the consumption period, regardless of the supplemented strains, the dose and duration or the method used for microbiome analysis[173,174,180]. Among the studies that report probiotics-associated microbiome alterations, it is difficult to point toward patterns of change in commonly altered microbes. While some works reported microbiome alterations to co-occur with health-promoting effects, none demonstrated causality, and it is thus far impossible to a priori claim that such microbiome alterations are beneficial.

## Safety

While the efficacy of probiotics in treating or preventing disease constitutes a decades-long ongoing debate, human supplementation with probiotic microorganisms is generally considered safe and is recognized as such for most probiotic strains by regulatory authorities[181]. This safety profile is mainly based on the history of safe use of probiotics in foods and on observations noted in clinical trials assessing probiotics efficacy, rather than safety, as the major readout[4]. While probiotics may be safe in healthy adults, their use has been associated with a higher risk of infection and/or morbidity in young infants[182] and neonates with very low birth weight[183]; critically ill adult and infant patients in intensive care units; and postoperative, hospitalized or immuno-compromised patients, in part due to bacteremia and fungemia[35,184–186]. Nonetheless, excluding trials in which the causative agent of bloodstream infection was the probiotic strain itself, this association between probiotics use and increased risk of infection remains to be causally validated. Of note, two large-scale systematic reviews of hundreds of probiotics trials concluded that adverse events and safety issues are poorly reported[187,188], calling for non-industry-sponsored, independent, high-quality, multicenter controlled trials assessing both efficacy and adverse effects in the above at-risk populations, preferentially coupled with assessment by regulatory bodies[189].

Interestingly, following antibiotic treatment of human individuals, enhanced colonic colonization by probiotic strains was associated with a persistent long-term probiotics-induced dysbiosis[116], which significantly delayed the reconstitution of both the fecal and the GI mucosal microbiome compared to no intervention following treatment with antibiotics. Soluble factors secreted from the administered *Lactobacillus* species were suggested to directly inhibit (at least ex vivo) human microbiome growth[116]. In agreement, two additional trials demonstrated that postantibiotic probiotic administration to individuals was associated with a lower number of observed species in the gut microbiome compared to no probiotic treatment[190,191], and a third trial reported no significant effect of probiotics on postantibiotics microbiome alpha and beta diversity compared to placebo[192]. Importantly, inhibition of reconstitution of microbiome quantity and diversity toward its preantibiotic configuration may result in significant long-term health effects. Persistent dysbiosis potentially hampers the colonization resistance to pathogens conferred by the microbiome, which may explain several associations made between probiotics use in individuals after antibiotics treatment and increased risk of communicable diseases[35,183,185,193–195], and might potentially contribute to the association between antibiotics and noncommunicable disease, such as type 1 and type 2 diabetes, obesity, idiopathic arthritis, asthma, allergies and inflammatory bowel disease[176]. Given these observations, it is crucial, in

our view, to better assess the long-term safety of probiotics in this context in future clinical trials, and in particular in children, immunosuppressed individuals and the critically ill.

## Future directions

With respect to probiotics data, personal beliefs, solid proof, intuition and commercial interests, coupled with lack of sufficient medical regulation, are often intermingled in ways that make objective interpretation close to impossible. With this unfortunate situation notwithstanding, we envision that recent discoveries in the microbiome field and the introduction of novel high-throughput sequencing and experimental techniques may allow us to revisit some elementary notions about probiotics and focus on biologically relevant questions to facilitate the transition from empiric into target-, disease- and patient-oriented therapeutics (Table 1). Instead of a 'black-box' modus operandi, that is, haphazardly administering one member or more of a limited array of bacteria with the intent to elicit health-promoting effects, a mechanism-oriented approach should be adopted in which probiotic preparations are devised ad hoc, following a set of meticulously established criteria. These may include careful consideration of the population to be treated and the medical indication to be targeted. The aim of microbial therapy should similarly be carefully determined, and a number of questions should be considered. Is the effect on the host mediated remotely or indirectly through secretion of molecules by allochthonous bacteria, by modulation of the indigenous microbiome, or by other putative contact-dependent mechanisms interlinking these bacteria to the intestinal epithelium? Are the intended probiotic effects strain-specific, or shared by many probiotic strains? Could a non-food-grade strain be suited to address a particular medical indication? For example, *A. muciniphila* supplementation in mice prevents diet-induced metabolic syndrome and protects against chemically induced colitis[11]. *Fecalibaterium prausnitzii* is inversely correlated with Crohn's disease activity, IBS and colorectal cancer, and has been suggested to protect mice from chemically induced colitis[11]. As with currently available commercial probiotics, it is important to deepen our understanding of the interactions between the host and its resident microbiome and these potential novel probiotic microorganisms.

Efficient probiotic therapy might require developing means to tackle colonization resistance. This may be achieved by developing predictive algorithms that assess colonization potential on the basis of baseline host and microbiome features[89,103,115,116] and may enable better patient stratification for a therapy[196] or generation of defined consortia fitting individualized patterns. Additional approaches may include rational co-administration of 'prebiotics'[12], colonization-modifying agents[197] or those tailored to support an administered strain[198] or counteract inhibitory mechanisms of commensals. The adverse effects of probiotics on postantibiotic reconstitution of the host transcriptome and the indigenous microbiome configuration need to be comprehensively assessed with more antibiotic regimens, probiotic strain combinations and modeled using human microbiome transfers into germ-free mice, allowing for the assessment of the potential long-term clinical consequences of probiotics-induced dysbiosis. However, the very same potentially negative impact of probiotics-associated dysbiosis, noted in the postantibiotic setting, may be harnessed as positive therapeutic means in other clinical contexts. As such, the apparent improved colonization of probiotics following 'niche freeing' induced by antibiotics may be used as means of potentiating the function of probiotics by allowing their colonization in a variety of microbiome-associated multifactorial disorders. Such a shift from the empiric 'one-size-fits-all' scheme into a person- and condition-tailored approach would inherently necessitate a better understanding of the forces shaping exogenous bacterial colonization and resistance to colonization along the human–gut interface. But it may hold promise in generating more robust and reproducible results in relation to utilization of specific strains, in specific human subpopulations and in specific clinical contexts while accounting for consumer safety.

Finally, diligently planned large-scale randomized and blinded clinical trials, preferentially devoid of commercial interests, should be the mainstay of evidence-based policy formulation. Endpoints should be objectively assessed and stratified to account for inter-individual differences that might mask effect sizes or confound desirable or undesirable outcomes. Adverse reactions should be better studied, reported and published. Unbiased risk and benefit assessment by treating physicians and consumers alike should be encouraged to improve accurate data-driven decision-making at various clinical settings. Data should be made readily accessible and shared to allow for a global collaborative effort to reproduce positive results before guidelines are drafted or modified. In light of the unfortunate historical lack of sufficient medical regulation for currently available probiotics, one cannot overemphasize the critical importance of a formal regulatory approval process to be used with next-generation probiotics, similarly to any other human medical intervention.

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## Author contributions

All authors have researched data for the article, made substantial contributions to discussion of content and wrote, reviewed and edited the manuscript before submission.

## Declaration of interests

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## Additional information

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**Correspondence** should be addressed to E.S. or E.E.

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