The pros, cons, and many unknowns of probiotics

The pros, the cons, and the many unknowns of probiotics

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

[1]Immunology Department, Weizmann Institute of Science, Rehovot, 7610001, Israel
[2]Digestive Center, Tel Aviv Sourasky Medical Center, Tel Aviv, 6423906, Israel
[3]Internal Medicine Department, Tel Aviv Sourasky Medical Center, Tel Aviv, 6423906, Israel
[4]Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, 7610001, Israel
[5]Department of Computer Science and Applied Mathematics, Weizmann Institute of Science, Rehovot, 7610001, Israel
* These authors contributed equally

All correspondence to:
Eran Segal, Ph.D.
Department of Computer Science and Applied Mathematics
Weizmann Institute of Science,
234 Herzl Street,
Rehovot, Israel, 7610001
(08) 934-4282 (phone)
eran.segal@weizmann.ac.il

Eran Elinav, M.D., Ph.D.
Immunology Department,
Weizmann Institute of Science,
234 Herzl Street,
Rehovot, Israel 7610001
(08) 934-4014 (phone)
eran.elinav@weizmann.ac.il

Keywords: probiotics; antibiotics; microbiome

Abstract

Consumption of over-the-counter probiotics has been globalized in recent years. Emerging health trends, extensive commercial endorsement, and conflicting clinical results have led to a highly polarized state, in which, on the one hand, probiotics use has been greatly popularized by the general public, but on the other hand many proposed probiotics health indications remain non-sufficiently substantiated, and are accompanied by a highly debated medical literature. Emerging insights from the microbiome field now enable a re-assessment of probiotics gut colonization, strain-level activity, interactions with the indigenous microbiome, safety and impacts on the eukaryotic host, in reaching more comprehensive conclusions on physiological effects and potentially useful medical indications. In this perspective, we will highlight key advances, challenges, and limitations in striving towards an unbiased interpretation of the large, but often debatable data regarding over-the-counter probiotics, and propose avenues to improve the quality of evidence, transparency, public awareness, and regulation of their use.

Introduction

The concept of oral consumption of microorganisms as means of inducing health benefits has intrigued humans for centuries. The term 'probiotics' first appeared in this context in 1974 and conceptually evolved to the current common definition suggested by the FAO/WHO in 2002: "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host"[1]. Nowadays, over-the-counter microbial therapy constitutes a constantly growing multi-billion-dollar industry[2] and is one of the most commonly consumed forms of food supplements worldwide[3]. Probiotics are supplemented to foods such as yogurt, cheese, ice cream, snacks and nutritional bars, breakfast cereals, and infant formulas, as well as cosmetic products, and are also commercialized in the form of lyophilized pills[4]. Probiotics consumption is widely supported by physicians[5], and specifically gastroenterologists[6]. This popularity notwithstanding, data from decades of research on the efficacy of probiotics in treating or preventing disease often points towards opposing conclusions, and remains conflicting, debated and confusing in many cases. Moreover, the major medical regulatory authorities, such as the European Food Safety Authority[7] or the US Food and Drug Administration[8], have yet to approve any probiotic formulation as a medical intervention modality. As a result, probiotics marketing as dietary supplements is often driven by properties such as safety, viability in the GI tract and lack of impact on food taste, rather than by unequivocal health-promoting effects[9]. This confusing state merits seeking better evidence-based proofs of probiotics impacts 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 probiotics, present challenges in interpretation of available data, and suggest possible strategies to clarify these issues and transform probiotics into a more reproducible and universally accepted measurement-based approach.

In providing this critical perspective, we would like to emphasize that the reviewed over-the-counter microbial interventions will be termed 'probiotics' regardless of their benefit, efficacy or lack thereof. Importantly, while aiming to offer a critical overview of the state of probiotics, we do not wish to 'throw the baby out with the bathwater'. The uncertainty created by some of the opposing evidence with regards to probiotics notwithstanding, we conceptually believe that rigorous research and regulation has a promising potential of materializing into an effective medical intervention in selected indications, some of which are exemplified below. Of note, this perspective is not aimed at reviewing investigational, non-commercially available "next generation" microbial therapy approaches that are being proposed as interventions in various medical indications. These are discussed elsewhere[11,12].

It is unrealistic to include all probiotics studies and their suggested indications in one perspective. Therefore, we will highlight notable examples to discuss **A.** The 'knowns' and challenges with respect to strength of evidence and clinical interpretation of studies assessing

health benefits of probiotics. **B.** Suggested mechanisms of probiotics, touching upon the gut colonization debate **C.** Interactions of probiotic strains with the gut microbiome. **D.** Safety, and **E.** 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, leading to multiple suggested prophylactic and therapeutic health indications and claims, including prevention or treatment of acute, antibiotic-associated, and *Clostridium difficile*-associated diarrhea, amelioration of inflammatory bowel disease and irritable bowel syndrome and risk reduction 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 cardiometabolic syndrome[13]. Regretfully, despite the fact that some clinical trials related to the above health claims are of high methodological quality and validity[14-18], careful examination of the large body of evidence reveals that, 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.

This confusing situation may stem from a number of reasons, including the fact that many of the probiotics trial readouts are based on empiric clinical data that varies in its collection methodology, clinical end-points, and analytical rigor. Many reports use of qualitative, self-reported parameters of "well-being"[19,20], others provide quantification of markers that do not necessarily have clinical significance, for example reduction of C-reactive protein (CRP) in healthy individuals[21], or elevation of glucose-stimulated glucagon-like peptide 1 (GLP-1) in glucose-tolerant individuals[22]. Likewise, a great variability exists as to the systems analyzed, ranging from cell cultures, *in vitro* studies, animal models and human studies spanning observational or randomized, placebo-controlled trials. At times, even within high quality placebo-controlled studies, probiotics putative benefits are conflicting between trials[23,24].

Another contributor to the variability between probiotics studies is the profusion of studied microorganisms. With observations made over a century ago[25,26], the dominant microorganisms used in the probiotics industry even nowadays belong to two genera: *Lactobacillus* and *Bifidobacterium.* Each of these genera includes multiple species, subspecies and strains that feature with both class effects and, in some cases, distinct strain-specific traits. Additional common microorganisms used in the probiotics industry include *Lactococcus spp., Streptococcus thermophilus*, *E. coli* Nissle 1917, and the yeast *Saccharomyces boulardii*[27]. Importantly, some health benefits may require interaction between different strains, contrasting with the current approach of considering probiotics as a homogenous therapeutic entity.

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[28], such as obscuring actual effects or their lack thereof by outlier studies that dominate the collective results and artificially resolving contradictory trends. In particular, meta-analyses concerning probiotics tend, at times, to group studies testing various unrelated supplemented bacteria under the same umbrella, thereby risking over- or misinterpretation of results[29,30]. Consequently, even meta-analyses addressing similar topics may conflict each other[31,32]. Thus, in our view, meta-analyses can complement, but not replace high-quality, large-scale, multi-center, randomized controlled clinical trials.

Moreover, unlike animal models, humans are highly heterogeneous in terms of diet, age range, genetic background and their gut microbiome configuration, and may therefore respond differently to the same intervention (**Fig. 1**). Nevertheless, these readily measurable personalization issues have not been sufficiently addressed in the probiotics literature. As described in the 'Gut colonization' section below, humans feature a differential and highly personalized gut colonization capacity for probiotics, which may drive differential probiotics effects on the host and/or on its indigenous gut microbiome.

Finally, many of the probiotics studies are linked, funded, initiated and endorsed by commercial entities of the probiotic industry or by professional lobby groups heavily associated and funded by the same industry[33]. 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 to be reproduced through non-affiliated research by scientific and medical entities. Examples of some of the most notable suggested probiotics indications include:

Acute gastroenteritis. Probiotics have been suggested to be effective as preventive or therapeutic means in various pediatric and adult etiologies manifesting as acute diarrhea. Several meta-analyses and systematic reviews indicate that some preparations[34], especially those containing *S. boulardii*[35], *Lactobacillus rhamnosus GG (LGG)*[36] and other strains within the *Lactobacillus* genus[37] may ameliorate acute diarrhea in children and shorten its duration by approximately one day. Likewise, probiotics have been shown effective in the prevention and treatment of acute diarrhea in adults, and various preparations, in particular *S. boulardii* and *L. rhamnosus*, have been suggested to improve antibiotic-associated diarrhea both in healthy children[38,39], adults[40,41], and in hospitalized patients[42]. In contrast, other studies and meta-analyses have shown contradictory results as for diarrhea prevention in children[43], adults[23], and in the elderly[41,44]. Notably, the results of two recent high-quality, large-scale, multi-center, randomized placebo-controlled trials assessing treatment with *L. rhamnosus (*LGG or R0011)

with or without *L. helveticus* R0052 in over 1800 children presenting to the emergency department with acute gastroenteritis demonstrated no clinical benefits[45,46]. One meta-analysis in children has noted that the quality of evidence with regard to this indication was low to very low[47], leading to the omission of probiotics from one clinical management guidelines[48], whereas another still advocates the use of *LGG* and *S. boulardii* while stating that the evidence upon which these recommendations are based is of low quality[49]. Notwithstanding the dispute, many parents "self-treat" their children, when contracted with gastroenteritis, with "functional foods" containing probiotics[50].

Clostridium difficile-associated diarrhea (CDAD). *Clostridium difficile* thrives in the gut when microbiome-conferred colonization resistance is compromised, such as upon antibiotics treatment in hospitalized patients, thereby causing a disease that can range in severity from mild diarrhea to a life-threatening condition termed pseudo-membranous colitis. Several meta-analyses have shown a cumulative beneficial outcome for probiotics in preventing *C. difficile* infection or its associated morbidity[51], especially when administered close to antibiotics exposure[52,]. A follow up 2017 meta-analysis further supported moderate beneficial evidence, but indicated a considerable heterogeneity between trials, and utilized a post-hoc analysis that suggested no significant effect to probiotics on CDAD in trials featuring low and moderate baseline CDAD risk[53]. Another meta-analysis concluded that of the various probiotic strains, only *S. boulardii* was effective against *C. difficile*[54], though a different meta-analysis relating specifically to *S. boulardii* found that it reduced CDAD risk in children, but not in adults[55], with low quality of evidence noted[56].

In taking a closer look on the individual studies forming the basis of these meta-analyses, *C. difficile* incidence was non-existent (8 trials, **Table 1**) or low in the majority of the trials regardless of treatment group, while the vast majority of trials included in meta-analyses (34 trials, **Table 1**) did not demonstrate a significant effect for probiotics of different strains on CDAD or *C. difficile* infection. While this may be related to insufficient power of these studies to demonstrate an effect in the context of low incidence of *C. difficile*, two 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[44,57]. Thus, the effects observed in meta-analyses are mostly contributed by a minority of works demonstrating a significant effect[18,42,58-61], of which two are non-peer-reviewed conference abstracts[62,63]. While *C. difficile* incidence in the placebo group was very high[18,42,62] in most works showing a beneficial effect[18,42,58,59,61], other works, in which CDAD was uncommon pointed towards a lower level of evidence with respect to probiotics efficacy in preventing CDAD[33,64]. Together, variable baseline risk of CDAD among cohorts may potentially explain the differences

in outcomes between studies, as well as the fact that the majority of meta-analyses aggregated studies testing a variety of probiotic strains, both fungal and bacterial[65].

Irritable Bowel Syndrome (IBS) 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 symptoms alleviation by probiotics is not equal or inferior to that of a placebo effect[66]. One recent meta-analysis has suggested that probiotics may be efficacious in treating symptoms of IBS[67], although it should be noted that none of the single strain preparations was proven effective for abdominal pain alleviation or for treatment of bloating, flatulence and urgency. Even within probiotic combinations some were found 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 various probiotic strains efficacy in IBS[68].

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 typically affecting premature newborns[69,70]. The protective mechanism against NEC may involve anti-pathogen 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[71,72]. Furthermore, a recent large-scale RCT strengthened these findings by showing that rural Indian infants who received a combination of oral preparation of *L. plantarum* PP 11-217 and fructooligosaccharide were protected from neonatal sepsis and death[14]. It still remains debated whether probiotics reduces the risk for late-onset sepsis in extremely low birth weight neonates[73-75], and whether milk-fed preterm infants feature a better response to this intervention as compared to formula-fed or infants kept on mixed feeding[76]. 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[77] warrants further studies.

Acute respiratory infection. Some systematic reviews and meta-analyses of studies empirically testing probiotic strains, suggest that they may be effective in reducing the severity, duration or incidence of common cold, respiratory infections and influenza-like symptoms in children, adults, the elderly, and even in athletes[78,79], however quality of evidence was stated as low to very-low and heterogeneity between studies 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[80]. These discrepancies may stem, at times, from reliance on subjective or indirect measures to assess infection, such as self-reporting[81-84], or antibiotic treatment and days of absence from work/daycare[78,85]. Discrepancies may also result from unadjusted results when treatment groups were different at baseline[85], subsampling with no clear clinical or biological justification[86,87], unexplained exclusion of trials from meta-analyses[78], and attributing an effect to treatment despite a counter-intuitive dose-response relationship[87]. On a causal level, there is a great need of a data-driven explanation of mechanisms by which gastrointestinal-localized probiotics would impact a disease involving a remote organ.

Gut colonization

An unresolved issue associated with probiotics mechanisms of action relates to the administered microorganisms capacity 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 host lining epithelial layer may be mechanistically crucial, as mucosal adhesion or even presence at low titers, may provide the micrometer distance of probiotics strains to the host gastrointestinal epithelium, which is a prerequisite for many activities including contact-dependent immune modulation[88,89], metabolite secretion in effective concentrations[90], and mucus layer modification[91]. This decades-long debate is comprised of two inherently distinct colonization-related questions, which have often been confusingly intermingled with each other, in the absence of concrete experimental data:

Question 1: 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 probiotics colonization claims have been extrapolated from assessment of their abundance in stool, without directly examining whether this actually reflects their colonization capacity, or merely a passage of non-engaging microbes across the GI tract and their excretion into stool[92]. Like stool assessment, probiotics adherence to human gastrointestinal cells *in vitro*[93,94] 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[95-98] and pigs[99,100] suggesting that probiotic bacteria could be universally isolated from various gastrointestinal organs during or even after supplementation, while others showing a highly limited and variable colonization patterns, observed in only a minority of tested individuals[101-104]. Noteworthy, the universal utilization of culturing or 16S rDNA techniques in these studies considerably limits the ability to distinguish between the administered probiotic strain and endogenous closely related commensals of the

same species/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 four most widely used probiotic genera (or placebo)[92] featured a significant 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 quantitative PCR. 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 a potential future prospect of probiotics tailoring to the individual. Interestingly, transplantation of fecal microbiome from 'resistant' or 'permissive' individuals into germ-free (GF) mice recapitulated the donor susceptibility to probiotics colonization, indicating a dominant microbiome-mediated colonization resistance mechanism[92]. Other 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[92] probiotic strains in 'resistant' individuals were not detected even in the gut lumen during active consumption (Gut Microbes, in press), suggesting that temporarily/persistently colonizing mucosa-associated probiotics may serve as an important reservoir for luminal bacteria.

Question 2: Do probiotics persistently colonize the gut mucosa, even after cessation of consumption? Even in 'permissive' individuals, it remains unclear whether probiotic colonization is maintained after supplementation ceases. In rats fed a fermented milk product (FMP) containing 5 probiotic strains, all strains were shed during feeding, but only a subset of rats continued to shed one of the five probiotics strains (*L. lactis* CNCM I-1631) two days following supplementation. Transferring the distinct microbiomes of 'permissive' or 'resistant' rats to GF rats replicated colonization permissiveness of the donors[105]. In humans, detectable shedding of probiotics in stool samples during supplementation that diminishes following cessation has been described for *Bifidobacterium* strains *infantis* 35624[106], *animalis* sbsp. *lactis* Bb-12[107], *Lactobacillus* strains *acidophilus* R52[108], *casei* DN-114 001[109], *johnsonii* La1[104,110], *plantarum* 299v[111], *reuteri* DSM17938[112,113], *rhamnosus* (LGG, R11, 19070-2)[103,108,113], and *salivarius* CECT5713[114] among others[115]. However, follow-up periods were limited to 1-2 weeks after cessation of consumption in most studies. Patterns emerging from longer follow-ups suggest both strain- and person-specific persistence variability. Two months following supplementation cessation, *L. rhamnosus* was detected only in 1/10 individuals[116], whereas one-third of *B. longum* AH1206 consumers continued to shed the probiotic species in stool up to 6 months after discontinuation[117]. Subject- and strain-specific post-cessation shedding were also noted in humans supplemented with the aforementioned 5-strains mix FMP, in which only *L. lactis* CNCM I-1631 was shed in stool samples five weeks following cessation, and only by a subset of individuals characterized by a distinct microbiome composition[105].

Mechanism of activity

Beneficial effects of probiotics have been postulated to occur through diverse mechanisms, including induction of immunomodulation, protection against physiological stress, suppression of pathogens, microbiome modulation and improvement of gut epithelium barrier function **(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 the myriad of crucial 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 stem from the poor colonization capacity of exogenous 'human compatible' probiotics in the murine GI mucosa, compared to that noted in humans[92,118]. Host discordance may be functionally significant, as administration of human commensals to mice can result in a markedly distinct effect on the immune system[119,120] or host metabolome[121] compared to mice harboring a murine microbiome. Importantly, some probiotic traits may represent class effects and be uniformly present between different members of the species or even the genus, for example both *Bifidobacterium spp.* and *Lactobacillus spp.* produce the enzyme beta-galactosidase, which may compensate in lactase insufficiency[122,123], while other traits may be species-[124] or even strain-specific[125], or require interaction between probiotic strains[126], as further discussed. Several major mechanisms have been suggested to be involved in probiotics effector functions:

Immunomodulation. Many probiotics studies suggested *in vitro* effects on expression of immune-related genes, inflammatory pathways activity and immune marker levels, including modulation of intestinal epithelial cell (IEC) NFκB, mitogen-activated protein kinase (MAPK), Akt / phosphoinositide 3-kinase (PI3K), peroxisome proliferator-activated receptor γ activity, CRP, IL-6, IL-8, tumor necrosis factor (TNF)-α, IL-1β, and interferon γ, through multiple, mostly contact-dependent mechanisms (reviewed in[127]). Interestingly in some studies, live and dead bacteria featured a differential effect on gene expression, suggesting that both cell surface and actively secreted molecules may affect intestinal transcriptome[128]. Additional examples of suggested immune impacts include *Lactobacillus-mediated* TLR2-dependent stimulation of TNF-α secretion through lipoteichoic acid (LTA)[129], *B. longum-mediated* contact-dependent IL-10 secretion[130], sortase-dependent pili in *Bifidobacterium* evoking a TNF-α response[93], cell surface exopolysaccharide (sEPS) in *B. longum* 36524 modulating proinflammatory cytokines and Th17 responses in the gut and the lung[131], and immuno-stimulatory cell surface appendages termed SpaCBA in *LGG*, mediating (*in vitro)* both binding to human intestinal mucus and TLR2-dependant modulation of TNF-α, IL-6, IL-10, and IL-12[132].

Additional examples of suggested *in vivo* mechanisms include *LGG* inducing the generation of reactive oxygen species and consequently inhibiting TNF-α-induced intestinal NFκB activation through SpaC-mediated adhesion to intestinal epithelium[133]; 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[124]; *L. acidophilus* L-92 binding to microfold ('M') cells mediated immune modulation by its surface layer protein A (SlpA)[134]; *B. infantis* 35624 inducing TLR2-depended T regulatory cells in humans[135]; and *B. animalis* sbsp. *lactis* Bb-12 inducing IgA secretion[136,137]. Collectively, most of the above examples point to a requirement of physical contact or proximity between host cells and probiotics to potentially induce both pro- and anti-inflammatory responses, highlighting the importance of the context in which they are administrated. 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 by attaching to epithelial cells and physically blocking the pathogen ability to adhere. This has been shown in culture[138] and indirectly in mice for *Salmonella* and *L. acidophilus* LAP5 or *L. fermentum* LF33[139]. *L. acidophilus* A4 can also antagonize adhesion of *E. coli* O157:H7 to IEC through up-regulation of MUC2, IL-8, IL-1β, and TNF-α[140]. Several *Bifidobacterium spp.* have been shown to produce acetate *in vivo,* consequently inhibiting Shiga toxin-producing *E. coli* (STEC) O157:H7 through acidity-related mechanisms[141,142]. Several lactic acid bacteria can produce bacteriocins, compounds that demonstrate antimicrobial activity[143]. For example, production of Abp118 bacteriocin by *L. salivarius* UCC118 protects mice from infection with *L. monocytogenes*[144]. 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*[145]; *L. acidophilus* GP1B inhibited AI-2 activity for *C. difficile in vitro* and its administration to mice with *C. difficile* infection improved their survival[146]; and *L. reuteri* RC-14 produced mediators to interfere with *S. aureus* QS and thus repressed its virulence, including the expression of toxic shock syndrome toxin-1[147]. Importantly, production and response to QS signals is a trait shared between pathogens and commensals[148], thus the complexity of QS signals and abundance of responders *in vivo* may differ from that of *in vitro* experiments[149], and QS manipulation *in vivo* can even result in inhibition of commensal bacteria[150].

Improved barrier function. Several underlying mechanisms have been suggested for probiotics stabilization of gut barrier function, and are reviewed elsewhere[151], including up-regulation of tight-junction (TJ) proteins (Claudin-1, Occludin, and ZO-1) and improved transepithelial

resistance, promotion of mucus secretion (by up-regulating MUC2, MUC3 and MUC1), 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 down-regulation of occludin, ZO-1, and claudin-1 induced by IFN-γ and TNF-α in culture, by regulating TNFR2 expression via the G protein-coupled receptor (GPR) 40/mitogen-activated protein kinase (MEK)/extracellular-signal-regulated kinase (ERK) pathway[152]. In mice, HYA decreased skin TNF-α and increased claudin-1 in a model of atopic dermatitis[153], and ameliorated pathogen-induced gingival epithelial barrier disruption in a GPR40-dependent manner[154]. 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[155]. Other effects may require direct mucosal adherence, as demonstrated for MUC3 mucin expression induced by *Lactobacillus* strains in HT29 cells[156], as well as MUC2 and *L. casei* GG in Caco-2 cells[91]. The requirement for adhesion may explain why VSL#3 supplementation *in vivo* results in conflicting findings regarding the ability to increase mucin secretion[157,158]. Importantly, when attempting to validate these findings in clinical trials the results were inconclusive, with probiotics-associated improvement observed in some trials[159-161], but not in others[162-165], across 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. Resistance to bile inhibition is one of the prerequisites for commercial probiotics. For example, *Lactobacillus* and *Bifidobacterium spp.* feature bile resistance by the production of bile salt hydrolases (BSH), which deconjugate glycine or taurine from the steroid core[166]. BSH activity has been associated with systemic beneficial metabolic effects, including reduction in mouse weight gain, plasma cholesterol, and liver triglycerides[167], as well as cholesterol lowering in humans[168]. Nonetheless, deconjugation of bile acids may lead to impaired digestion of dietary lipids and the formation of gallstones[166], as well as impaired glucose tolerance[169].

Probiotics were also suggested to affect signaling to the enteric and central nervous systems, and conduce anxiolytic, antidepressant, and ant nociceptive effects on the host[170]. Mice fed with *L. rhamnosus* JB-1 experience specific regional changes in mRNA for γ-aminobutyric acid (GABA)-A and -B receptor in the brain, associated with attenuation of the corticosterone response to stress and an anxiolytic phenotype, which was not observed in vagotomized animals[171]. Nonetheless, the same strain failed to modulate stress or cognitive performance in humans[172]. In mice, maternal high-fat diet results in dysbiosis of both the dam and the offspring, which has a causal role (as demonstrated by transplantations to GF mice) in impairing social behavior in 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[173]. *L. reuteri* DSM 17938 may also present an ant nociceptive effect in rats in a transient receptor potential vanilloid 1 (TRPV1)-dependent manner[174]. *L. acidophilus* NCFM induced expression of μ-opioid and cannabinoid receptors in intestinal epithelial cells, and had an analgesic effect in rats[175]. With the potential of beneficially influencing the gut-brain axis by probiotics notwithstanding, key molecular players are still unknown and will be critical for proper translation of findings in animal models to human-relevant therapies.

Interactions with the indigenous microbiome

While probiotics impact 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 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 intestinal microbiota in healthy individuals remains highly debated, as highlighted by a 2015 systematic review that reported lack of evidence for probiotics effect on the microbiota in 6/7 analyzed studies[176], as well as an earlier systematic review analyzing different trials, of which only 21% resulted in microbiome alterations[177]. Presumable effects on the microbiome may stem from analytical biases **(Box 1)**. In all, the majority of studies on probiotics in healthy adults, children, and elderly individuals reported no effect of probiotics on the fecal microbiota composition, regardless of the supplemented strains, dose, duration or microbiome analysis method **(Table 2)**. Importantly, 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 pre-exposure assembly, which may differ between individuals. Antibiotics significantly perturb the microbiome[178], thus relieving colonization resistance to probiotics[118], but also to pathogens[179]. In this context, probiotics are postulated to serve as placeholders in the cleared niche, preventing pathogen colonization and antibiotic-associated diarrhea[38], or as means of correcting antibiotic-associated dysbiosis[1], but evidence to support an ability of probiotics to facilitate reconstitution of the gut microbiome following antibiotics perturbation is often based on bacterial cultures or specific FISH or qPCR probes, which represent only a minimal fraction of the perturbed microbiome, and even using this methodology, the restoration reported may be partial[180,181] or minimal[182] and is highly debated[177]. 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 **(Table 2)**[176,177]. Among the studies that report probiotics-associated microbiome alterations, it is

difficult to point towards commonly altered microbial patterns of change **(Box 1)**. 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[183]. This safety profile is mainly based on history of safe use 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 higher risk for infections and/or morbidity in young infants[184] and very low birth weight neonates[185], critically ill adult and infant patients in intensive care units, and postoperative, hospitalized or immuno-compromised patients, in part due to bacteremia and fungemia[38,186-188]. Of note, two large-scale systematic reviews of hundreds of probiotics trials concluded that adverse events and safety issues are poorly reported[189,190], calling for the performance of non-industry sponsored independent, high quality, multi-centered controlled trials assessing both efficacy and adverse effects in the above at-risk populations, preferentially coupled with regulatory body assessment[191].

Interestingly, following antibiotics treatment, enhanced probiotics colonic colonization was associated with a persistent long-term probiotics-induced dysbiosis[118], which significantly delayed the reconstitution of both the fecal and the GI mucosal microbiome compared to no post-antibiotics intervention. Soluble factors secreted from the administered *Lactobacillus* species were suggested (at least *ex-vivo*) to directly inhibit human microbiome growth[118]. In agreement, two additional trials demonstrated post-antibiotics probiotics administration to be associated with a lower number of observed species compared to no probiotic treatment[192,193]. Importantly, inhibiting reconstitution of the microbiome quantity and diversity towards its pre-antibiotic configuration may result in significant long-term health effects. Such persistent dysbiosis hampers the colonization resistance to pathogens conferred by the microbiome, which may potentially explain several associations made between probiotics use after antibiotics and increased risk of communicable[38,185,187,194,195,196], and non-communicable disease such as type 1 and type 2 diabetes, obesity, idiopathic arthritis, asthma and allergies, and IBD[179]. Given these observations, it is crucial, in our view, to better assess probiotics long-term safety in this context in future clinical trials, and in particular in children, immunosuppressed individuals, and the critically ill.

Future directions

The probiotics field is one of the most opinionated and polarized disciplines in biomedical sciences. Data, personal beliefs, solid proof, intuition and commercial interests, coupled with lack of medical regulation, are often intermingled in ways making 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 to revisit some elementary notions about probiotics and focus on biologically relevant questions to facilitate the transition from empirical into target-, disease- and patient-oriented therapeutics **(Fig. 3)**. 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 be similarly carefully determined: 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 inter-linking these bacteria to the intestinal epithelium? Are the intended probiotic effects strain-specific or represent a class effect? Could a nonfood-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]. *Faecalibaterium prausnitzii* is inversely correlated with Crohn's disease activity, IBS, and colorectal cancer, and suggested to protect mice from chemically-induced colitis[11]. As with currently available commercial probiotics, it would be important to deepen our understanding of the interactions between these novel potential microorganisms, the host and its resident microbiome, when administered exogenously.

Development of means of tackling colonization resistance may be necessary in many instances, and should require careful patient-subset selection[197], development of predictive algorithms assessing colonization potential based on baseline host and microbiome features[92,105,117,118], rational co-administration of "prebiotics"[14], colonization modifying agents[198], or those tailored to support an administered strain[199], generation of defined consortia fitting individualized patterns, and counteracting commensal-generated inhibitory mechanisms. The adverse effects of probiotics on post-antibiotic host and indigenous microbiome reconstitution need to be comprehensively assessed with more antibiotic regimens, probiotic strain combinations, and modeled using human microbiome transfers into GF 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 post-antibiotic 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 utilized as means of potentiating probiotics function, by allowing their colonization in a variety of microbiome-associated multi-factorial disorders. Such 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. However, it may hold promise in generating more robust and reproducible results in relation to specific strains utilization, in specific human subpopulations, 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 outcome. Adverse reactions should be better studied, reported, and published. Unbiased risk and benefit assessment by treating physicians and consumers alike should be encouraged, in improving 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 contrast to the unfortunate historical lack of sufficient medical regulation for currently available probiotics, one cannot underscore the critical importance of a formal regulatory approval process to be utilized with 'next generation' probiotics, similarly to any other human medical intervention.

Figure legends

Figure 1. Precision aspects of probiotics. Distinct initial host and microbiome conditions and environmental exposures can result in different outcomes when supplemented with the same probiotic preparation. Probiotic bacteria isolated from distinct host populations may present with differential properties, such as adhesion, hydrophobicity and autoaggregation[197,200]. Underlying medical conditions, such as atopic dermatitis[201] or milk hypersensitivity[202], modified the effects probiotics exerted 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 were associated with ameliorated clinical responses in women with IBS[203] and murine models of colitis[204] and depression[205]. These 'permissive' microbiomes were also more prone to compositional and functional alterations in response to probiotics, and their hosts' gut epithelium exhibited enrichment in distinct pathways compared to 'resistant' microbiomes[92]. Pre-supplementation butyrate levels were associated with a differential effect of probiotics on the microbiome and butyrate[206]. Diet may also affect properties of probiotics, as dietary polyunsaturated fatty acids (PUFA) modulated probiotics adhesion in vitro. Similarly, it may

affect clinical outcome, as preterm infants fed with human milk showed a reduced risk of late-onset sepsis and a shorter time to achieve full enteral feeding, while formula-fed infants did not[76].

Figure 2. Mechanistic interactions of probiotics with the host and its microbiome. Probiotic may have several effects on the host, including metabolism of nutrients to improve digestion (lactose) or produce systemic effects (bile salts), direct and indirect pathogen antagonism (but potentially also promoting virulence), improved barrier function, altering the microbiome, affecting signaling to the nervous system, and immunomodulation. These may be contact-dependent and/or mediated by surface molecules (such as LTA, sEPS, SpaCBA, and sortase-dependent pili), or by secreted molecules (such as SCFA, bacteriocins, p40 and p75). Dashed lines represent putative mechanisms. BSH, bile salt hydrolase; B-gal, beta-galactosidase; QS, quorum sensing; SlpA, S-layer protein A; sIgA, secreted immunoglobulin A; M-Cell, microfold cell; DC, dendritic cell; MOR, mu-opioid receptor; GABA, Gamma-Aminobutyric Acid; PVN, paraventricular nucleus of the hypothalamus; TGF-b, Transforming growth factor beta; LTA, lipoteichoic acid; TLR, toll-like receptor; IFNg, interferon gamma; TNFa, tumor necrosis factor alpha; HYA, 10-Hydroxy-cis-12-octadecenoic acid; GPR40, G-protein-coupled receptor 40; Akt, Protein kinase B; LPS, lipopolysaccharide; ROS, reactive oxygen species.

Figure 3. Common limitations of the current approach to probiotic research and proposed strategies to overcome them. Translating the large body of probiotic research into clinical guidelines can sometimes be challenging due to inconclusive or conflicting evidence deriving from suboptimal study conduct and data analysis methodology. A novel perspective to probiotics may include expanding the variety of administered strains and examining them separately per-strain and per-individual according to personalized considerations, such as baseline host and microbiome parameters, the medical condition to treat and the specific aim of treatment. This will require a mechanism-based approach, implemented through meticulously planned high-quality studies in humans, preferably regulated by health authorities, which directly assess the organ of interest and do not overlook long-term safety.

Table 1. Individual trials included in meta-analyses addressing a role for probiotics in *C. difficile* diarrhea, infection or recurrence. Trials with more than one intervention arm appear as separate rows with the difference indicated in the "probiotics intervention" column. Eight trials had a significant effect, and 34 trials did not. P-values and confidence intervals (CI) are taken from the published works, NA indicates that these values were not calculated as part of the work. CDAD, *Clostridium difficile*-associated diarrhea.

Table 2. Probiotics supplementation effect on fecal microbiome composition of healthy individuals. *, Significant taxonomic differences relative to the control group / baseline, excluding the administered strains; N.D., not determined / no data; MZ, monozygotic; CFU, colony forming units. S, Streptococcus; L, Lactobacillus; B, Bifidobacterium.

Box 1: microbiome analysis strategies in probiotics research

Advances in the field of microbiome research now offer implementing 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 abundances (RA) in stool samples. As supplemented probiotic bacteria are excreted in stool, increase in their RA concomitantly leads to a spurious reduction in RA of other community members, sometimes misleadingly interpreted as microbiota modification[207]. Thus, an increase in the RA of the administered probiotic strain should not be interpreted as a *bone-fide* effect on the microbiome[208]. Interestingly, even introduction of heat-killed bacteria was suggested to result in supposed microbiome alterations[209]. Utilization of culture-based methods or species-specific probes can overcome this caveat by describing probiotics-associated changes in their absolute abundances[210], while accounting for viability[211], but cannot describe global shifts in microbiome configuration compared to pre-supplementation or placebo (beta diversity) or alterations in species richness (alpha diversity). While shotgun metagenomic sequencing may also result in conflicting reports[212,213], it offers the advantage of strain-level resolution and characterizing potential probiotics effect on microbiome function. Interestingly, several studies have reported probiotics-related effects on microbiota-encoded function or its associated metabolites, despite no apparent effect on global composition, although these functional microbiome alterations may represent genes contributed by the supplemented probiotic strain, rather than global modulation[117,214,215]. An additional limitation concerns the definition of the sought "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 (**Table 2**), and reports of microbiome changes induced by probiotics are in many times conflicting, for example in the case of *Clostridium perfringens*[209,210,216] or *Escherichia*[211,216,217], and in various clinical contexts[177]. 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)[212], and mirrored (for *B. wadsworthia)* in a separate cohort of individuals (n=107) in a subset of "responders", which experienced alleviation of symptoms following the intervention[203] but was not reproduced by a third RCT (n=55)[218]. Importantly,

even in cases in which probiotics administration was associated with microbiome changes, these changes could be stemming 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 modulations in improving a disease phenotype.

Box 2: quantifying probiotics effect on the gastrointestinal microbiome *in situ*.

While stool samples may not accurately represent the GI mucosa-adherent microbiome[219], 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[102]. Likewise, no significant alterations at the lower GI luminal or mucosal microbiome was noted in probiotics-supplemented humans, compared either to their own baseline or to placebo-administered individuals[92]. 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[220]. 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[221]. Conflicting results regarding probiotics-related microbiome modulation were also observed in patients with pouchitis[159,222], although the reported alterations may be merely stemming from the introduction of the VSL#3 bacteria into the niche[222].

Acknowledgements

We thank the members of the Elinav and Segal laboratories for discussions and apologize to authors whose work was not included due to space constraints. J.S. is the recipient of the Strauss Institute research fellowship. N.Z. is supported by the Gilead Sciences International Research Scholars Program in Liver Disease. E.S. is supported by the Crown Human Genome Center; the Else Kroener Fresenius Foundation; Donald L. Schwarz, Sherman Oaks, CA; Jack N. Halpern, NY, NY; Leesa Steinberg, Canada; and grants funded by the European Research Council and the Israel Science Foundation. E.E. is supported by Y. and R. Ungar, the Abisch Frenkel Foundation for the Promotion of Life Sciences, the Gurwin Family Fund for Scientific Research, the Leona M. and Harry B. Helmsley Charitable Trust, the Crown Endowment Fund for Immunological Research, the estate of J. Gitlitz, the estate of L. Hershkovich, the Benoziyo Endowment Fund for the Advancement of Science, the Adelis Foundation, J.L. and V. Schwartz, A. and G. Markovitz, A. and C. Adelson, the French National Center for Scientific Research (CNRS), D.L. Schwarz, the V.R. Schwartz Research Fellow Chair, L. Steinberg, J.N. Halpern, A. Edelheit, grants funded by the

European Research Council, a Marie Curie Integration grant, the German-Israeli Foundation for Scientific Research and Development, the Israel Science Foundation, the Minerva Foundation, the Rising Tide Foundation, the Helmholtz Foundation, and the European Foundation for the Study of Diabetes. E.E. is a senior fellow of the Canadian Institute of Advanced Research (CIFAR) and an international scholar of the Bill and Melinda Gates Foundation and Howard Hughes Medical Institute (HHMI).

Author contributions

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

Declaration of interests

ES & EE are paid consultants at DayTwo and BiomX. None of their work on microbial therapies is related to, funded or endorsed by, shared or discussed with or licensed to any commercial entity.

References

1 Hill, C. *et al.* Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* **11**, 506-514, doi:10.1038/nrgastro.2014.66 (2014).

2 Research, Z. M. *Global Probiotic Market Is Set For Rapid Growth and is Expected To Reach Value Around USD 65.87 Billion by 2024*, <https://www.zionmarketresearch.com/news/probiotics-market> (2018).

3 Clarke, T. C., Black, L. I., Stussman, B. J., Barnes, P. M. & Nahin, R. L. Trends in the use of complementary health approaches among adults: United States, 2002-2012. *Natl Health Stat Report*, 1-16 (2015).

4 Hoffmann, D. E. *et al.* Probiotics: achieving a better regulatory fit. *Food and drug law journal* **69**, 237-272, ii (2014).

5 Draper, K., Ley, C. & Parsonnet, J. Probiotic guidelines and physician practice: a cross-sectional survey and overview of the literature. *Benef Microbes* **8**, 507-519, doi:10.3920/BM2016.0146 (2017).

6 Williams, M. D., Ha, C. Y. & Ciorba, M. A. Probiotics as therapy in gastroenterology: a study of physician opinions and recommendations. *Journal of clinical gastroenterology* **44**, 631-636, doi:10.1097/MCG.0b013e3181d47f5b (2010).

7 Rijkers, G. T. *et al.* Health benefits and health claims of probiotics: bridging science and marketing. *Br J Nutr* **106**, 1291-1296, doi:10.1017/S000711451100287X (2011).

8 Saldanha, L. G. US Food and Drug Administration regulations governing label claims for food products, including probiotics. *Clin Infect Dis* **46 Suppl 2**, S119-121; discussion S144-151, doi:10.1086/523328 (2008).

9 Degnan, F. H. Clinical studies involving probiotics: when FDA's investigational new drug rubric applies-and when it may not. *Gut Microbes* **3**, 485-489, doi:10.4161/gmic.22158 (2012).

10 Rijkers, G. T. *et al.* Guidance for substantiating the evidence for beneficial effects of probiotics: current status and recommendations for future research. *J Nutr* **140**, 671s-676s, doi:10.3945/jn.109.113779 (2010).

11 El Hage, R., Hernandez-Sanabria, E. & Van de Wiele, T. Emerging Trends in "Smart Probiotics": Functional Consideration for the Development of Novel Health and Industrial Applications. *Frontiers in microbiology* **8**, 1889, doi:10.3389/fmicb.2017.01889 (2017).

12 Cani, P. D. Human gut microbiome: hopes, threats and promises. *Gut* **67**, 1716-1725, doi:10.1136/gutjnl-2018-316723 (2018).

13 Sniffen, J. C., McFarland, L. V., Evans, C. T. & Goldstein, E. J. C. Choosing an appropriate probiotic product for your patient: An evidence-based practical guide. *PLoS One* **13**, e0209205, doi:10.1371/journal.pone.0209205 (2018).

14 Panigrahi, P. *et al.* A randomized synbiotic trial to prevent sepsis among infants in rural India. *Nature* **548**, 407-412, doi:10.1038/nature23480 (2017).

15 Kruis, W. *et al.* Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. *Gut* **53**, 1617-1623, doi:10.1136/gut.2003.037747 (2004).

16 Canani, R. B. *et al.* Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations. *Bmj* **335**, 340, doi:10.1136/bmj.39272.581736.55 (2007).

17 Ruszczynski, M., Radzikowski, A. & Szajewska, H. Clinical trial: effectiveness of Lactobacillus rhamnosus (strains E/N, Oxy and Pen) in the prevention of antibiotic-associated diarrhoea in children. *Aliment Pharmacol Ther* **28**, 154-161, doi:10.1111/j.1365-2036.2008.03714.x (2008).

18 Gao, X. W., Mubasher, M., Fang, C. Y., Reifer, C. & Miller, L. E. Dose-response efficacy of a proprietary probiotic formula of Lactobacillus acidophilus CL1285 and Lactobacillus casei LBC80R for antibiotic-associated diarrhea and Clostridium difficile-associated diarrhea prophylaxis in adult patients. *Am J Gastroenterol* **105**, 1636-1641, doi:10.1038/ajg.2010.11 (2010).

19 Fujimori, S. *et al.* A randomized controlled trial on the efficacy of synbiotic versus probiotic or prebiotic treatment to improve the quality of life in patients with ulcerative colitis. *Nutrition (Burbank, Los Angeles County, Calif.)* **25**, 520-525, doi:10.1016/j.nut.2008.11.017 (2009).

20 Benton, D., Williams, C. & Brown, A. Impact of consuming a milk drink containing a probiotic on mood and cognition. *European journal of clinical nutrition* **61**, 355-361, doi:10.1038/sj.ejcn.1602546 (2007).

21 Kekkonen, R. A. *et al.* Probiotic intervention has strain-specific anti-inflammatory effects in healthy adults. *World journal of gastroenterology* **14**, 2029-2036 (2008).

22 Simon, M. C. *et al.* Intake of Lactobacillus reuteri improves incretin and insulin secretion in glucose-tolerant humans: a proof of concept. *Diabetes care* **38**, 1827-1834, doi:10.2337/dc14-2690 (2015).

23 Pereg, D. *et al.* The effect of fermented yogurt on the prevention of diarrhea in a healthy adult population. *American journal of infection control* **33**, 122-125, doi:10.1016/j.ajic.2004.11.001 (2005).

24 Dietrich, C. G., Kottmann, T. & Alavi, M. Commercially available probiotic drinks containing Lactobacillus casei DN-114001 reduce antibiotic-associated diarrhea. *World journal of gastroenterology* **20**, 15837-15844, doi:10.3748/wjg.v20.i42.15837 (2014).

25 Metchnikoff, I. I. *The prolongation of life: optimistic studies*. (Springer Publishing Company, 2004).

26 Tissier, H. *Traitement des infections intestinales par la méthode de transformation de la flore bactérienne de l'intestin*. (1907).

27 Gareau, M. G., Sherman, P. M. & Walker, W. A. Probiotics and the gut microbiota in intestinal health and disease. *Nat Rev Gastroenterol Hepatol* **7**, 503-514, doi:10.1038/nrgastro.2010.117 (2010).

28 de Vrieze, J. The metawars. *Science* **361**, 1184-1188, doi:10.1126/science.361.6408.1184 (2018).

29 Moayyedi, P. *et al.* The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Gut* **59**, 325-332, doi:10.1136/gut.2008.167270 (2010).
30 Shimizu, M., Hashiguchi, M., Shiga, T., Tamura, H. O. & Mochizuki, M. Meta-Analysis: Effects of Probiotic Supplementation on Lipid Profiles in Normal to Mildly Hypercholesterolemic Individuals. *PLoS One* **10**, e0139795, doi:10.1371/journal.pone.0139795 (2015).
31 Lu, C. *et al.* Probiotic supplementation does not improve eradication rate of Helicobacter pylori infection compared to placebo based on standard therapy: a meta-analysis. *Sci Rep* **6**, 23522, doi:10.1038/srep23522 (2016).
32 Lu, M. *et al.* Efficacy of Probiotic Supplementation Therapy for Helicobacter pylori Eradication: A Meta-Analysis of Randomized Controlled Trials. *PLoS One* **11**, e0163743, doi:10.1371/journal.pone.0163743 (2016).
33 Kolber, M. R., Vandermeer, B. & Allan, G. M. Funding may influence trial results examining probiotics and Clostridium difficile diarrhea rates. *Am J Gastroenterol* **109**, 1081-1082, doi:10.1038/ajg.2014.109 (2014).
34 Allen, S. J., Martinez, E. G., Gregorio, G. V. & Dans, L. F. Probiotics for treating acute infectious diarrhoea. *Cochrane Database Syst Rev*, Cd003048, doi:10.1002/14651858.CD003048.pub3 (2010).
35 Feizizadeh, S., Salehi-Abargouei, A. & Akbari, V. Efficacy and safety of Saccharomyces boulardii for acute diarrhea. *Pediatrics* **134**, e176-191, doi:10.1542/peds.2013-3950 (2014).
36 Szajewska, H., Skorka, A., Ruszczynski, M. & Gieruszczak-Bialek, D. Meta-analysis: Lactobacillus GG for treating acute gastroenteritis in children--updated analysis of randomised controlled trials. *Aliment Pharmacol Ther* **38**, 467-476, doi:10.1111/apt.12403 (2013).
37 Van Niel, C. W., Feudtner, C., Garrison, M. M. & Christakis, D. A. Lactobacillus therapy for acute infectious diarrhea in children: a meta-analysis. *Pediatrics* **109**, 678-684 (2002).
38 Goldenberg, J. Z. *et al.* Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database Syst Rev*, CD004827, doi:10.1002/14651858.CD004827.pub4 (2015).
39 Szajewska, H. & Kolodziej, M. Systematic review with meta-analysis: Lactobacillus rhamnosus GG in the prevention of antibiotic-associated diarrhoea in children and adults. *Aliment Pharmacol Ther* **42**, 1149-1157, doi:10.1111/apt.13404 (2015).
40 Hempel, S. *et al.* Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. *Jama* **307**, 1959-1969, doi:10.1001/jama.2012.3507 (2012).
41 Jafarnejad, S. *et al.* Probiotics Reduce the Risk of Antibiotic-Associated Diarrhea in Adults (18-64 Years) but Not the Elderly (>65 Years): A Meta-Analysis. *Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition* **31**, 502-513, doi:10.1177/0884533616639399 (2016).
42 Hickson, M. *et al.* Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. *BMJ* **335**, 80, doi:10.1136/bmj.39231.599815.55 (2007).
43 Olek, A. *et al.* Efficacy and Safety of Lactobacillus plantarum DSM 9843 (LP299V) in the Prevention of Antibiotic-Associated Gastrointestinal Symptoms in Children-Randomized, Double-Blind, Placebo-Controlled Study. *J Pediatr* **186**, 82-86, doi:10.1016/j.jpeds.2017.03.047 (2017).
44 Allen, S. J. *et al.* Lactobacilli and bifidobacteria in the prevention of antibiotic-associated diarrhoea and Clostridium difficile diarrhoea in older inpatients (PLACIDE): a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* **382**, 1249-1257, doi:10.1016/S0140-6736(13)61218-0 (2013).
45 Freedman, S. B. *et al.* Multicenter Trial of a Combination Probiotic for Children with Gastroenteritis. *N Engl J Med* **379**, 2015-2026, doi:10.1056/NEJMoa1802597 (2018).

46 Schnadower, D. *et al.* Lactobacillus rhamnosus GG versus Placebo for Acute Gastroenteritis in Children. *N Engl J Med* **379**, 2002-2014, doi:10.1056/NEJMoa1802598 (2018).
47 Freedman, S. B. *et al.* Gastroenteritis Therapies in Developed Countries: Systematic Review and Meta-Analysis. *PLoS One* **10**, e0128754, doi:10.1371/journal.pone.0128754 (2015).
48 Khanna, R., Lakhanpaul, M., Burman-Roy, S. & Murphy, M. S. Diarrhoea and vomiting caused by gastroenteritis in children under 5 years: summary of NICE guidance. *Bmj* **338**, b1350, doi:10.1136/bmj.b1350 (2009).
49 Szajewska, H. *et al.* Use of probiotics for management of acute gastroenteritis: a position paper by the ESPGHAN Working Group for Probiotics and Prebiotics. *Journal of pediatric gastroenterology and nutrition* **58**, 531-539, doi:10.1097/mpg.0000000000000320 (2014).
50 Li, S. T., Klein, E. J., Tarr, P. I. & Denno, D. M. Parental management of childhood diarrhea. *Clinical pediatrics* **48**, 295-303, doi:10.1177/0009922808327057 (2009).
51 Goldenberg, J. Z. *et al.* Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children. *Cochrane Database Syst Rev*, Cd006095, doi:10.1002/14651858.CD006095.pub3 (2013).
52 Shen, N. T. *et al.* Timely Use of Probiotics in Hospitalized Adults Prevents Clostridium difficile Infection: A Systematic Review With Meta-Regression Analysis. *Gastroenterology* **152**, 1889-1900 e1889, doi:10.1053/j.gastro.2017.02.003 (2017).
53 Goldenberg, J. Z. *et al.* Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children. *Cochrane Database Syst Rev* **12**, Cd006095, doi:10.1002/14651858.CD006095.pub4 (2017).
54 McFarland, L. V. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. *Am J Gastroenterol* **101**, 812-822, doi:10.1111/j.1572-0241.2006.00465.x (2006).
55 Szajewska, H. & Kolodziej, M. Systematic review with meta-analysis: Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea. *Aliment Pharmacol Ther* **42**, 793-801, doi:10.1111/apt.13344 (2015).
56 Szajewska, H. *et al.* Probiotics for the Prevention of Antibiotic-Associated Diarrhea in Children. *Journal of pediatric gastroenterology and nutrition* **62**, 495-506, doi:10.1097/mpg.0000000000001081 (2016).
57 Georgieva, M. *et al.* Use of the probiotic lactobacillus reuteri dsm 17938 in the prevention of antibiotic-associated infections in hospitalIzed Bulgarian children: a randomized, controlled trial. *Journal of IMAB–Annual Proceeding Scientific Papers* **21**, 895-900 (2015).
58 Ouwehand, A. C. *et al.* Probiotics reduce symptoms of antibiotic use in a hospital setting: a randomized dose response study. *Vaccine* **32**, 458-463, doi:10.1016/j.vaccine.2013.11.053 (2014).
59 Klarin, B. *et al.* Lactobacillus plantarum 299v reduces colonisation of Clostridium difficile in critically ill patients treated with antibiotics. *Acta anaesthesiologica Scandinavica* **52**, 1096-1102, doi:10.1111/j.1399-6576.2008.01748.x (2008).
60 Morrow, L. E., Kollef, M. H. & Casale, T. B. Probiotic prophylaxis of ventilator-associated pneumonia: a blinded, randomized, controlled trial. *American journal of respiratory and critical care medicine* **182**, 1058-1064, doi:10.1164/rccm.200912-1853OC (2010).
61 Shan, L. S. *et al.* Prevention and treatment of diarrhoea with Saccharomyces boulardii in children with acute lower respiratory tract infections. *Benef Microbes* **4**, 329-334, doi:10.3920/bm2013.0008 (2013).
62 Rafiq, R. *et al.* in *Gastroenterology.* A187-A187 (WB SAUNDERS CO-ELSEVIER INC 1600 JOHN F KENNEDY BOULEVARD, STE 1800, PHILADELPHIA, PA 19103-2899 USA).
63 Lemann, M., Cezard, J., Ruemmele, F. & Turck, D. European Society for Paediatric Gastroenterology, Hepatology, and Nutrition Annual Meeting June 3–6, 2009

Budapest, Hungary. *Journal of pediatric gastroenterology and nutrition* **48**, E1-E149 (2009).
64 Viggars, A. P., Gracie, D. J. & Ford, A. C. Use of Probiotics in Hospitalized Adults to Prevent Clostridium difficile Infection: DownGRADE the Quality of Evidence? *Gastroenterology* **153**, 1451-1452, doi:10.1053/j.gastro.2017.05.067 (2017).
65 McFarland, L. V. Deciphering meta-analytic results: a mini-review of probiotics for the prevention of paediatric antibiotic-associated diarrhoea and Clostridium difficile infections. *Benef Microbes* **6**, 189-194, doi:10.3920/bm2014.0034 (2015).
66 Guyonnet, D. *et al.* Effect of a fermented milk containing Bifidobacterium animalis DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicentre, randomized, double-blind, controlled trial. *Aliment Pharmacol Ther* **26**, 475-486, doi:10.1111/j.1365-2036.2007.03362.x (2007).
67 Ford, A. C., Harris, L. A., Lacy, B. E., Quigley, E. M. M. & Moayyedi, P. Systematic review with meta-analysis: the efficacy of prebiotics, probiotics, synbiotics and antibiotics in irritable bowel syndrome. *Aliment Pharmacol Ther* **48**, 1044-1060, doi:10.1111/apt.15001 (2018).
68 McKenzie, Y., Thompson, J., Gulia, P. & Lomer, M. British Dietetic Association systematic review of systematic reviews and evidence- based practice guidelines for the use of probiotics in the management of irritable bowel syndrome in adults (2016 update). *Journal of human nutrition and dietetics* **29**, 576-592 (2016).
69 Olsen, R., Greisen, G., Schroder, M. & Brok, J. Prophylactic Probiotics for Preterm Infants: A Systematic Review and Meta-Analysis of Observational Studies. *Neonatology* **109**, 105-112, doi:10.1159/000441274 (2016).
70 Rao, S. C., Athalye-Jape, G. K., Deshpande, G. C., Simmer, K. N. & Patole, S. K. Probiotic Supplementation and Late-Onset Sepsis in Preterm Infants: A Meta-analysis. *Pediatrics* **137**, e20153684, doi:10.1542/peds.2015-3684 (2016).
71 Ganguli, K. *et al.* Probiotics prevent necrotizing enterocolitis by modulating enterocyte genes that regulate innate immune-mediated inflammation. *American journal of physiology. Gastrointestinal and liver physiology* **304**, G132-141, doi:10.1152/ajpgi.00142.2012 (2013).
72 Yan, F. *et al.* Neonatal colonization of mice with LGG promotes intestinal development and decreases susceptibility to colitis in adulthood. *Mucosal Immunol* **10**, 117-127, doi:10.1038/mi.2016.43 (2017).
73 Zhang, G. Q., Hu, H. J., Liu, C. Y., Shakya, S. & Li, Z. Y. Probiotics for Preventing Late-Onset Sepsis in Preterm Neonates: A PRISMA-Compliant Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Medicine* **95**, e2581, doi:10.1097/md.0000000000002581 (2016).
74 Dermyshi, E. *et al.* The "Golden Age" of Probiotics: A Systematic Review and Meta-Analysis of Randomized and Observational Studies in Preterm Infants. *Neonatology* **112**, 9-23, doi:10.1159/000454668 (2017).
75 Sun, J. *et al.* Effects of Probiotics on Necrotizing Enterocolitis, Sepsis, Intraventricular Hemorrhage, Mortality, Length of Hospital Stay, and Weight Gain in Very Preterm Infants: A Meta-Analysis. *Advances in nutrition (Bethesda, Md.)* **8**, 749-763, doi:10.3945/an.116.014605 (2017).
76 Aceti, A. *et al.* Probiotics Prevent Late-Onset Sepsis in Human Milk-Fed, Very Low Birth Weight Preterm Infants: Systematic Review and Meta-Analysis. *Nutrients* **9**, doi:10.3390/nu9080904 (2017).
77 Sommer, F. & Backhed, F. The gut microbiota--masters of host development and physiology. *Nature reviews. Microbiology* **11**, 227-238, doi:10.1038/nrmicro2974 (2013).
78 King, S. *et al.* Does probiotic consumption reduce antibiotic utilization for common acute infections? A systematic review and meta-analysis. *European journal of public health*, doi:10.1093/eurpub/cky185 (2018).

79 Hao, Q., Dong, B. R. & Wu, T. Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database Syst Rev*, Cd006895, doi:10.1002/14651858.CD006895.pub3 (2015).

80 Vouloumanou, E. K., Makris, G. C., Karageorgopoulos, D. E. & Falagas, M. E. Probiotics for the prevention of respiratory tract infections: a systematic review. *Int J Antimicrob Agents* **34**, 197.e191-110, doi:10.1016/j.ijantimicag.2008.11.005 (2009).

81 Merenstein, D. *et al.* Use of a fermented dairy probiotic drink containing Lactobacillus casei (DN-114 001) to decrease the rate of illness in kids: the DRINK study. A patient-oriented, double-blind, cluster-randomized, placebo-controlled, clinical trial. *European journal of clinical nutrition* **64**, 669-677, doi:10.1038/ejcn.2010.65 (2010).

82 de Vrese, M. *et al.* Effect of Lactobacillus gasseri PA 16/8, Bifidobacterium longum SP 07/3, B. bifidum MF 20/5 on common cold episodes: a double blind, randomized, controlled trial. *Clinical nutrition (Edinburgh, Scotland)* **24**, 481-491, doi:10.1016/j.clnu.2005.02.006 (2005).

83 Smith, T. J., Rigassio-Radler, D., Denmark, R., Haley, T. & Touger-Decker, R. Effect of Lactobacillus rhamnosus LGG(R) and Bifidobacterium animalis ssp. lactis BB-12(R) on health-related quality of life in college students affected by upper respiratory infections. *Br J Nutr* **109**, 1999-2007, doi:10.1017/s0007114512004138 (2013).

84 Shinkai, S. *et al.* Immunoprotective effects of oral intake of heat-killed Lactobacillus pentosus strain b240 in elderly adults: a randomised, double-blind, placebo-controlled trial. *Br J Nutr* **109**, 1856-1865, doi:10.1017/s0007114512003753 (2013).

85 Hatakka, K. *et al.* Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. *Bmj* **322**, 1327 (2001).

86 West, N. P. *et al.* Lactobacillus fermentum (PCC(R)) supplementation and gastrointestinal and respiratory-tract illness symptoms: a randomised control trial in athletes. *Nutrition journal* **10**, 30, doi:10.1186/1475-2891-10-30 (2011).

87 Murata, M. *et al.* Effects of paraprobiotic Lactobacillus paracasei MCC1849 supplementation on symptoms of the common cold and mood states in healthy adults. *Benef Microbes*, 1-10, doi:10.3920/bm2017.0197 (2018).

88 Atarashi, K. *et al.* Th17 Cell Induction by Adhesion of Microbes to Intestinal Epithelial Cells. *Cell* **163**, 367-380, doi:10.1016/j.cell.2015.08.058 (2015).

89 Thaiss, C. A. *et al.* Microbiota Diurnal Rhythmicity Programs Host Transcriptome Oscillations. *Cell* **167**, 1495-1510.e1412, doi:10.1016/j.cell.2016.11.003 (2016).

90 Uchimura, Y. *et al.* Antibodies Set Boundaries Limiting Microbial Metabolite Penetration and the Resultant Mammalian Host Response. *Immunity* **49**, 545-559.e545, doi:10.1016/j.immuni.2018.08.004 (2018).

91 Mattar, A. F. *et al.* Probiotics up-regulate MUC-2 mucin gene expression in a Caco-2 cell-culture model. *Pediatric surgery international* **18**, 586-590, doi:10.1007/s00383-002-0855-7 (2002).

92 Zmora, N. *et al.* Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features. *Cell* **174**, 1388-1405.e1321, doi:10.1016/j.cell.2018.08.041 (2018).

93 Turroni, F. *et al.* Role of sortase-dependent pili of Bifidobacterium bifidum PRL2010 in modulating bacterium-host interactions. *Proc Natl Acad Sci U S A* **110**, 11151-11156, doi:10.1073/pnas.1303897110 (2013).

94 Van Tassell, M. L. & Miller, M. J. Lactobacillus adhesion to mucus. *Nutrients* **3**, 613-636, doi:10.3390/nu3050613 (2011).

95 Fujimura, S. *et al.* Detection of Lactobacillus gasseri OLL2716 strain administered with yogurt drink in gastric mucus layer in humans. *Letters in applied microbiology* **43**, 578-581, doi:10.1111/j.1472-765X.2006.02017.x (2006).

96 Valeur, N., Engel, P., Carbajal, N., Connolly, E. & Ladefoged, K. Colonization and immunomodulation by Lactobacillus reuteri ATCC 55730 in the human gastrointestinal tract. *Appl Environ Microbiol* **70**, 1176-1181 (2004).

97 Johansson, M. L. *et al.* Administration of different Lactobacillus strains in fermented oatmeal soup: in vivo colonization of human intestinal mucosa and effect on the indigenous flora. *Appl Environ Microbiol* **59**, 15-20 (1993).
98 Shibahara-Sone, H. *et al.* Living cells of probiotic Bifidobacterium bifidum YIT 10347 detected on gastric mucosa in humans. *Benef Microbes* **7**, 319-326, doi:10.3920/bm2015.0138 (2016).
99 Yang, Y., Galle, S., Le, M. H., Zijlstra, R. T. & Ganzle, M. G. Feed Fermentation with Reuteran- and Levan-Producing Lactobacillus reuteri Reduces Colonization of Weanling Pigs by Enterotoxigenic Escherichia coli. *Appl Environ Microbiol* **81**, 5743-5752, doi:10.1128/aem.01525-15 (2015).
100 Riboulet-Bisson, E. *et al.* Effect of Lactobacillus salivarius bacteriocin Abp118 on the mouse and pig intestinal microbiota. *PLoS One* **7**, e31113, doi:10.1371/journal.pone.0031113 (2012).
101 Crittenden, R. *et al.* Lactobacillus paracasei subsp. paracasei F19: Survival, ecology and safety in the human intestinal tract-A survey of feeding studies within the PROBDEMO project. *Microbial ecology in health and disease* **14**, 22-26 (2002).
102 Goossens, D. A., Jonkers, D. M., Russel, M. G., Stobberingh, E. E. & Stockbrugger, R. W. The effect of a probiotic drink with Lactobacillus plantarum 299v on the bacterial composition in faeces and mucosal biopsies of rectum and ascending colon. *Aliment Pharmacol Ther* **23**, 255-263, doi:10.1111/j.1365-2036.2006.02749.x (2006).
103 Alander, M. *et al.* Persistence of colonization of human colonic mucosa by a probiotic strain, Lactobacillus rhamnosus GG, after oral consumption. *Appl Environ Microbiol* **65**, 351-354 (1999).
104 Gianotti, L. *et al.* A randomized double-blind trial on perioperative administration of probiotics in colorectal cancer patients. *World journal of gastroenterology* **16**, 167-175 (2010).
105 Zhang, C. *et al.* Ecological robustness of the gut microbiota in response to ingestion of transient food-borne microbes. *Isme j* **10**, 2235-2245, doi:10.1038/ismej.2016.13 (2016).
106 Charbonneau, D., Gibb, R. D. & Quigley, E. M. Fecal excretion of Bifidobacterium infantis 35624 and changes in fecal microbiota after eight weeks of oral supplementation with encapsulated probiotic. *Gut Microbes* **4**, 201-211, doi:10.4161/gmic.24196 (2013).
107 Alander, M. *et al.* Effect of galacto-oligosaccharide supplementation on human faecal microflora and on survival and persistence of Bifidobacterium lactis Bb-12 in the gastrointestinal tract. *International Dairy Journal* **11**, 817-825 (2001).
108 Firmesse, O., Mogenet, A., Bresson, J. L., Corthier, G. & Furet, J. P. Lactobacillus rhamnosus R11 consumed in a food supplement survived human digestive transit without modifying microbiota equilibrium as assessed by real-time polymerase chain reaction. *J Mol Microbiol Biotechnol* **14**, 90-99, doi:10.1159/000106087 (2008).
109 Rochet, V. *et al.* Effects of orally administered Lactobacillus casei DN-114 001 on the composition or activities of the dominant faecal microbiota in healthy humans. *Br J Nutr* **95**, 421-429 (2006).
110 Garrido, D., Suau, A., Pochart, P., Cruchet, S. & Gotteland, M. Modulation of the fecal microbiota by the intake of a Lactobacillus johnsonii La1-containing product in human volunteers. *FEMS microbiology letters* **248**, 249-256, doi:10.1016/j.femsle.2005.05.045 (2005).
111 Goossens, D. *et al.* The effect of Lactobacillus plantarum 299v on the bacterial composition and metabolic activity in faeces of healthy volunteers: a placebo-controlled study on the onset and duration of effects. *Aliment Pharmacol Ther* **18**, 495-505 (2003).
112 Smith, T. J., Anderson, D., Margolis, L. M., Sikes, A. & Young, A. J. Persistence of Lactobacillus reuteri DSM17938 in the human intestinal tract: response to consecutive and alternate-day supplementation. *Journal of the American College of Nutrition* **30**, 259-264 (2011).

113 Jacobsen, C. N. *et al.* Screening of probiotic activities of forty-seven strains of Lactobacillus spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans. *Appl Environ Microbiol* **65**, 4949-4956 (1999).

114 Sierra, S. *et al.* Intestinal and immunological effects of daily oral administration of Lactobacillus salivarius CECT5713 to healthy adults. *Anaerobe* **16**, 195-200, doi:10.1016/j.anaerobe.2010.02.001 (2010).

115 Frese, S. A., Hutkins, R. W. & Walter, J. Comparison of the colonization ability of autochthonous and allochthonous strains of lactobacilli in the human gastrointestinal tract. *Advances in Microbiology* **2**, 399 (2012).

116 Tannock, G. W. *et al.* Analysis of the fecal microflora of human subjects consuming a probiotic product containing Lactobacillus rhamnosus DR20. *Appl Environ Microbiol* **66**, 2578-2588 (2000).

117 Maldonado-Gomez, M. X. *et al.* Stable Engraftment of Bifidobacterium longum AH1206 in the Human Gut Depends on Individualized Features of the Resident Microbiome. *Cell Host Microbe* **20**, 515-526, doi:10.1016/j.chom.2016.09.001 (2016).

118 Suez, J. *et al.* Post-Antibiotic Gut Mucosal Microbiome Reconstitution Is Impaired by Probiotics and Improved by Autologous FMT. *Cell* **174**, 1406-1423.e1416, doi:10.1016/j.cell.2018.08.047 (2018).

119 Chung, H. *et al.* Gut immune maturation depends on colonization with a host-specific microbiota. *Cell* **149**, 1578-1593, doi:10.1016/j.cell.2012.04.037 (2012).

120 Dogi, C. A. & Perdigon, G. Importance of the host specificity in the selection of probiotic bacteria. *The Journal of dairy research* **73**, 357-366, doi:10.1017/s0022029906001993 (2006).

121 Marcobal, A. *et al.* A metabolomic view of how the human gut microbiota impacts the host metabolome using humanized and gnotobiotic mice. *Isme j* **7**, 1933-1943, doi:10.1038/ismej.2013.89 (2013).

122 de Vrese, M. *et al.* Probiotics--compensation for lactase insufficiency. *Am J Clin Nutr* **73**, 421s-429s, doi:10.1093/ajcn/73.2.421s (2001).

123 Bonder, M. J. *et al.* The effect of host genetics on the gut microbiome. *Nature genetics* **48**, 1407-1412, doi:10.1038/ng.3663 (2016).

124 Macho Fernandez, E. *et al.* Anti-inflammatory capacity of selected lactobacilli in experimental colitis is driven by NOD2-mediated recognition of a specific peptidoglycan-derived muropeptide. *Gut* **60**, 1050-1059, doi:10.1136/gut.2010.232918 (2011).

125 Lin, Y. P., Thibodeaux, C. H., Pena, J. A., Ferry, G. D. & Versalovic, J. Probiotic Lactobacillus reuteri suppress proinflammatory cytokines via c-Jun. *Inflammatory bowel diseases* **14**, 1068-1083, doi:10.1002/ibd.20448 (2008).

126 Lavasani, S. *et al.* A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated by IL-10 producing regulatory T cells. *PLoS One* **5**, e9009, doi:10.1371/journal.pone.0009009 (2010).

127 Thomas, C. M. & Versalovic, J. Probiotics-host communication: Modulation of signaling pathways in the intestine. *Gut Microbes* **1**, 148-163 (2010).

128 van Baarlen, P. *et al.* Differential NF-kappaB pathways induction by Lactobacillus plantarum in the duodenum of healthy humans correlating with immune tolerance. *Proc Natl Acad Sci U S A* **106**, 2371-2376, doi:10.1073/pnas.0809919106 (2009).

129 Matsuguchi, T. *et al.* Lipoteichoic acids from Lactobacillus strains elicit strong tumor necrosis factor alpha-inducing activities in macrophages through Toll-like receptor 2. *Clinical and diagnostic laboratory immunology* **10**, 259-266 (2003).

130 Medina, M., Izquierdo, E., Ennahar, S. & Sanz, Y. Differential immunomodulatory properties of Bifidobacterium logum strains: relevance to probiotic selection and clinical applications. *Clinical and experimental immunology* **150**, 531-538, doi:10.1111/j.1365-2249.2007.03522.x (2007).

131 Schiavi, E. *et al.* The Surface-Associated Exopolysaccharide of Bifidobacterium longum 35624 Plays an Essential Role in Dampening Host Proinflammatory Responses and Repressing Local TH17 Responses. *Appl Environ Microbiol* **82**, 7185-7196, doi:10.1128/aem.02238-16 (2016).

132 von Ossowski, I. *et al.* Using recombinant Lactococci as an approach to dissect the immunomodulating capacity of surface piliation in probiotic Lactobacillus rhamnosus GG. *PLoS One* **8**, e64416, doi:10.1371/journal.pone.0064416 (2013).
133 Ardita, C. S. *et al.* Epithelial adhesion mediated by pilin SpaC is required for Lactobacillus rhamnosus GG-induced cellular responses. *Appl Environ Microbiol* **80**, 5068-5077, doi:10.1128/aem.01039-14 (2014).
134 Yanagihara, S. *et al.* Uromodulin-SlpA binding dictates Lactobacillus acidophilus uptake by intestinal epithelial M cells. *International immunology* **29**, 357-363, doi:10.1093/intimm/dxx043 (2017).
135 Konieczna, P. *et al.* Bifidobacterium infantis 35624 administration induces Foxp3 T regulatory cells in human peripheral blood: potential role for myeloid and plasmacytoid dendritic cells. *Gut* **61**, 354-366, doi:10.1136/gutjnl-2011-300936 (2012).
136 Fukushima, Y., Kawata, Y., Hara, H., Terada, A. & Mitsuoka, T. Effect of a probiotic formula on intestinal immunoglobulin A production in healthy children. *International journal of food microbiology* **42**, 39-44 (1998).
137 Galdeano, C. M. & Perdigon, G. The probiotic bacterium Lactobacillus casei induces activation of the gut mucosal immune system through innate immunity. *Clinical and vaccine immunology : CVI* **13**, 219-226, doi:10.1128/cvi.13.2.219-226.2006 (2006).
138 Gueimonde, M., Margolles, A., de los Reyes-Gavilan, C. G. & Salminen, S. Competitive exclusion of enteropathogens from human intestinal mucus by Bifidobacterium strains with acquired resistance to bile--a preliminary study. *International journal of food microbiology* **113**, 228-232, doi:10.1016/j.ijfoodmicro.2006.05.017 (2007).
139 Tsai, C. C. *et al.* Antagonistic activity against Salmonella infection in vitro and in vivo for two Lactobacillus strains from swine and poultry. *International journal of food microbiology* **102**, 185-194, doi:10.1016/j.ijfoodmicro.2004.12.014 (2005).
140 Kim, Y., Kim, S. H., Whang, K. Y., Kim, Y. J. & Oh, S. Inhibition of Escherichia coli O157:H7 attachment by interactions between lactic acid bacteria and intestinal epithelial cells. *Journal of microbiology and biotechnology* **18**, 1278-1285 (2008).
141 Asahara, T. *et al.* Probiotic bifidobacteria protect mice from lethal infection with Shiga toxin-producing Escherichia coli O157:H7. *Infection and immunity* **72**, 2240-2247 (2004).
142 Fukuda, S. *et al.* Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* **469**, 543-547, doi:10.1038/nature09646 (2011).
143 Cotter, P. D., Hill, C. & Ross, R. P. Bacteriocins: developing innate immunity for food. *Nature reviews. Microbiology* **3**, 777-788, doi:10.1038/nrmicro1273 (2005).
144 Corr, S. C. *et al.* Bacteriocin production as a mechanism for the antiinfective activity of Lactobacillus salivarius UCC118. *Proc Natl Acad Sci U S A* **104**, 7617-7621, doi:10.1073/pnas.0700440104 (2007).
145 Medellin-Pena, M. J., Wang, H., Johnson, R., Anand, S. & Griffiths, M. W. Probiotics affect virulence-related gene expression in Escherichia coli O157:H7. *Appl Environ Microbiol* **73**, 4259-4267, doi:10.1128/aem.00159-07 (2007).
146 Yun, B., Oh, S. & Griffiths, M. W. Lactobacillus acidophilus modulates the virulence of Clostridium difficile. *Journal of dairy science* **97**, 4745-4758, doi:10.3168/jds.2014-7921 (2014).
147 Li, J., Wang, W., Xu, S. X., Magarvey, N. A. & McCormick, J. K. Lactobacillus reuteri-produced cyclic dipeptides quench agr-mediated expression of toxic shock syndrome toxin-1 in staphylococci. *Proc Natl Acad Sci U S A* **108**, 3360-3365, doi:10.1073/pnas.1017431108 (2011).
148 Miller, M. B. & Bassler, B. L. Quorum sensing in bacteria. *Annual review of microbiology* **55**, 165-199, doi:10.1146/annurev.micro.55.1.165 (2001).
149 Lagrafeuille, R. *et al.* Opposing effect of Lactobacillus on in vitro Klebsiella pneumoniae in biofilm and in an in vivo intestinal colonisation model. *Benef Microbes* **9**, 87-100, doi:10.3920/bm2017.0002 (2018).

150 Thompson, J. A., Oliveira, R. A., Djukovic, A., Ubeda, C. & Xavier, K. B. Manipulation of the quorum sensing signal AI-2 affects the antibiotic-treated gut microbiota. *Cell Rep* **10**, 1861-1871, doi:10.1016/j.celrep.2015.02.049 (2015).
151 Ohland, C. L. & Macnaughton, W. K. Probiotic bacteria and intestinal epithelial barrier function. *American journal of physiology. Gastrointestinal and liver physiology* **298**, G807-819, doi:10.1152/ajpgi.00243.2009 (2010).
152 Miyamoto, J. *et al.* A gut microbial metabolite of linoleic acid, 10-hydroxy-cis-12-octadecenoic acid, ameliorates intestinal epithelial barrier impairment partially via GPR40-MEK-ERK pathway. *The Journal of biological chemistry* **290**, 2902-2918, doi:10.1074/jbc.M114.610733 (2015).
153 Kaikiri, H. *et al.* Supplemental feeding of a gut microbial metabolite of linoleic acid, 10-hydroxy-cis-12-octadecenoic acid, alleviates spontaneous atopic dermatitis and modulates intestinal microbiota in NC/nga mice. *International journal of food sciences and nutrition* **68**, 941-951, doi:10.1080/09637486.2017.1318116 (2017).
154 Yamada, M. *et al.* A bacterial metabolite ameliorates periodontal pathogen-induced gingival epithelial barrier disruption via GPR40 signaling. *Sci Rep* **8**, 9008, doi:10.1038/s41598-018-27408-y (2018).
155 Yan, F. *et al.* Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. *Gastroenterology* **132**, 562-575, doi:10.1053/j.gastro.2006.11.022 (2007).
156 Mack, D. R., Ahrne, S., Hyde, L., Wei, S. & Hollingsworth, M. A. Extracellular MUC3 mucin secretion follows adherence of Lactobacillus strains to intestinal epithelial cells in vitro. *Gut* **52**, 827-833 (2003).
157 Gaudier, E., Michel, C., Segain, J. P., Cherbut, C. & Hoebler, C. The VSL# 3 probiotic mixture modifies microflora but does not heal chronic dextran-sodium sulfate-induced colitis or reinforce the mucus barrier in mice. *J Nutr* **135**, 2753-2761, doi:10.1093/jn/135.12.2753 (2005).
158 Caballero-Franco, C., Keller, K., De Simone, C. & Chadee, K. The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. *American journal of physiology. Gastrointestinal and liver physiology* **292**, G315-322, doi:10.1152/ajpgi.00265.2006 (2007).
159 Persborn, M. *et al.* The effects of probiotics on barrier function and mucosal pouch microbiota during maintenance treatment for severe pouchitis in patients with ulcerative colitis. *Aliment Pharmacol Ther* **38**, 772-783, doi:10.1111/apt.12451 (2013).
160 Jones, C. *et al.* Modulation of gut barrier function in patients with obstructive jaundice using probiotic LP299v. *Eur J Gastroenterol Hepatol* **25**, 1424-1430, doi:10.1097/MEG.0b013e328363e26e (2013).
161 Zeng, J. *et al.* Clinical trial: effect of active lactic acid bacteria on mucosal barrier function in patients with diarrhoea-predominant irritable bowel syndrome. *Aliment Pharmacol Ther* **28**, 994-1002, doi:10.1111/j.1365-2036.2008.03818.x (2008).
162 Sabico, S. *et al.* Effects of a multi-strain probiotic supplement for 12 weeks in circulating endotoxin levels and cardiometabolic profiles of medication naive T2DM patients: a randomized clinical trial. *Journal of translational medicine* **15**, 249, doi:10.1186/s12967-017-1354-x (2017).
163 Wilms, E. *et al.* Effects of Supplementation of the Synbiotic Ecologic(R) 825/FOS P6 on Intestinal Barrier Function in Healthy Humans: A Randomized Controlled Trial. *PLoS One* **11**, e0167775, doi:10.1371/journal.pone.0167775 (2016).
164 Horvath, A. *et al.* Randomised clinical trial: the effects of a multispecies probiotic vs. placebo on innate immune function, bacterial translocation and gut permeability in patients with cirrhosis. *Aliment Pharmacol Ther* **44**, 926-935, doi:10.1111/apt.13788 (2016).
165 Stadlbauer, V. *et al.* Lactobacillus casei Shirota Supplementation Does Not Restore Gut Microbiota Composition and Gut Barrier in Metabolic Syndrome: A Randomized Pilot Study. *PLoS One* **10**, e0141399, doi:10.1371/journal.pone.0141399 (2015).

166 Begley, M., Hill, C. & Gahan, C. G. Bile salt hydrolase activity in probiotics. *Appl Environ Microbiol* **72**, 1729-1738, doi:10.1128/aem.72.3.1729-1738.2006 (2006).
167 Joyce, S. A. *et al.* Regulation of host weight gain and lipid metabolism by bacterial bile acid modification in the gut. *Proc Natl Acad Sci U S A* **111**, 7421-7426, doi:10.1073/pnas.1323599111 (2014).
168 Costabile, A. *et al.* An in vivo assessment of the cholesterol-lowering efficacy of Lactobacillus plantarum ECGC 13110402 in normal to mildly hypercholesterolaemic adults. *PLoS One* **12**, e0187964, doi:10.1371/journal.pone.0187964 (2017).
169 Sun, L. *et al.* Gut microbiota and intestinal FXR mediate the clinical benefits of metformin. *Nature medicine*, doi:10.1038/s41591-018-0222-4 (2018).
170 Sarkar, A. *et al.* Psychobiotics and the Manipulation of Bacteria-Gut-Brain Signals. *Trends in neurosciences* **39**, 763-781, doi:10.1016/j.tins.2016.09.002 (2016).
171 Bravo, J. A. *et al.* Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci U S A* **108**, 16050-16055, doi:10.1073/pnas.1102999108 (2011).
172 Kelly, J. R. *et al.* Lost in translation? The potential psychobiotic Lactobacillus rhamnosus (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. *Brain, behavior, and immunity* **61**, 50-59, doi:10.1016/j.bbi.2016.11.018 (2017).
173 Buffington, S. A. *et al.* Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. *Cell* **165**, 1762-1775, doi:10.1016/j.cell.2016.06.001 (2016).
174 Perez-Burgos, A. *et al.* The TRPV1 channel in rodents is a major target for antinociceptive effect of the probiotic Lactobacillus reuteri DSM 17938. *The Journal of physiology* **593**, 3943-3957, doi:10.1113/jp270229 (2015).
175 Rousseaux, C. *et al.* Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. *Nature medicine* **13**, 35-37, doi:10.1038/nm1521 (2007).
176 Kristensen, N. B. *et al.* Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials. *Genome medicine* **8**, 52 (2016).
177 McFarland, L. V. Use of probiotics to correct dysbiosis of normal microbiota following disease or disruptive events: a systematic review. *BMJ Open* **4**, e005047, doi:10.1136/bmjopen-2014-005047 (2014).
178 Jakobsson, H. E. *et al.* Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. *PLoS One* **5**, e9836, doi:10.1371/journal.pone.0009836 (2010).
179 Sommer, F., Anderson, J. M., Bharti, R., Raes, J. & Rosenstiel, P. The resilience of the intestinal microbiota influences health and disease. *Nature reviews. Microbiology* **15**, 630-638, doi:10.1038/nrmicro.2017.58 (2017).
180 Bruzzese, E. *et al.* Disrupted intestinal microbiota and intestinal inflammation in children with cystic fibrosis and its restoration with Lactobacillus GG: a randomised clinical trial. *PLoS One* **9**, e87796, doi:10.1371/journal.pone.0087796 (2014).
181 Zoppi, G., Cinquetti, M., Benini, A., Bonamini, E. & Minelli, E. B. Modulation of the intestinal ecosystem by probiotics and lactulose in children during treatment with ceftriaxone. *Current therapeutic research* **62**, 418-435 (2001).
182 Wang, Z. J. *et al.* Effects of anti-Helicobacter pylori concomitant therapy and probiotic supplementation on the throat and gut microbiota in humans. *Microbial pathogenesis* **109**, 156-161, doi:10.1016/j.micpath.2017.05.035 (2017).
183 Hazards, E. P. o. B. *et al.* Update of the list of QPS- recommended biological agents intentionally added to food or feed as notified to EFSA 8: suitability of taxonomic units notified to EFSA until March 2018. *EFSA Journal* **16**, e05315 (2018).
184 Quin, C. *et al.* Probiotic supplementation and associated infant gut microbiome and health: a cautionary retrospective clinical comparison. *Sci Rep* **8**, 8283, doi:10.1038/s41598-018-26423-3 (2018).

185 Topcuoglu, S., Gursoy, T., Ovali, F., Serce, O. & Karatekin, G. A new risk factor for neonatal vancomycin-resistant Enterococcus colonisation: bacterial probiotics. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet* **28**, 1491-1494, doi:10.3109/14767058.2014.958462 (2015).
186 Didari, T., Solki, S., Mozaffari, S., Nikfar, S. & Abdollahi, M. A systematic review of the safety of probiotics. *Expert opinion on drug safety* **13**, 227-239, doi:10.1517/14740338.2014.872627 (2014).
187 Carvour, M. L. *et al.* Predictors of Clostridium difficile infection and predictive impact of probiotic use in a diverse hospital-wide cohort. *American journal of infection control*, doi:10.1016/j.ajic.2018.07.014 (2018).
188 Besselink, M. G. *et al.* Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. *Lancet* **371**, 651-659, doi:10.1016/s0140-6736(08)60207-x (2008).
189 Hempel, S. *et al.* Safety of probiotics used to reduce risk and prevent or treat disease. *Evidence report/technology assessment*, 1-645 (2011).
190 Bafeta, A., Koh, M., Riveros, C. & Ravaud, P. Harms Reporting in Randomized Controlled Trials of Interventions Aimed at Modifying Microbiota: A Systematic Review. *Annals of internal medicine*, doi:10.7326/m18-0343 (2018).
191 Administration, U. S. F. a. D. *Development & Approval Process (Drugs)*, <https://www.fda.gov/drugs/developmentapprovalprocess/default.htm> (2018).
192 Grazul, H., Kanda, L. L. & Gondek, D. Impact of probiotic supplements on microbiome diversity following antibiotic treatment of mice. *Gut Microbes* **7**, 101-114, doi:10.1080/19490976.2016.1138197 (2016).
193 Kabbani, T. A. *et al.* Prospective randomized controlled study on the effects of Saccharomyces boulardii CNCM I-745 and amoxicillin-clavulanate or the combination on the gut microbiota of healthy volunteers. *Gut Microbes* **8**, 17-32, doi:10.1080/19490976.2016.1267890 (2017).
194 Brecht, M., Garg, A., Longstaff, K., Cooper, C. & Andersen, C. Lactobacillus Sepsis following a Laparotomy in a Preterm Infant: A Note of Caution. *Neonatology* **109**, 186-189, doi:10.1159/000441965 (2016).
195 Spinler, J. K. *et al.* Administration of probiotic kefir to mice with Clostridium difficile infection exacerbates disease. *Anaerobe* **40**, 54-57, doi:10.1016/j.anaerobe.2016.05.008 (2016).
196 Oliveira, B. C. M. & Widmer, G. Probiotic product enhances susceptibility of mice to cryptosporidiosis. *Appl Environ Microbiol*, doi:10.1128/aem.01408-18 (2018).
197 He, F. *et al.* Differences in composition and mucosal adhesion of bifidobacteria isolated from healthy adults and healthy seniors. *Current microbiology* **43**, 351-354 (2001).
198 Kankaanpaa, P. E., Salminen, S. J., Isolauri, E. & Lee, Y. K. The influence of polyunsaturated fatty acids on probiotic growth and adhesion. *FEMS microbiology letters* **194**, 149-153 (2001).
199 Shepherd, E. S., DeLoache, W. C., Pruss, K. M., Whitaker, W. R. & Sonnenburg, J. L. An exclusive metabolic niche enables strain engraftment in the gut microbiota. *Nature* **557**, 434-438, doi:10.1038/s41586-018-0092-4 (2018).
200 Andriantsoanirina, V., Teolis, A. C., Xin, L. X., Butel, M. J. & Aires, J. Bifidobacterium longum and Bifidobacterium breve isolates from preterm and full term neonates: comparison of cell surface properties. *Anaerobe* **28**, 212-215, doi:10.1016/j.anaerobe.2014.07.002 (2014).
201 Roessler, A. *et al.* The immune system in healthy adults and patients with atopic dermatitis seems to be affected differently by a probiotic intervention. *Clin Exp Allergy* **38**, 93-102, doi:10.1111/j.1365-2222.2007.02876.x (2008).
202 Pelto, L., Isolauri, E., Lilius, E. M., Nuutila, J. & Salminen, S. Probiotic bacteria down-regulate the milk-induced inflammatory response in milk-hypersensitive subjects but

have an immunostimulatory effect in healthy subjects. *Clin Exp Allergy* **28**, 1474-1479 (1998).

203 Hod, K. *et al.* The effect of a multispecies probiotic on microbiota composition in a clinical trial of patients with diarrhea- predominant irritable bowel syndrome. *Neurogastroenterology & Motility*, e13456 (2018).

204 Suwal, S. *et al.* The Probiotic Effectiveness in Preventing Experimental Colitis Is Correlated With Host Gut Microbiota. *Frontiers in microbiology* **9**, 2675, doi:10.3389/fmicb.2018.02675 (2018).

205 Abildgaard, A. *et al.* The antidepressant-like effect of probiotics and their faecal abundance may be modulated by the cohabiting gut microbiota in rats. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology*, doi:10.1016/j.euroneuro.2018.10.011 (2018).

206 Ferrario, C. *et al.* Modulation of fecal Clostridiales bacteria and butyrate by probiotic intervention with Lactobacillus paracasei DG varies among healthy adults. *J Nutr* **144**, 1787-1796, doi:10.3945/jn.114.197723 (2014).

207 Degirolamo, C., Rainaldi, S., Bovenga, F., Murzilli, S. & Moschetta, A. Microbiota modification with probiotics induces hepatic bile acid synthesis via downregulation of the Fxr-Fgf15 axis in mice. *Cell Rep* **7**, 12-18, doi:10.1016/j.celrep.2014.02.032 (2014).

208 Ouwehand, A. C. *et al.* Bifidobacterium microbiota and parameters of immune function in elderly subjects. *FEMS immunology and medical microbiology* **53**, 18-25, doi:10.1111/j.1574-695X.2008.00392.x (2008).

209 Garcia-Albiach, R. *et al.* Molecular analysis of yogurt containing Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus in human intestinal microbiota. *Am J Clin Nutr* **87**, 91-96, doi:10.1093/ajcn/87.1.91 (2008).

210 Wang, C. *et al.* Intestinal Microbiota Profiles of Healthy Pre-School and School-Age Children and Effects of Probiotic Supplementation. *Ann Nutr Metab* **67**, 257-266, doi:10.1159/000441066 (2015).

211 Mohan, R. *et al.* Effects of Bifidobacterium lactis Bb12 supplementation on intestinal microbiota of preterm infants: a double-blind, placebo-controlled, randomized study. *J Clin Microbiol* **44**, 4025-4031, doi:10.1128/jcm.00767-06 (2006).

212 Veiga, P. *et al.* Changes of the human gut microbiome induced by a fermented milk product. *Sci Rep* **4**, 6328, doi:10.1038/srep06328 (2014).

213 Brahe, L. K. *et al.* Dietary modulation of the gut microbiota--a randomised controlled trial in obese postmenopausal women. *Br J Nutr* **114**, 406-417, doi:10.1017/s0007114515001786 (2015).

214 McNulty, N. P. *et al.* The impact of a consortium of fermented milk strains on the gut microbiome of gnotobiotic mice and monozygotic twins. *Sci Transl Med* **3**, 106ra106, doi:10.1126/scitranslmed.3002701 (2011).

215 Eloe-Fadrosh, E. A. *et al.* Functional dynamics of the gut microbiome in elderly people during probiotic consumption. *MBio* **6**, doi:10.1128/mBio.00231-15 (2015).

216 Martin, F. P. *et al.* Probiotic modulation of symbiotic gut microbial-host metabolic interactions in a humanized microbiome mouse model. *Mol Syst Biol* **4**, 157, doi:10.1038/msb4100190 (2008).

217 Burton, K. J. *et al.* Probiotic yogurt and acidified milk similarly reduce postprandial inflammation and both alter the gut microbiota of healthy, young men. *Br J Nutr* **117**, 1312-1322, doi:10.1017/s0007114517000885 (2017).

218 Kajander, K. *et al.* Effects of multispecies probiotic supplementation on intestinal microbiota in irritable bowel syndrome. *Aliment Pharmacol Ther* **26**, 463-473, doi:10.1111/j.1365-2036.2007.03391.x (2007).

219 Donaldson, G. P., Lee, S. M. & Mazmanian, S. K. Gut biogeography of the bacterial microbiota. *Nature reviews. Microbiology* **14**, 20-32, doi:10.1038/nrmicro3552 (2016).

220 Uronis, J. M. *et al.* Gut microbial diversity is reduced by the probiotic VSL#3 and correlates with decreased TNBS-induced colitis. *Inflammatory bowel diseases* **17**, 289-297, doi:10.1002/ibd.21366 (2011).

221 Arthur, J. C. *et al.* VSL#3 probiotic modifies mucosal microbial composition but does not reduce colitis-associated colorectal cancer. *Sci Rep* **3**, 2868, doi:10.1038/srep02868 (2013).
222 Kuehbacher, T. *et al.* Bacterial and fungal microbiota in relation to probiotic therapy (VSL# 3) in pouchitis. *Gut* **55**, 833-841 (2006).