Review

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Fate, activity, and impact of ingested bacteria within the human gut microbiota

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The human gut contains a highly diverse microbial community that is essentially an open ecosystem, despite being deeply embedded within the human body. Food-associated fermentative bacteria, including probiotics, are major sources of ingested bacteria that may temporarily complement resident microbial communities, thus forming part of our transient microbiome. Here, we review data on the fate and activity of ingested bacteria and, in particular, lactobacilli and bifidobacteria in the gastrointestinal (GI) tract and their impact on the composition and metabolism of the gut microbiome with a focus on data from clinical studies. In addition, we discuss the mechanisms involved and the potential impact on the host's health.

Human gut microbiota

The gut microbiome comprises a diverse and abundant (10^{14} cells) microbial community consisting of bacteria, archaea, and eukaryotes that live in an intimate relationship with the host [1]. There has been a dramatic increase in interest regarding the composition and function of the gut microbiome over the past 20 years, largely driven by the rapid improvement and wide availability of sequencing-based analysis techniques. Today, a large body of evidence indicates that the gut microbiome plays a key role in shaping host physiology and the maintenance of gut and immune homeostasis. Furthermore, the gut microbiome broadly influences host physiology, both locally in the gut and remotely at other organs such as the brain and liver [2,3]. Conversely, the host immune system and secreted molecules, such as mucus, shape the gut microbiome, and recent advances in postgenomic and high-throughput techniques have elucidated a myriad of molecular and cellular mechanisms involved [4,5]. Hence, the gut microbiome is increasingly recognized as an intervention target with important potential in the management of health and disease [6].

Though deeply embedded in, and closely associated with, the human body, the GI tract essentially comprises an open ecosystem. Host genetics plays a role in shaping gut microbiota, but its influence appears to be limited compared to that of environmental factors [7–10], the strongest of these being diet, medication, and age [11–13]. The microbial communities in the GI tract are continually exposed to allochthonous bacteria primarily originating from dietary intake. Despite the resistance of the resident communities to colonization by ingested organisms, many food-ingested bacteria are capable of transient integration into the gut microbiota where they may impact the composition and activity of the resident gut communities. Hence, they can be regarded as part of our 'transient microbiome' that is both highly dynamic and individualized. Its composition at any time is the result of recent exposure, the gut ecosystem conditions, diet, and the fitness of the ingested organisms.

In this review we discuss what is known about the impact of food-derived bacteria on gut microbial communities with a focus primarily on bifidobacteria, lactic acid bacteria (LAB), and propionibacteria; due to their application in fermented foods, and as probiotics, they are quantitatively the most important ingested bacteria. Specifically, due to their use as probiotics, many research groups have initiated studies in order to examine how organisms in these taxa impact the gut microbiota and the host's health. Hence, they serve as valuable models for studying the fate of environmental bacteria in the gut microbiome [14]. Here, studies on food-borne pathogens are excluded as these have been reviewed elsewhere and are often associated with pathological host responses that indirectly but strongly impact the gut microbiome and therefore fall outside the scope of this review [15].

Gut microbiome community structure: core, variable, and transient communities

Following birth, microbial colonization of the gut involves exogenous bacteria that originate either from the mother's microbiota (mainly from the intestine and vagina) or from other environmental sources. Early colonizers consist mainly of facultative anaerobes that create a favorable niche for more strictly anaerobic bacteria that subsequently dominate the microbiota within a few weeks [16–20]. During the first 3 years of life, radical dietary changes related to weaning, antibiotic use, and modifications in host

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physiology all contribute to the highly dynamic nature of the gut microbiota [21]. The absence of a stable microbiota configuration contributes to a high susceptibility to gut infections [22]. Around 2–3 years of age, the gut microbial communities have developed a richness and diversity that is characteristic of a healthy adult gut. Further information about the composition, diversity, and function of the gut microbiome can be found in other recent reviews [1,23].

Diet is also a major determinant that shapes the gut microbiome in adults (for reviews, see [24–27]). Healthy, adult-gut communities respond to dietary challenge but typically revert to a stable configuration. High bacterial richness and functional redundancy in gene function contribute to community resilience. In line with these observations, ingested bacteria typically integrate transiently into resident communities. Interestingly, commensal community configurations in the elderly appear to be more susceptible to environmental challenges. Together with decreased efficacy of the aging immune system, a more vulnerable microbiota configuration may increase the chances of gut pathogenic infections [1,28].

Initial comparative studies revealed large interpersonal differences in the composition of the gut microbiota. Some bacterial species are present in the vast majority of the human population across continents, and these account for approximately one-half of the number of species identified in a given individual [29,30]. Recently, it was shown that some gut species are either abundant or nearly absent in most individuals. This bimodal distribution is not affected by short-term diet interventions, and this ecological concept has been referred to as tipping elements, essentially alternative stable states [31]. Additionally, each individual harbors a similar number of species that are rare and which therefore vary greatly among the population; this is referred to as a 'variable microbiome'. Many of these species appear to be true commensals that stably colonize the intestine [32]. In a recent study, David *et al.* elegantly demonstrated that some of the species in the variable microbiome can be traced back to dietary constituents [13,33,34]. Several of these were LAB and were re-isolated from fecal samples. Fecal transcripts of food-ingested bacteria made up more than 1% of the fecal transcriptome in some conditions. Taken together, these data indicate that the human gut microbiome is comprised of a core and a variable commensal community that is likely specific and retained in each individual over prolonged periods. This community is amended with a transient community depending on recent diet and environmental exposure.

Most of the data discussed above derive from studies on fecal material, which is believed to be representative of the colonic microbial populations. Much less is known about the microbiome of the small intestine, due mainly to the fact that it is accessible only with invasive sampling. The small intestine is the primary site of food digestion, nutrient absorption, and metabolic signaling. Additionally, the presence of Peyer's patches and Paneth cells contributes to interaction between the luminal microbiota and the host immune system [35]. The small intestine is populated by distinct microbial communities that are less diverse, compared to colonic communities, and are dominated by *Veillonella*, *Streptococcus*, *Lactobacillus*, and *Clostridium*

clusters [36–38]. It is noteworthy to mention, in the context of this review, that these communities are highly specialized in the utilization of simple dietary carbohydrates, much like food-fermenting bacteria (see below) – which may therefore compete for the same niche in the small intestine. Microbial population densities in the small intestine are much lower than in the colon, ranging from 10^4 cells per gram in the duodenum to 10^8 cells per gram in the terminal ileum. Hence, the consumption of a dose of 10^{10} ingested bacterial cells is predicted to induce a dramatic population shift that temporarily overcrowds resident communities and which is likely to impact the host's immune and neuroendocrine functions [35] (Box 1).

Major groups of ingested bacteria that can complement the gut microbiome

Fermented foods and beverages are estimated to make up approximately one-third of the human diet and are the major sources of those environmental bacteria that enter the GI tract [39]. Food fermentations are ancient processes that date back to the introduction of agriculture and animal husbandry, approximately 10 000 years ago, when they were used as a means of conserving foods [40]. A short description of the major bacteria derived from food, and the history of their discovery, is given in Box 2. Artisanal fermentation methodologies, based on serial inoculation in a process known as back-slopping, have been increasingly replaced by highly controlled, reproducible, and safe industrial processes using fermenting strains that are specifically selected for technologic, organoleptic or health-beneficial properties [41,42]. LAB are most widely applied and used to produce fermented plant- and animal-derived foods (milk and meat) but are also frequently found on (decaying) plant materials [43]. LAB are also found in the GI tract, and the *Lactobacillus*/*Enterococcus* group typically constitutes around 1% of the fecal bacterial population [44]. Some *Lactobacillus* species, notably *Lactobacillus gasseri* and *Lactobacillus reuteri*, are thought to be true GI commensals while other species, such as *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, and *Lactobacillus paracasei*, appear to be transient passengers [45,46].

Metabolism in LAB is optimized for the conversion of simple carbohydrates to primarily lactic acid and, in some cases, a mixture of acids. Growth is characterized by high carbon fluxes, rapid acidification, and low growth yield ([47] and references therein). Species such as *Lactobacillus delbrueckii* and *Streptococcus thermophilus* are highly adapted to the fermentation of dairy substrates and they display remnants of genome decay indicating advanced specialization towards the utilization of lactose and the utilization of dairy proteins as amino acid sources. LAB that frequently inhabit niches rich in plant-derived carbohydrates can utilize building blocks of complex plant polymers such as (hemi-) cellulose and xylans [48,49]. These enzyme systems may also contribute to fitness in the gut environment where plant polymers serve as an important growth substrate, which may explain the frequent detection of such species in fecal samples.

Bifidobacteria are Gram-positive bacteria with a high GC content; they belong to the Actinobacteria phylum and are the dominant members of the microbiota of breast-fed
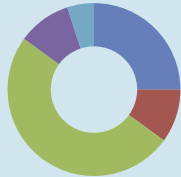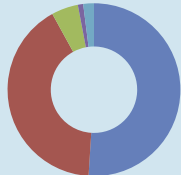
Box 1. Ingested bacteria-mediated microbiome alteration along the GI tract

Information about changes in the composition of the gastrointestinal (GI) microbiome upon ingestion of bacteria is often derived from fecal samples. Typically, this reveals small changes, of the order of 1% or less, which may mask large shifts occurring in the upper GI tract. Table I shows the taxonomic composition of the microbiome in the stomach, ileum, and feces, the total number of resident bacteria, and the predicted population shifts taking into account the volume and population densities along the GI tract. In this example we assumed a typical food-microbial exposure of 10^{10} cells, 10% survival, and no active replication – resulting in a dose of 10^9 cells.

The results indicate that major changes occur in the stomach and small intestine. These are important not only quantitatively, they may also dramatically alter the relative abundance of major phyla. This is likely to impact metabolic capacities, microbe–host crosstalk, and host physiology, and it should provide an important area for future research.

Table I. Bacterial community along the human GI tract

	Taxonomy (phylum level)	Resident bacteria (number/ml or g)	Transit time[a]	Relative abundance of ingested bacteria compared to resident bacteria[b]
Stomach[c]		10^2–10^4	15 min–3 h	100 to 10 000-fold
Small intestine (ileum)[d]		10^6–10^8	2–5 h	0.01 to 1-fold
Colon (feces)[e]		10^{10}–10^{11}	12–24 h	0.0001 to 0.00001-fold

■ Firmicutes ■ Bacteroidetes ■ Proteobacteria ■ Actinobacteria ■ Other

[a]From [133].

[b]For several well-studied ingested bacteria the impact may be underestimated as survival rates may be higher (see text) and growth may occur in the lower GI tract. Calculations assumed an average ileal volume of 0.5 l and a colonic volume of 2 l [134].

[c]Data from [135].

[d]Data from [136].

[e]Data from [137].

babies. The successful colonization of the infant gut by bifidobacteria is due to the ability of some species to utilize human milk oligosaccharides [50]. The bifidobacterial population in adults typically accounts for more than 1% of total gut bacteria and is dominated by other species that harbor an extensive repertoire of enzymes dedicated to the degradation of host-derived glycans and dietary carbohydrates [44,51]. Bifidobacteria convert carbohydrates to organic acids via the 'bifid-shunt', yielding acetic acid and lactic acid. Finally, *Bifidobacterium animalis* subsp. *lactis* is frequently used as a probiotic. It exhibits an unusually high oxygen tolerance and is capable of growing in dairy substrates. It is less frequently recovered than other species of *Bifidobacterium* (e.g., *longum*, *bifidum*, *adolescentis*) in human fecal samples [52], and its presence is directly correlated with recent ingestion [53]. Hence, *B. animalis* is a typical constituent of the transient microbiome.

The propionibacteria, forming another genus in the Actinobacteria, are commonly ingested microbes as they are used to produce Swiss-type cheeses. Their main fermentation products are propionate, acetate, succinate, and CO_2 [54].

For all the species described above, a few key traits are important for survival in the GI tract. These include factors contributing to adhesion to the host (e.g., pili, adhesins, and other binding proteins) as well as efflux systems and hydrolases that confer bile-salt tolerance ([55] and reference therein).

Fate and activity of ingested strains

The abundance of orally ingested strains from fermented foods and probiotics ranges between 10^8 and 10^{12} CFU per day. After consumption, ingested bacteria enter a hostile environment where subsequent passage through the stomach and duodenum exposes them to highly stressful physicochemical and biological conditions. Typical stressors in the stomach are the low pH (<3) and high levels of pepsin, which are detrimental to most ingested bacteria, leading to cell inactivation and death. Upon entry into the small intestine, pH values rise to >6 but cells are exposed to bile, pancreatin, and lipase. Here, recovery, and even
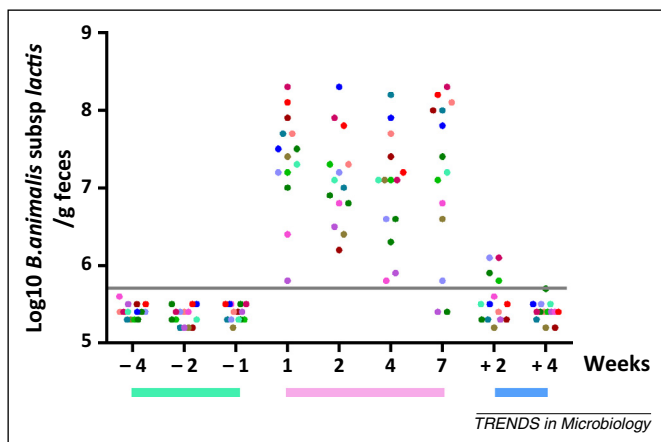
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Box 2. Bacteria hunting: early discoveries and isolation of common probiotic, intestinal, and dietary bacteria

Ever since the initial discovery of the microbial world, in 1674, by Antonie van Leeuwenhoek in Delft, The Netherlands, food and intestinal microbes have been an important focus of pioneering microbiologists. *Lactococcus lactis* (formerly named *Bacterium lactis*) was isolated from milk in England by Joseph Lister in 1873 [138]. This discovery was soon followed by isolation of the first *Bifidobacterium* (originally *Bacillus bifidus*), which was obtained, in 1900, from the feces of breast-fed infants by the French pediatrician Henry Tissier (H. Tissier, thesis, University of Paris, 1900). The universal model organism, *Escherichia coli* (formerly *Bacterium coli commune*), was first described by Theodor Escherich in 1885 and isolated from the feces of newborns in Germany [139]. *Escherichia coli* Nissle 1917, a strain marketed as a probiotic, was isolated during the first world war by a German, Alfred Nissle, from the feces of an army officer [140]. *Lactobacillus acidophilus* (*Bacillus acidophilus*) was isolated by the Austrian pediatrician Ernst Moro in 1900 from the stool of an infant [141]. *Propionibacterium* species (originally named *Bacterium acidi-propionici* or *Bacillus acidi-propionici*) were isolated in 1906 from Emmental Swiss cheese by von Freudenreich and Orla-Jensen [142].

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growth, of some strains may occur, which can continue in the colon.

Most studies in humans rely on fecal quantification of ingested strains, also referred to as 'persistence'. Essentially, this reflects the dose of ingested strains, the extent of cell death (mainly in the upper GI tract), and subsequent replication of surviving cells. Recovery of bacteria in fecal samples, either measured by cultivation or molecular approaches, consistently shows variable recovery between individuals. In most cases, ingested strains are still detected after a few days but rarely after 1 week [56,57]. Figure 1 illustrates this concept and depicts the variable and transient integration of *B. animalis* subsp. *lactis* CNCM I-2494 in stool from healthy females who consumed it as part of a fermented milk product [53].

Very few studies have been performed with the aim of determining the fate of ingested strains in the upper GI tract of humans. A mix of 19 *Lactobacillus* strains (10^8 of each per day) given to 13 healthy volunteers for 10 days increased total *Lactobacillus* counts in both jejunal and rectal biopsies, and strains could still be detected 11 days after discontinuation of consumption [58]. *In vitro* models have been successfully used to mimic passage through the GI tract, aiming to reveal the impact of GI stresses on both survival and activity of bacterial strains. In some cases, these results are supported by validation in human subjects [59]. A recent study investigated survival and persistence in the GI tract of 42 *L. plantarum* strains in an *in vitro* system and in human subjects; it was found that survival involved a high strain specificity [60]. Strain-specific persistence profiles in the GI tract, as defined by recovery in feces, were highly consistent across humans, and the qualitative ranking of persistence in human subjects and in *in vitro* systems was highly correlated. It should be stressed that some of the aforementioned studies relied on quantification of bacteria by cultivation. Because bacteria may enter into a viable-but-non-culturable (VBNC) state after stress exposure, quantification of survival and fecal persistence may be underestimated in some cases [61].

Few human studies have attempted to investigate the activity and physiologic response of ingested strains in the GI tract. McNulty and coworkers demonstrated that *B. animalis* subsp. *lactis* strain CNCM I-2494, in humanized mice, strongly upregulates genes required for the utilization of xylo-oligosaccharides, which are important building blocks of dietary hemicellulose [53]. Similarly, using comparative transcriptome analysis, *L. plantarum* 299 v was shown to specifically adapt its metabolic capacity in the human intestine for carbohydrate acquisition and expression of exopolysaccharide (EPS) and proteinaceous cell-surface compounds [62]. Interestingly, these adaptive responses converged in both mice and humans.

In conclusion, robustness is highly variable among species, with strains of *B. animalis, L. casei, L. rhamnosus* and *L. plantarum* ranking among the most robust. As much as 50% of the orally ingested cells of these strains can survive gastric passage [63]. Additionally, there is a high strain-to-strain variation within species, and animal experiments have shown that survival is influenced further by the co-ingested matrix, gastric pH, and length of exposure. Finally, some ingested strains have been shown to be metabolically active in the ileum and colon [64,65].

Human trials

Once ingested strains have entered the gut ecosystem, the question arises as to whether and how these transiting bacteria impact resident communities. Here, we catalog the major findings of clinical studies on probiotics and fermented foods with respect to their impact on the microbiota (Table 1, and see Table S1 in the supplementary material online), identify whether common trends occur, and interpret these findings in the context of preclinical studies that have revealed potential mechanisms of interaction between ingested bacteria and commensals.

Several challenges exist in drawing conclusions based on clinical studies of probiotic effects. First, clinical studies have been highly heterogeneous, with wide taxonomic diversity, and heterogeneity in dosage and duration of consumption of the organisms administered. Second, the matrix in which the probiotic was delivered has been

Figure 1. Intra- and inter-individual variability in the recovery of ingested *Bifidobacterium animalis* subsp. *lactis* CNCM I-2494 in fecal samples from 14 healthy female twins who consumed a fermented-milk product over a period of 7 weeks [53]. Green: preconsumption period; pink: consumption period; and blue: postconsumption period. The gray line represents the detection limit threshold. Colors indicate samples taken from the same individual. Adapted from [53].

Table 1. Overview of clinical studies that used next-generation sequencing approaches or metabolomics to study the impact of ingested bacteria on the human gut microbiota

Tested strain	Cohort description (status, gender, age)	Type of study design and country of investigation	Test and control groups	Intake (duration form, daily dose)	Gut microbiota analysis approach	Observed results in test groups (composition, metabolites, enzymes)	Refs
16S rRNA-based approaches							
Lactobacillus rhamnosus GG	Healthy subjects (M[a], F[b]) 23–55 years	RDBPC[c], Finland	Test: $n = 9$ Control: $n = 13$	3 weeks; MP[d]; 10^{10} CFU	16S phylogenetic microarray	No significant impact of gut microbiota composition and stability	[67]
	Infants 6 months	Open, USA	Test: $n = 16$	Birth until 6 months; form unknown; 10^9 CFU	16S phylogenetic microarray	No difference in gut microbiota	[74]
Lactobacillus paracasei Zhang	Healthy subjects (M, F) 24–68 years	Open, China	Test: $n = 24$	28 days; chewable tablet; 10^{10} CFU	16S sequencing	Difference in the composition and diversity of intestinal microbiota compared to baseline; positive correlation of *L. paracasei* Zhang with *Prevotella, Lactobacillus., Faecalibacterium, Propionibacterium,* and *Bifidobacterium*; negative correlation of *L. paracasei* Zhang with *Clostridium, Phascolarctobacterium, Serratia, Enterococcus, Shigella,* and *Shewanella.*	[143]
Lactobacillus reuteri DSM17938	Cystic fibrosis patients (M, F) 8–44 years	RDBPC-CO[e], Spain	Test: $n = 24$	6 months; chewable tablet; 10^8 CFU	16S sequencing	Decreased total bacterial load; decreased γ-Proteobacteria	[66]
	Colicky breast-fed infants (M, F) 10–60 days old	RDBPC, Italy	Test: $n = 15$ Placebo: $n = 14$	21 days; freeze-dried; 10^8 CFU	16S sequencing	No change to the overall composition of the microbiota	[68]
L. reuteri NCIMB 30242	Hypercholesterolemic (M, F) 20–75 years	Randomized, UK	Test: $n = 10$	4 weeks; capsule; 3×10^9 to 1.8×10^{10} CFU	16S sequencing	Trend to increase Firmicutes/Bacteroidetes ratio	[122]
L. paracasei DG	Healthy subjects (M, F) 23–55 years	RDBPC-CO, Italy	Group A: $n = 14$ Group B: $n = 16$	4 weeks; capsule; 2.4×10^{10} CFU	16S sequencing	Increase in Proteobacteria and *Coprococcus*; decrease in *Blautia*	[144]
Bifidobacterium animalis subsp. *lactis* CNCM I-2494	Healthy subjects (F) 18–55 years	RDBPC, USA	MP: $n = 12$ Control: $n = 11$ No product: $n = 13$	4 weeks; MP; 2.5×10^{10} CFU	16S sequencing	No change in overall structure	[69]
	Healthy subjects (F) 21–32 years	Open, USA	Test: $n = 14$	7 weeks; MP; 2.5×10^{10} CFU	16S sequencing	No change in dominant microbiota	[53]
Bifidobacterium breve M-16V + *Bifidobacterium longum* BB536	Mothers, 4 weeks before delivery, and infants, from birth to 6 months	Open, Japan	Test: $n = 49$ Control: $n = 15$	Mothers: 4 weeks; infants: birth to 6 months; capsule; 5×10^9 CFU each strain	16S sequencing	Decrease of Proteobacteria in mothers; for infants at 4 months there was an increase in Bacteroidetes	[76]
Six commercially available probiotics containing either *Bifidobacterium* or *Lactobacillus* strains	18 healthy adults (M, F) 19–33 years	Open, Japan	Six groups (three individuals/group)	8 weeks; MP; 10^8–10^{10} CFU	16S sequencing	No significant changes in the overall structure of gut microbiota; changes in some operational taxonomic units	[77]

Table 1 (*Continued*)

Tested strain	Cohort description (status, gender, age)	Type of study design and country of investigation	Test and control groups	Intake (duration form, daily dose)	Gut microbiota analysis approach	Observed results in test groups (composition, metabolites, enzymes)	Refs
VSL#3[f]	IBS[g] subjects (M, F) 31–60 years	Open, Hong-Kong	Test: *n* = 10	4 weeks; lyophilized; 1.8 x 10^{12} CFU	16S sequencing	Decrease in *Bacteroides*	[78]
Lactobacillus acidophilus NCFM or *Bifidobacterium lactis* Bi-07	Children with atopic dermatitis 7–24 months	RPC[h], Denmark	Test: *Lactobacillus acidophilus*: *n* = 3 Test: *B. lactis*: *n* = 3	8 weeks; freeze-dried capsule; 10^{10} CFU	16S sequencing	No change in composition and diversity of the main bacterial populations in feces; trends in increased *Faecalibacterium* and *Bifidobacterium*	[70]
Bifidobacterium longum Bar33 + *Lactobacillus helveticus* Bar13	32 healthy subjects (M, F) 71–88 years	RDBPC, Italy	Test: *n* = 16 Placebo: *n* = 16	1 month; lyophilized biscuit; 10^9 CFU each strain	16S phylogenetic microarray	No increase in *Clostridium* cluster XI, *Clostridium difficile*, *Clostridium perfringens*, *Enterococcus faecium*, or *Campylobacter*	[71]
Lactobacillus rhamnosus GG, *Lactobacillus rhamnosus* Lc705, *Propionibacterium freudenreichii subsp. shermanii* JS *and Bifidobacterium animalis subsp. lactis* Bb12	IBS subjects (M, F) 20–65 years	RDBPC, Finland	Test: *n* = 12 Control: *n* = 8	5 months; MP; 1.2 x 10^9 CFU each	16S phylogenetic microarray	Stabilization of the microbiota	[72]
Metagenomics							
Bifidobacterium animalis subsp. *lactis* CNCM I-2494	Healthy subjects (F) 21–32 years	Open, USA	Test: *n* = 14	7 weeks; MP; 2.5 x 10^{10} CFU	Shotgun sequencing	No change in microbiota	[53]
	IBS subjects (F) 20–69 years	RDBPC, UK	Test: *n* = 13 Placebo: *n* = 15	4 weeks; MP; 2.5 x 10^{10} CFU	Quantitative metagenomics	Increase in species capable of butyrate production; decrease in *Bilophila*	[19]
Metatranscriptomics							
B. animalis subsp. *lactis* CNCM I-2494	Seven pairs of healthy twins (F) 21–32 years	Open, USA	Test: *n* = 14	7 weeks; MP; 2.5 x 10^{10} CFU	RNA sequencing	Increase in expression of genes involved in polysaccharide degradation by resident microbes	[53]
Metabolomics							
Lactobacillus sp. HY7801 *Bifidobacterium longum* HY8004 *Lactobacillus brevis* HY7401	74 IBS subjects (M, F) 21–55 years	RDBPC, Korea	Test: *n* = 37 Control: *n* = 36	8 weeks; MP; 1.2 x 10^{10} CFU	Nuclear magnetic resonance	No difference in fecal metabolites; normalization of serum glucose and tyrosine in probiotic compared to healthy individuals	[73]

[a]M, male.

[b]F, female.

[c]RDBPC, randomized, double-blind, placebo-controlled clinical trial.

[d]MP, milk-product matrix.

[e]RDBPC-CO, randomized, double-blind, placebo-controlled, crossover clinical trial.

[f]Consists of *Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus plantarum, Lactobacillus paracasei*, and *Streptococcus thermophilus*.

[g]IBS, irritable-bowel syndrome.

[h]RPC, randomized, placebo-controlled clinical trial.

variable, with bacteria administered either in a dairy matrix or as a powder or capsule. Third, the reference samples for analysis of gut microbiota have varied; while many studies have used a placebo control to determine the effect of an ingested microbe on microbiota composition [66–73], other studies have used a baseline sample for comparison [53,74–78]. Fourth, most studies have been conducted in the adult population. Finally, the evolution of analytic techniques is apparent, as early studies examined changes in the gut microbiota by cultivation, fluorescent *in situ* hybridization, and/or quantitative PCR, giving a less-complete overview of gut microbiota composition (Table S1).

Several recent studies have used high-resolution next-generation sequencing approaches (Table 1). It is apparent that there is no major alteration in the dominant fecal gut microbiota. However, some studies report an interesting global impact on the gut microbiota or changes in specific bacterial groups. For example, Kajander *et al.* report a stabilization of the microbiota in an irritable bowel syndrome (IBS) cohort, as deduced from an increased similarity index at three time points during the intervention after consumption of multispecies (five strains) probiotic supplementation [72]. In another study, IBS patients were given a fermented milk product containing *B. animalis* subsp. *lactis* CNCM I-2494 [19]. Using quantitative metagenomics, the fermented milk was reported to reduce the pathobiont *Bilophila wadsworthia*, a δ-proteobacterium that is increasingly correlated with impaired health. It also increased the butyrate-producing potential of commensal communities [19], which corroborates previous studies in the TRUC mouse model of colitis using the same fermented milk product; this demonstrates that carbohydrate-metabolizing potential and short-chain fatty acid (SCFA) production are stimulated by this product [79].

Stimulation of known butyrate producers belonging to the *Clostridium cluster* IV (Ruminococcaceae) and *Clostridium* cluster XIV (Lachnospiraceae) has also been observed in other studies, suggesting that it may be a common result of supplementation with LAB or bifidobacteria [75,80,81]. In addition, the inhibition and decrease in abundance of members of the Proteobacteria, either facultative anaerobes (Enterobacteriaceae) or strict anaerobes (*Bilophila, Desulfovibrio*, and others), is reported in recent metagenomic surveys [19] as well as in older studies [58,66,82,83].

As the composition of the microbiota in infants and young children is distinct, relatively unstable, and less diverse than that of adults, bacteria that are ingested either during the prenatal period or in the first weeks after birth may have a large impact on the gut microbiota. While an increase in the overall richness of the microbiota has been correlated with improvement in metabolic parameters in adults [84], breast-fed babies typically harbor a low overall diversity of microbiota but a high abundance of *Bifidobacterium*. Clinical trials with probiotics in this population have been focused mostly on stimulating the development of a microbiota like that in breast-fed infants in formula-fed infants, or focused on decreasing specific symptoms, such as allergy or colic. In this respect, a desirable outcome of probiotic supplementation would be

an increase in the number and diversity of *Bifidobacterium* spp. and/or a decrease in the number of potential pathogens. Probiotic supplementation with LGG in infants, and even short-term prenatal consumption of LGG by mothers, has been shown to increase endogenous *Bifidobacterium* colonization in infants [85,86].

While adult and infant microbiota have received most attention, modulation of microbiota from elderly subjects has also gained interest in the last decade owing to large and well-characterized cohorts [28]. Ingested bacteria have been suggested to revert an age-related increase in pathogens [71].

Mechanisms by which ingested bacteria impact resident communities

Ingested bacteria can impact resident communities via at least three different mechanisms: through trophic interactions, a direct alteration in fitness, or an indirect alteration in fitness through altered production of host-derived molecules (Figure 2).

Many ingested bacteria have the ability to rapidly metabolize simple carbohydrates to lactic acid, acetic acid, or propionic acid. These bacteria may influence and integrate into trophic networks of dietary carbohydrate degradation, thereby altering metabolic outputs. *In vitro* models of GI fermentation that mimic the complexity of the colonic ecosystem allow the analysis of the impact of ingested bacteria on microbial interaction networks to be carried out independently of host inputs. The more advanced models may include beads coated with mucins [87], mucosal biofilms [88], or the addition of polymer beads [89] to better simulate the GI tract and avoid rapid washout. These models may also allow sampling at different colonic compartments (ascending, transverse, and descending colon) that are otherwise poorly accessible (for reviews see [90,91]). A number of studies report increased production of SCFA, which in some cases coincides with an increase in LAB and/or bifidobacteria (Table S2). For instance, administration of *Enterococcus faecium* CRL 183 increased bifidobacteria and SCFA levels, whereas *Lactobacillus acidophilus* CRL 1014 increased *Bifidobacterium* and *Lactobacillus* as well as acetate in the simulator of human intestinal microbial ecosystem (SHIME) *in vitro* GI model [92]. Similarly, the stimulation of butyrate production by a fermented milk product containing *B. animalis* subsp. *lactis* CNCM I-2494 [19] might be due to the fact that bacteria producing lactic and acetic acids stimulate SCFA production through increased cross-feeding with commensal SCFA producers [93]. Co-culture experiments have shown that supplementation with *Bifidobacterium* can directly stimulate butyrate producers that utilize acetate or lactate [93,94]. In addition to direct mechanisms, effects on trophic networks may also include more upstream conversion steps catalyzed by resident communities. For example, in a clinical study, consumption of a fermented milk product containing *B. animalis* subsp. *lactis* CNCM I-2494 increased the expression of genes involved in metabolism of complex plant polysaccharides, an effect that was confined to the period of fermented milk consumption [53].

The trophic impact may not be restricted to the metabolism of dietary carbohydrates – it may also be extended to
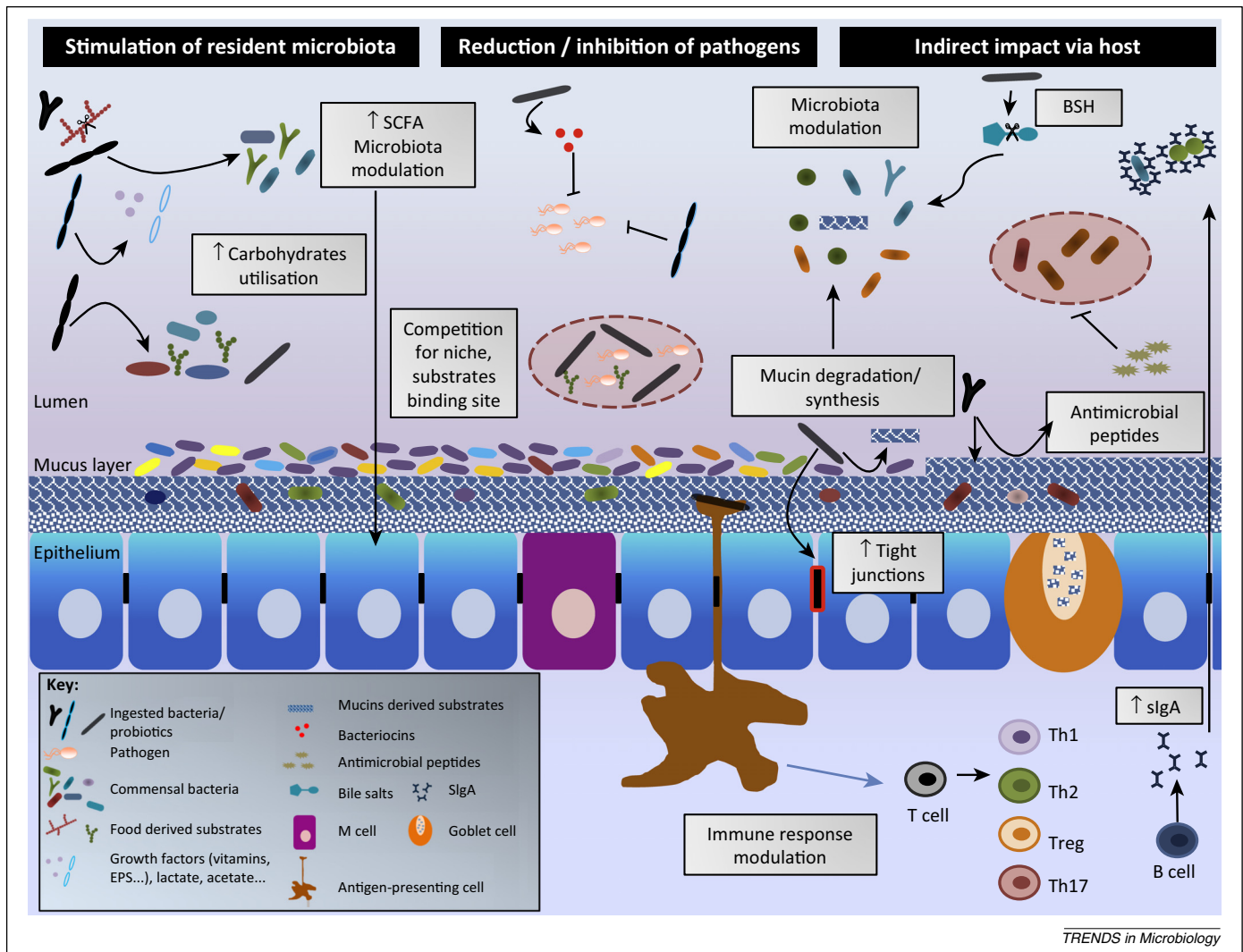
Figure 2. Different mechanisms by which ingested bacteria can impact the resident microbiota. First, ingested bacteria may stimulate growth of the resident bacteria by supplying metabolites such as acetate, lactate, and propionate. Through cross-feeding with resident commensal bacteria, this can lead to the production of propionate and butyrate. Some ingested bacteria produce growth factors, including vitamins and exopolysaccharide (EPS). In addition, some strains stimulate carbohydrate metabolism by resident bacteria. Mucin degradation can liberate sugars, amino acids, sialic acids, and sulfate that serve as substrates for the resident commensals. Second, probiotics can directly impact the abundance of pathogens through a decrease in pH resulting from the production of lactate and short-chain fatty acids (SCFA), niche competition, or through EPS and bacteriocins. Finally, some strains may impact the resident bacteria indirectly by interaction with host epithelium and the epithelial immune system. Stimulation of mucin production can also impact mucosa-associated bacteria. Ingested strains may also impact the gut microbiota indirectly through secretory IgA (sIgA), epithelial structure, and inflammatory pathways that, in turn, impact the resident bacteria. Abbreviation: BSH, bile salt hydrolase.

EPS, produced by ingested bacteria, that may potentially serve as a growth substrate for resident commensals (for a recent review, see [95]). For example, a cell-surface-associated EPS from *Bifidobacterium breve* UCC2003 was demonstrated to suppress the murine pathogen *Citrobacter rodentium* [96]. In another study, purified EPS from *Pediococcus parvulus* 2.6 reduced microbial diversity significantly [97].

A second mechanism by which ingested bacteria can impact the host microbiome is by direct stimulatory or inhibitory effects that alter the presence of certain members of resident gut communities. Essentially, modifications of the physicochemical conditions in the gut environment can change the fitness of its microbial inhabitants. Substrate competition may decrease levels of specific resident bacteria. As an example, *B. breve* UCC2003 can use sialic acids [98], and, as a result, it may outcompete

opportunist pathogens such as *Salmonella enterica* serovar Typhimurium or *Clostridium difficile* [99]. Competitive exclusion can also occur through physical displacement of commensals or pathogens from adherence sites [100], which is the case for mannose adhesion [101]. Conversely, some ingested bacteria produce vitamins, such as vitamin B12, which is a growth-limiting factor for *Bacteroides thetaiotaomicron* [102]. Other studies have identified metabolites, such as precursors of menaquinone (vitamin K) produced by *Propionibacterium freudenreichii* ET-3, that stimulate some strains of bifidobacteria *in vitro* [103]. Stimulation of bifidobacteria is commonly observed after ingestion of propionibacteria [104] or lactobacilli [75,105,106], potentially through vitamins or undocumented mechanisms.

LAB, in particular, have been extensively studied for their ability to produce bacteriocins, which can be

broad-range and contribute to major shifts in community composition, as demonstrated by *in vitro* studies with gut simulators [107]. Hence, some groups have successfully selected bacteriocins, aiming to control pathogens such as *C. difficile*. A comparative analysis of the bacteriocin-producing *Lactobacillus salivarius* UCC 118 and a bacteriocin-knockout mutant revealed a bacteriocin-dependent modulation of gut microbiota at the genus level in mice and pigs [108].

Due to the high acidification activity of ingested LAB, these organisms may decrease local pH, which might favor certain taxa such as butyrate producers [109] or acetogens [110].

A third mechanism by which ingested bacteria alter resident communities is indirect, involving a host response to ingested bacteria that, in turn, modifies the composition or activity of the microbiota. This has been reviewed extensively by others [111–113] and is therefore discussed only briefly here. The intestinal epithelium is protected from luminal bacteria, pathogens and antigens by several host-produced components. Among them, secretory IgA (sIgA), mucus, and antimicrobial peptides are the first line of defense in protecting the intestinal epithelium from microbial invasion. sIgA secreted by B cells can coat the luminal microbiota [114] and maintain homeostasis in the mucosal barrier. A study in healthy humans, using fluorescent probes, indicated that 24–74% of fecal bacteria are coated with sIgA [115]. Consumption of some probiotics was shown to increase fecal sIgA in humans [82,116,117]. Mucins are the major glycoproteins of the mucus layer which forms the physical barrier between intestinal cells and the lumen. The thin and firm inner mucus layer is mostly devoid of bacteria, whereas the loose and thick outer layer is colonized by bacteria [118]. *In vitro* studies using cell lines have shown that some probiotics can increase mucin synthesis (for a review see [113]). Administration of the probiotic mixture VSL#3 in rats increased the luminal mucin content [119]. Moreover, mucins can be nutrients for intestinal bacteria because they are composed of amino acids and oligosaccharides. Some intestinal bacteria possess the glycosyl hydrolases necessary for the degradation of mucin oligosaccharides, which can be further metabolized by resident microbiota. Notably, *Bifidobacterium bifidum* PRL2010 can grow on gastric mucin as a sole carbon source, and genome analysis has revealed that this bacterium can use host mucins [120]. Antimicrobial peptides (including defensins) are mostly produced by Paneth cells in the small intestine. Notably, consumption of *Escherichia coli* Nissle 1917 or *Bifidobacterium lactis* Bb12 by healthy individuals led to enhanced fecal human β-defensins [117,121]. Bile salts are also key determinants of the fitness landscape. Numerous ingested LAB and bifidobacteria express bile-salt hydrolases which, through deconjugation, can potentially modify bile-salt profiles in the gut lumen – but surprisingly, only a few studies have described the impact of bile-salt-hydrolase-expressing ingested bacteria on community structure [122].

Other mechanisms, such as stimulation of the immune response and barrier integrity, have been extensively reviewed elsewhere [123,124].

Concluding remarks and future outlook

Here, we have reviewed data from clinical studies that describe the impact of ingested bacteria on the resident microbiota. There is compelling evidence that some ingested bacteria can reach the small intestine and colon alive and metabolically active. Here, they make up an important part of our transient microbiome which, in turn, is part of the variable microbiome that is repeatedly identified in comparative microbiome studies.

Microbiota analysis in most clinical studies on adults has focused on composition profiling on fecal samples. Despite the large heterogeneity in design and analysis tools, it is evident that the impact of interventions is relatively small when compared to dietary or antibiotic interventions. Multiple studies have reported an impact on the resident microbiota, and especially an increase in SCFA production potential and a decrease in members of the Proteobacteria, in particular, species of the Enterobacteriaceae. Moreover, in early life and preterm infants, the development of the gut microbiota seems to have a large impact on host health [125]. Although very few studies have attempted to analyze the impact on microbiota functionality, the available studies have revealed elevated expression of genes encoding carbohydrate utilization enzymes and an increase in SCFA production potential [19,53]. Future studies using these techniques on larger cohorts should be used to validate such findings. In addition, the impact on communities in the small intestine has been largely ignored, though ingested bacteria will cause major population shifts that are likely to be highly relevant for host physiology. Dedicated studies combining a controlled intervention in healthy adults or ileostomy patients with concomitant microbiota profiling should be highly instrumental in this respect [35].

Most studies were designed for examining a clinical endpoint where microbiota analysis was added as a secondary endpoint and, in many cases, only *post-hoc*. Considering the high diversity and high interpersonal and temporal variation in the composition of the microbiota, future studies should preferably be designed specifically for analyzing an impact on the microbiota [53]. Specific points of attention should be the control of environmental factors (in particular, diet), sampling at multiple time points, and the use of sufficient power to enable meaningful statistical analysis of the compositional analysis involving such large numbers of microbial species and strains. In addition, stratification of individuals based on their baseline microbiota may be helpful to elucidate generic and specific modulation by ingested microbes.

Ultimately, a key question is whether the impact of ingested bacteria reaches beyond pure ecology of the gut microbiome and impacts host health (Box 3). The clinical efficacy of ingested bacteria in decreasing the risk of necrotizing enterocolitis in preterm babies by preventing a bloom of pathogens is an obvious example of the latter [125]. In this context, the finding that some probiotics stimulate butyrate production and decrease Proteobacteria is encouraging. A decrease in butyrate producers and a bloom of Proteobacteria is frequently associated with a state of impaired health or even disease [126]. This includes bowel disorders such as IBS [127] and

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Box 3. Outstanding questions

- How can lactobacilli, bifidobacteria and other food-associated bacteria be selected for their ability to target modulation of the microbiome?
- What level of microbiome modulation is expected, and necessary, to impact the host's health?
- Is there a need for 'personalized' or 'stratified' probiotics taking into account the variability of the microbiome across the human population?
- Can baseline variation in the microbiome explain responders and non-responders in clinical studies with ingested bacteria?
- What will be the role of specific gut commensals and 'synthetic microbiomes' as next-generation probiotics and therapeutic microbes?

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inflammatory bowel disease [128] as well as metabolic diseases such as type 2 diabetes [129,130]. Clearly, ingested bacteria that target the restoration of butyrate production, and prevent Proteobacteria blooms, provide an interesting area for future research. The identification of other microbial metabolites, such as trimethylamine, which has been associated with an increased risk of cardiovascular disease [131], and amino-acid-derived metabolites [132], hold promise as additional intervention targets to improve the host's health. Rationalizing strain selection and subsequent clinical validation by monitoring such key marker metabolites may enable the development of a new generation of ingested bacteria that target microbiome functions important in the prevention and management of major health concerns.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tim.2015.03.002.

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