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Bifidobacterium and the intestinal mucus layer

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Abstract

Bifidobacterium species are integral members of the human gut microbiota and these microbes have significant interactions with the intestinal mucus layer. This review delves into *Bifidobacterium*-mucus dynamics, shedding light on the multifaceted nature of this relationship. We cover conserved features of *Bifidobacterium*-mucus interactions, such as mucus adhesion and positive regulation of goblet cell and mucus production, as well as species and strain-specific attributes of mucus degradation. For each interface, we explore the molecular mechanisms underlying these interactions and their potential implications for human health. Notably, we emphasize the ability of *Bifidobacterium* species to positively influence the mucus layer, shedding light on its potential as a mucin-builder and a therapeutic agent for diseases associated with disrupted mucus barriers. By elucidating the complex interplay between *Bifidobacterium* and intestinal mucus, we aim to contribute to a deeper understanding of the gut microbiota-host interface and pave the way for novel therapeutic strategies.

Keywords: Bifidobacterium, mucus, intestine, probiotic

INTRODUCTION

Intestinal mucus

The intestine is continually exposed to a multitude of luminal antigens and bacterial components. To protect itself, the intestinal epithelium harbors specialized cells known as goblet cells, which synthesize and secrete mucus. The structure of intestinal mucus is intricately designed to form a protective barrier. The

primary structural component of intestinal mucus is a gel-forming glycoprotein called MUC2. MUC2 is a large, heavily glycosylated protein that forms disulfide-bonded dimers. These dimers undergo further polymerization and crosslinking, resulting in the formation of a gel-like network that constitutes the mucus layer.

In addition to the gel-forming MUC2, intestinal mucus contains a diverse array of compounds that contribute to its composition and functionality. Mucus harbors Antimicrobial Peptides (AMPs): small cationic peptides that possess antimicrobial properties. AMPs in the mucus layer help to maintain the balance of microbial populations by inhibiting the growth of pathogenic bacteria and promoting the growth of beneficial commensal bacteria. Immunoglobulin A (IgA) antibodies are also abundant in the mucus layer of the gut. They are produced by specialized immune cells called plasma cells and secreted into the mucus, where they play a crucial role in immune defense by neutralizing pathogens, preventing their adherence to the intestinal epithelium, and promoting their clearance from the gut. In addition to MUC2, goblet cells also secrete trefoil factors, a family of small peptides that contribute to the maintenance of mucosal integrity and repair by promoting epithelial cell migration, enhancing wound healing, and providing protection against injury and inflammation. Other mucus-associated proteins, such as FCGBP, metalloenzyme CLCA1, ZG16, Lypd8, glycosaminoglycans, and chitinases, contribute to the structural organization, hydration, and stability of the mucus layer[1]. These compounds play various roles in shaping the mucus layer and modulating host-microbe interactions within the gut.

The structural organization of intestinal mucus is highly dynamic, exhibiting regional variations along the gastrointestinal tract. In the small intestine, the mucus layer is thinner and less firmly attached to the epithelium, allowing for efficient absorption of nutrients. In contrast, the mucus layer in the colon is thicker and firmly adheres to the epithelial surface, serving as a physical barrier that limits direct contact between luminal contents and the epithelium. In the colon, the mucus layer is stratified, consisting of two distinct regions: the inner mucus layer and the outer mucus layer. The inner mucus layer, also known as the firmly adherent mucus layer, is in direct contact with the intestinal epithelium. It is tightly packed with MUC2, forming a dense and organized matrix that provides a protective barrier against luminal contents. The outer mucus layer, also referred to as the loose mucus layer, is less compact and acts as a reservoir for commensal bacteria and other luminal components. This outer layer is more penetrable and allows for the establishment of symbiotic interactions between the gut microbiota and the host.

One feature of mucus that makes it amenable to microbe interactions is the structure of the mucin proteins. The MUC2 protein is extensively O-glycosylated with branched oligosaccharides[2-7]. O-glycans are attached at serine and threonine residues in the MUC2 protein and consist of core structures of α- and β-linked N-acetyl-glucosamine, N-acetyl-galactosamine, and galactose. The core structures are then elongated and generally modified by α-linked fucose, sialic acid, and sulfate residues[4]. Mucin glycoproteins serve as both an adhesion site and nutrient source for the resident gut microbes, providing an array of complex microbe-host interactions.

Bifidobacteria and mucus

Among the bacteria found in the gut microbiota, *Bifidobacterium* species are known to reside within the intestinal mucus layer[8-14] and exert multiple beneficial effects on the host[15-20]. Bifidobacteria are Gram-positive anaerobic bacteria from the phylum Actinobacteria that can have a rod or a distinctive bifid (i.e., Y) shape. There are currently 55 recognized species and subspecies of *Bifidobacterium*[21-23]. These species can be grouped into seven phylogenetic clusters: *B. longum*, *B. adolescentis*, *B. pseudolongum*, *B. boum*, *B. asteroides*, *B. pullorum*, and *B. bifidum*.

Bifidobacteria are predominant in the healthy breast-fed infant gut due to the presence of human milk oligosaccharides (HMOs), which these bacteria are adept at utilizing[24-27]. Studies have suggested that *Bifidobacterium* species make up ~80% of a breast-fed infant gut microbiota[28-32]. The benefits of *Bifidobacterium* strains are especially pronounced in early life, encompassing epithelial maturation, immune cell activation, and gut-brain-axis crosstalk[33-39]. Upon the introduction of solid food and weaning, the level of intestinal bifidobacteria continually decreases until adulthood, at which point bifidobacteria are maintained at a relative abundance of about ~10% throughout adult life[31,40-42]. In the elderly, the level of bifidobacteria further diminishes to about 0%-5% relative abundance[42]. This reduction in bifidobacteria levels in the elderly has been linked to age-related alterations in lifestyle and environment. Interestingly, this decline in *Bifidobacterium* abundance coincides with a simultaneous decrease in the thickness of intestinal mucus and an increase in its permeability[43-45]. It remains uncertain whether there is a direct link between decreased *Bifidobacterium* and decreased mucus, but this interesting observation suggests a relationship. Independent of age, *Bifidobacterium* species can be found in both the small intestine and colon, although they exhibit a higher abundance in the colon. Several *Bifidobacterium* species have been observed to interact with intestinal mucus, colonize the mucus layer, consume mucus glycans, and exert strain-specific modulatory effects on the mucus layer. This review covers the existing literature for the following *Bifidobacterium*-mucus interactions: (1) mucus adhesion; (2) mucin glycan degradation; (3) positive modulation of goblet cell cells; (4) goblet cell retention during inflammation; and (5) suppression of pro-inflammatory cytokines and production of anti-inflammatory IL-10.

MUCUS ADHESION BY BIFIDOBACTERIUM SPECIES

Multiple studies have demonstrated the ability of *Bifidobacterium* species to adhere to mucus [Table 1]. *B. adolescentis*, *B. angulatum*, *B. bifidum*, *B. breve*, *B. catenulatum*, *B. infantis*, *B. longum*, *B. infantis*, *B. animalis* subsp. *lactis*, and *B. pseudocatenulatum* have all been shown to bind to mucus isolated from the stool of human infants and/or adults[46-51]. *B. bifidum*, *B. breve*, *B. animalis*, *B. animalis* subsp. *lactis*, *B. longum*, *B. longum* subsp. *infantis*, and *B. catenulatum* have also been demonstrated to bind to intestinal mucus isolated from the healthy part of resected colonic tissue[52-58].

Interestingly, *Bifidobacterium animalis* subsp. *lactis* and unclassified *Bifidobacterium* species were shown to adhere well to mucus isolated from the feces of newborns, 2-month-old infants, 6-month-old infants, and adults (25 to 52 years), but had substantially lower adhesion to mucus derived from the feces of elderly individuals (74 to 93 years)[41]. It was also found that *B. animalis* subsp. *lactis* had diminished adhesion to mucus isolated during episodes of diarrhea[50]. These findings point to the integrity of mucus for adhesion.

In addition to human stool and tissue derived mucus, *B. dentium*, *B. bifidum*, *B. adolescentis*, *B. breve*, *B. pseudocatenulatum*, *B animalis* subsp. *lactis*, *B. longum*, and *B. infantis* have been shown to bind to human mucus-producing HT29-MTX, Caco-2, INT-407, and LS-174T cells[53,59-69] as well as to cecal mucus from germ-free mice and rats[18,65] [Table 1]. *B. adolescentis*, *B. angulatum*, *B. longum*, *B. infantis*, *B. pseudocatenulatum*, *B. bifidum*, *B. breve*, *B. catenulatum*, and *B. animalis* subsp. *lactis* were also found to bind to pig stomach mucus[48,70], and *B. animalis* subsp. *lactis* was reported to bind to pig intestinal mucus[71]. In agreement with these findings, *Bifidobacterium* species were found to have widespread adhesion to mucin gels created with pig stomach mucus in a bioreactor model[72]. These studies indicate that mucus adhesion is widely conserved among *Bifidobacterium* species.

The binding of *Bifidobacterium* to intestinal mucus is regulated by diverse adhesins [Figure 1]. *Bifidobacterium* species employ pili, surface adhesion proteins, moonlighting proteins, and other surface-anchored proteins to adhere to intestinal mucus [Table 2][73-75]. For example, *B. bifidum* has several known

Table 1. Literature review of mucus adhering *Bifidobacterium* species and strains

Bifidobacterium species	Mucus type	Ref.
B. animalis subsp. Bb12	Human stool mucus	[41]
Bifidobacterium 420		
Bifidobacterium BF1100		
Bifidobacterium 913		
B. adolescentis JCMI275T	Human stool mucus	[46]
B. adolescentis JCM7042		
B. adolescentis JCM7046		
B. angulatum JCM7096T		
B. animalis JCM 1190T		
B. animalis JCM 1253		
B. animalis JCM 7117		
B. animalis JCM 7124		
B. bifidum JCM 1254T		
B. bifidum JCM 1255		
B. bifidum JCM 7004		
B. breve JCM1192T		
B. breve JCM7016		
B. catenulatum ATCC 27675		
B. catenulatum JCM 7131T		
B. infantis JCM 1210		
B. infantis JCM 1222T		
B. infantis JCM 1272		
B. animalis subsp. *BbI2*		
B. lactis JCM 10140T		
B. longum JCM 127F		
B. longum JCM 7052		
B. longum JCM 7054		
B. pseudocatenulatum JCM 1200T		
B. animalis sbusp. lactis Bb12	Human stool mucus	[47]
B. adolescentis JCM 2701T	Human stool mucus	[48]
B. angulatum ATCC *27678 T*		
B. longum subsp. infantis JCM 1222 T		
B. pseudocatenulatum JCM 1200 T		
B. bifidum JCM 1255 T		
B. breve JCM 1192 T		
B. catenulatum JCM 1194 T		
B. longum subsp. longum JCM 1217 T		
B. animalis subsp. lactis Bb12		
B. bifidum TMC3115		
B. bifidum TMC3103		
B. bifidum TMC3104		
B. bifidum TMC3108		
B. bifidum TMC3110		
B. bifidum TMC3112		
B. bifidum TMC3116		
B. bifidum TMC3119		
B. bifidum TMC3120		
B. bifidum TMC3121		
B. bifidum TMC3122		

B. animalis subsp. lactis Bb12	Human stool mucus	[49]
B. lactis Bb12	Human stool mucus	[50]
B. lactis Bb12	Human stool mucus	[51]
B. longum BIF9s	Colonic tissue mucus	[52]
B. longum BIF12s		
B. longum BIF13s		
B. catenulatum BIF31s		
B. breve 99 (DSM 13692)	Colonic tissue mucus	[54]
B. lactis Bb12 (DSM 10140)		
B. breve 99 (DSM 13692)	Colonic tissue mucus	[55]
B. bifidum M6	Colonic tissue mucus	[56]
B. bifidum A1		
B. infantis BIR-0304	Colonic tissue mucus	[57]
B. infantis BIR-0307		
B. infantis BIR-0312		
B. catenulatum BIR-0324		
B. bifidum BIR-0326		
B. infantis BIR-0349		
B. breve BIR-0350		
B. longum BIR-BPD1		
B. longum BIR-BPD3		
B. longum BIR-BPG1		
B. longum BIR-BPG4		
B. bifidum M6	Colonic tissue mucus	[58]
B. bifidum M6dCo		
B. bifidum PBT		
B. bifidum PBTdOx		
B. animalis IPLA 658		
B. animalis 658dOx		
B. bifidum A8		
B. bifidum A8dOx		
B. bifidum A1		
B. bifidum A1dOx		
B. longum NIZO B667		
B. longum B667dCo		
B. animalis IPLA 4549		
B. animalis 4549dCo		
B. animalis 4549dOx		
B. bifidum DSM20456	Colonic tissue mucus, Caco-2 cells	[53]
B. bifidum MIMBb75		
B. animalis subsp. lactis Bb12	Porcine intestinal mucus	[71]
B. dentium ATCC 27678	Germ-free mouse cecal mucus, HT29-MTX cells	[18]
B. longum subsp. infantis ATCC 15697		
B. longum subsp. longum ATCC 55813		
B. breve ATCC 15698		
B. longum BIF 53	Porcine stomach mucus	[70]
B. lactis Bb 12		
B. longum BB 536		
B. longum NCC 2705		
B. longum W 11		
B. longum SP 07/3		

B. longum NCIMB 8809		
B. longum ATCC 15707		
B. longum BIR 324		
B. longum BIF 53		
B. animalis subsp. lactis IPLA4549	HT29-MTX cells	[60]
B. animalis subsp. lactis 4549dOx		
B. animalis subsp. lactis A1		
B. animalis subsp. lactis A1dOx		
B. animalis subsp. lactis A1dOx-R1		
B. longum NB667		
B. longum 667Co		
B. animalis subsp. *lactis CCDM 374*	Caco-2 cells, HT29-MTX cells	[61]
B. breve 4	Caco-2 cells, HT29-MTX cells	[62]
B. breve 5		
B. breve 25		
B. longum 4		
B. longum 16		
B. longum 18		
B. longum 22		
B. bifidum 8		
B. bifidum 7		
B. infantis 1		
B. animalis IATA-A2	Caco-2 cells, HT29-MTX cells	[64]
B. bifidum IATA-ES2		
Bifidobacterium animalis subsp. lactis Bb12		
B. bifidum DSM 20082	Caco-2 cells, HT29-MTX cells; rat cecal mucus	[65]
B. breve DSM 20213		
B. longum DSM 20219		
B. animalis DSM 20104		
B. longum CSCC 5089	Caco-2 cells	[63]
B. bifidum DNG6	Caco-2 cells	[66]
B. lactis NCC362	Caco-2 cells	[67]
B. longum NCC 490		
B. adolescentis NCC251		
B. bifidum NCC 189		
B. breve MB226		
B. bifidum S16		
B. bifidum S17		
B. infantis E18		
B. adolescentis ATCC 15706	Caco-2 cells	[68]
B. adolescentis TMC 2704		
B. adolescentis TMC 2705		
B. animalis TMC 5101		
B. infantis TMC 2906		
B. infantis TMC 2908		
B. longum TMC 2607		
B. longum TMC 2608		
B. longum TMC 2609		
B. bifidum TMC 3101		
B. bifidum TMC 3108		
B. bifidum TMC 3115		

B. bifidum TMC 3116		
B. bifidum TMC 3117		
B. breve TMC 3207		
B. breve TMC 3217		
B. breve TMC 3218		
B. breve TMC 3219		
B. infantis ATCC 15697	Glycan array	[86]

Table 2. Literature review of mucus and cell binding adhesins in *Bifidobacterium* species and strains

***Bifidobacterium* species**	**Adhesin**	**Ref.**
B. bifidum PRL2010	Sortase-dependent pili	[76]
B. bifidum ATCC 15696	Extracellular sialidase	[77]
B. bifidum A8	Extracellular transaldolase	[79]
B. longum NCC2705	Extracellular transaldolase	[78]
B. longum BBMN68	Putative adhesion proteins	[75]
B. longum BBMN68	FimM	[75]
B. bifidum 85B	FimM homologs	[75]
B. gallinarum CACC 514	FimM homologs	[75]
B. longum NCC2705	Type 2 glycoprotein-binding fimbriae homolog	[80]
B. longum NCC2705	EF-Tu	[81]
B. longum NCC2705	Enolase	[81]
B. animalis subsp. *lactis* BI07	Enolase	[82]
B. animalis subsp. *lactis* KLDS 2.0603	GroEL	[83]
B. longum VMKB44	Blap-1	[84]
B. longum JCM1217	Endo-α-N-acetylgalactosaminidase	[85]
B. longum subsp. *infantis* ATCC 15697	Family 1 of solute binding proteins (F1SBPs)	[86,87]
B. breve UCC2003	Type IVb pilus-type proteins	[82,83,88]
B. longum NCC2705	Extracellular vesicles	[89]

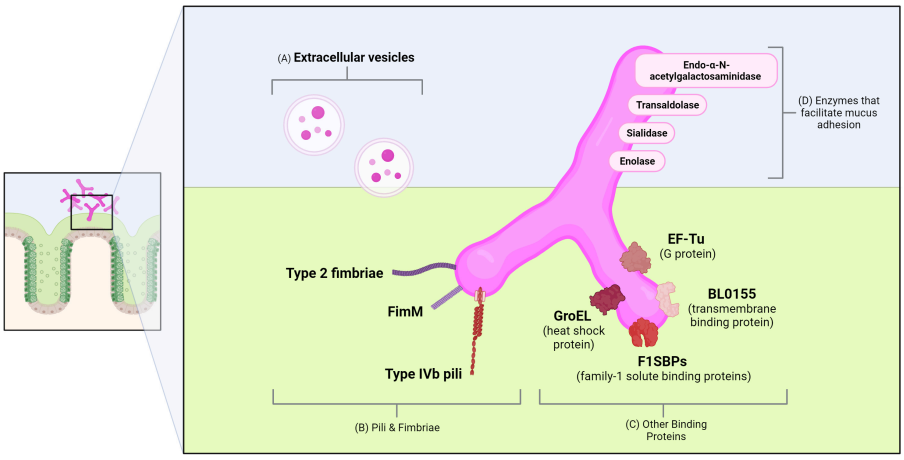

Figure 1. Schematic outlining examples of the various mechanisms by which *Bifidobacterium* adhere to mucus. (A) Extracellular vesicles released by *Bifidobacterium* can bind to mucus, and in turn, this binding can inhibit pathogen colonization; (B) *Bifidobacterium* possess a wide array of pili and fimbriae, including FimM and its homologs, type 2 fimbriae, and type IVb pili, which bind to mucus; (C) Other proteins such as F1SBPs (family-1 binding proteins), BL0155 (a type of ABC transport transmembrane protein), GroEL (a heat shock protein), and EF-Tu (Elongation Factor Tu) are involved in mucus binding; (D) Endo-α-N-acetylgalactosaminidase, transaldolase, sialidase, and enolase are enzymes that facilitate mucus adhesion.

mucin-binding partners. *B. bifidum* possesses two sortase-dependent pili that promote bacterial coaggregation and bind to mucus-producing Caco-2 cells[76]. Another study found that *B. bifidum* produces an extracellular sialidase that mediates adhesion to mucus via a conserved sialidase domain peptide that interacts with mucin carbohydrates[77]. Similar to *B. bifidum*, *B. longum* also expresses multiple mucus-binding proteins. *B. bifidum* and *B. longum* both have been shown to express extracellular transaldolases that function as an adhesin that is capable of binding mucin[78,79]. A recent study found that *B. longum* harbors 21 putative adhesion proteins[75]. Using an overexpression system in a heterologous host, it was found that FimM exhibited significant adhesion to mucus-producing LS174T goblet cells, and it was further found that mucin was one of the major adhesion receptors for the FimM protein[75]. Homologs of FimM were also identified in *B. bifidum*, *B. gallinarum*, and 23 other *B. longum* strains by sequence similarity analysis. Another study found that *B. longum* harbors a protein with high homology to type 2 glycoprotein-binding fimbriae that may mediate mucus adhesion[80]. *B. longum* additionally produces the moonlighting proteins EF-Tu and enolase, which indirectly promote adhesion to mucus-producing Caco-2 cells through interactions with host plasminogen[81]. Likewise, enolase plays the role of an adhesion factor in *B. lactis* Bl07[82], and GroEL is another moonlighting protein that has been indicated as an adhesion factor for *B. animalis* subsp. *lactis*[83].

As another example of the various adhesins employed by *Bifidobacterium* species, *B. longum* was found to possess a 26-amino-acid peptide called Blap-1 that mediates adhesion to HT-29 cells. Interestingly, genomic analysis revealed that Blap-1 was an identical match to a site in a large extracellular transmembrane protein encoded by the BL0155 open reading frame of *B. longum* NCC2705[84]. Additionally, *B. longum* possesses an endo-α-N-acetylgalactosaminidase that contains binding sites specific to the protein core of mucin glycoproteins[85]. Furthermore, the genome of *B. longum* subsp. *infantis* encodes several family 1 of solute binding proteins (F1SBPs), and these proteins were shown to bind and transport mucin oligosaccharides[86,87]. In addition to *B. bifidum* and *B. longum*, *B. breve* has type IVb pilus-type proteins that facilitate colonization in the host gut[82,83,88]. Interestingly, it has also been shown that *B. longum* produces extracellular vesicles that export mucin-binding cytoplasmic proteins, and these proteins promote the adhesion of *B. longum* to mucus[89]. It has also been recently shown that the polyamine Spermidine significantly increased the adhesion of *B. bifidum* Bb12 to mucus isolated from healthy infants[90], suggesting that secreted factors could also influence the adherence of *Bifidobacterium* to mucus. Together, these studies indicate that although multiple *Bifidobacterium* species can bind to mucus, the mechanisms of adhesion appear to be diverse, even among strains of the same species.

The structure of mucus likely dictates the consequences of mucus binding for *Bifidobacterium* species. In the small intestine, the mucus is loose and not attached to the epithelium. As a result, mucus adhesion likely does not promote persistent colonization of the small intestine. In contrast, in the colon, the mucus is highly organized and adhesion to colonic mucus most likely allows *Bifidobacterium* species to persist and colonize the colon. The adhesion of *Bifidobacterium* to colonic mucus is also thought to increase the transit time of the bacteria in the gut, thereby maximizing its beneficial properties[91,92]. It has also been shown that colonization of the mucus layer by *Bifidobacterium* species positively regulates goblet cells. These interactions are all viewed as beneficial for the host. As a result of these positive attributes, the ability to adhere to human intestinal mucus is a commonly employed criterion for the selection of probiotic organisms[75,93,94].

The binding of *Bifidobacterium* to intestinal mucus extends beyond a mere physical attachment; it serves as a gateway for host-microbe crosstalk. By positioning themselves within the mucus, *Bifidobacterium* strains gain proximity to host cells, enabling the effective delivery of health-promoting molecules, metabolites, and

signaling compounds[18,95,96]. Furthermore, the presence of *Bifidobacterium* within the mucus layer influences the spatial organization and composition of the gut microbiota, thereby impacting the overall microbial ecosystem. In several studies, the ability to bind to the mucus layer allowed *Bifidobacterium* species to create a niche and exclude pathogens[54,56,57,62,64,92,97]. One study found that a probiotic containing *Bifidobacterium* could inhibit pathogenic colonization of *Escherichia coli*, and this protective effect was dependent on MUC2 expression by Caco-2 cells[98]. This data suggests that mucus adhesion is critical for excluding pathogens. In addition to excluding pathogens, *Bifidobacterium* species likely have synergistic interactions with other commensal microbes in the mucus layer. *Bifidobacterium* has been shown to cross-fed commensal *Eubacterium rectale*[99], *E. hallii*[100,101], and *Faecalibacterium prausnitzii*[102]. In each of these scenarios, *Bifidobacterium*-commensal co-cultures generated elevated levels of butyrate, a beneficial short-chain fatty acid, compared to the mono-cultures. The literature clearly indicates that *Bifidobacterium* species readily bind to mucus, and this mucus adhesion likely sets the stage for a range of beneficial effects on both the host and the gut microbial community.

MUCUS DEGRADATION BY *BIFIDOBACTERIUM* SPECIES

In addition to serving as a binding site for bacteria, mucus can act as a nutrient source. The mucin protein is heavily O-glycosylated and has multiple structures of repeating α- and β-linked N-acetyl-galactosamine (GalNAc), N-acetyl-glucosamine (GlcNAc), and galactose (Gal) residues, terminated with α-linked fucose (Fuc), and sialic acid (Neu5Ac) residues[103]. Mucus-degrading bacteria harbor specific glycosyl hydrolases (GHs) that enzymatically degrade mucin glycans[3,4,103-106]. After cleavage, the released glycan oligosaccharides can feed the bacteria or other microbes in the vicinity[3,107]. In order to degrade mucin glycans, intestinal bacteria must possess GH33 sialidases (also known as neuraminidases), which cleave terminal sialic acid residues. For efficient glycan cleavage, bacteria can also generate GH29 or GH95 to remove fucose residues. Once the terminal sugars are removed, the underlying GalNAc, GlcNAc, and galactose residues can be removed. Bacteria can have GH101 or GH129 to remove GalNAc, GH84, GH85, GH89, or GH20 to remove GlcNAc, or GH2, GH35, GH42, and GH98 to remove galactose residues. Some bacteria also encode for GH16, endo-acting O-glycanases that remove larger glycan structures. A recent genome analysis confirmed that *B. bifidum* harbored the largest repertoire of mucus-degrading GHs among the *Bifidobacterium* species[103]. All *B. bifidum* genomes had GH33, GH29, GH95, GH20, GH2, GH42, GH101, GH129, GH89, and GH84[103], suggesting that this species was capable of cleaving sialic acid, fucose, GalNAc, GlcNAc, and galactose from mucus glycans. *B. breve*, *B. longum*, and *B. scardovii* were also found to possess multiple mucus-associated GHs. This finding is consistent with other genome studies and *in vitro* studies, which report that *B. bifidum*, *B. longum*, and *B. breve* can degrade mucus[19,100,103,108-112]. In contrast, *B. adolescentis*, *B. angulatum*, *B. animalis*, *B. dentium*, *B. pseudolongum*, and *B. thermophilum* possessed few mucus-associated GHs[103]. *In vitro* work confirmed that *B. dentium* and *B. angulatum* were unable to grow on pig colonic mucus as the sole carbon source[103]. Separate studies have also found that *B. animalis* subsp. *lactis* and *B. pseudolongum* do not degrade mucus[100,113-115]. These studies suggest that, unlike mucus adhesion, mucus degradation is not conserved in *Bifidobacterium* species[103].

Mucin degradation is considered to be a normal process of intestinal mucus turn-over[116] and begins within the first few months of life[117,118]. Infants are commonly colonized with mucin-degrading *B. bifidum*, *B. longum* subsp. *infantis*, and *B. breve*[28-30,118], as well as *Akkermansia muciniphila* and *Bacteroides* species[116]. Interestingly, breast-fed babies that are dominated by *Bifidobacterium* species exhibit a delay in the mucin degradation profile as compared with babies fed with formula milk[118]. Consistent with this notion, Karav *et al.* found that supplementation of *B. longum* subsp. *infantis* EVC001 to healthy breast-fed infants significantly reduced the proportion of free colonic mucin-derived O-glycans in the total glycan pool to 1.87% compared to 37.68% in the control infants who did not receive supplemented *B. longum*[119]. The

level of freed mucin-derived O-glycans was negatively correlated with populations of *Bifidobacteriaceae*, indicating that mucus degradation was not occurring at the same level in *B. longum* supplemented infants[119]. Along the same lines, genes involved in mucus-degrading pathways, particularly in carbohydrate metabolism, in *Bifidobacterium* species were found to be expressed to a greater degree in formula-fed infants than in breast-fed infants[120]. It has been speculated that HMOs, which are similar to mucus in some of the glycan structures[120,121], or other mucin-like glycoproteins present in breast milk, may compete with intestinal mucus as a substrate[118].

In addition to being found in infants, mucus-degrading *Bifidobacterium* species are present in adults and have been linked to the suppression of detrimental mucus degradation. One example of excessive mucus degradation that may be prevented by Bifidobacteria is in the context of a Westernized diet, a diet characterized by low fiber but high fat and sugar. It has been demonstrated in mice harboring defined microbial communities that consuming a Westernized diet leads to an expansion of mucin-degrading bacteria such as *Akkermansia muciniphila* and *Bacteroides caccae*, and this shift enables the bacterial community to target the mucus layer for digestion in lieu of dietary fibers[122]. In a model with complex native gut microbiota, mice fed a Westernized diet similarly exhibited an expansion of *Akkermansia* and a corresponding decrease in *Bifidobacterium* species[123] and increased susceptibility to pathogens and inflammation. In this setting, the addition of *B. longum* NCC 2705 or the prebiotic inulin resulted in elevated levels of endogenous *Bifidobacterium* species, reduced mucus degradation, and restored the mucus barrier. In a similar vein, *B. bifidum* G9-1 was shown to protect against mucus degradation by *A. muciniphila* following small intestine injury caused by a proton pump inhibitor and aspirin[124]. Another study found that the administration of *B. pseudolongum* Patronus increased mucosal thickness in rats and decreased the levels of *A. muciniphila*[125]. These data suggest that mucus degradation by *Bifidobacterium* species is not detrimental to the host and that *Bifidobacterium* species keep mucus degradation in check.

MUCUS MODULATION BY *BIFIDOBACTERIUM* SPECIES

Modulation of mucus by Bifidobacteria in homeostasis

Although some bifidobacteria have mucolytic properties, they generally have an overall positive net effect in regulating intestinal mucus. Several studies have found that *Bifidobacterium* species elevate mucus levels *in vitro* and *in vivo* [Table 3 and Figure 2]. *In vitro*, *B. infantis*, *B. breve*, *B. longum* and a probiotic cocktail containing these microbes and others (VSL#3) was found to stimulate mucus secretion in human mucus-producing LS174T cells[126]. The probiotic cocktail was also found to increase MUC2 expression and secretion in rat colonic loops[126]. In another study, *B. dentium* was reported to increase MUC2 in human mucus-producing T84 cells[18]. Short-chain fatty acids (SCFA) have been demonstrated to increase MUC2 expression[127], and *Bifidobacterium* species are known to produce high levels of SCFA acetate. The application of acetate was likewise able to increase MUC2 gene and protein levels in T84 cells[18]. *In vivo*, *B. dentium* was found to colonize germ-free mice, elevate intestinal acetate levels, and increase MUC2 at the gene and protein levels[18]. An elevated number of goblet cells and goblet cell-specific genes were observed in *B. dentium* mono-associated mice, as well as increased mucin glycosylation[18]. In this model, it was speculated that *B. dentium*-generated gamma-aminobutyric acid (GABA) was able to activate autophagy and calcium signaling to stimulate the release of mucus from goblet cells and bolster the mucus barrier[18]. In addition to B. *dentium*, *B. bifidum* and *B. longum* colonize germ-free mice and increase intestinal mucin glycoproteins[128,129]. These studies using mono-associated gnotobiotic animals provide very powerful evidence that *B. dentium*, *B. bifidum* and *B. longum* can modulate goblet cell function and increase mucus production. In mice with complex gut microbiota, *B. breve* supplementation led to 3,996 upregulated and 465 downregulated genes in supplemented neonatal mice relative to the untreated group[35]. Upregulated genes in the neonatal mice encoded multiple mucus layer-associated proteins such as MUC2. These data suggest that *B. breve* in early life modulates goblet cells. In adult mice, administration of a probiotic cocktail

Table 3. Literature review of the positive effects of Bifidobacteria on mucus expression, mucin levels and mucus expulsion

***Bifidobacterium* species**	**Finding**	**Experimental model**	**Ref.**
B. infantis	Increased mucus secretion	LS174T cells	[126]
B. breve	Increased mucus secretion	LS174T cells	[126]
B. longum	Increased mucus secretion	LS174T cells	[126]
VSL#3	Increased mucus secretion	LS174T cells	[126]
VSL#3	Increased MUC2 expression and secretion	Rat colonic loops	[126]
B. dentium ATCC 27678	Increased mucus expression and secretion	T84 cells	[18]
B. dentium ATCC 27678	Increased MUC2 expression and mucus levels	Adult gnotobiotic mice	[18]
B. bifidum FPLC AA22	Increased mucus levels	Adult gnotobiotic mice	[129]
B. longum FPLC 117	Increased mucus levels	Adult gnotobiotic mice	[128]
B. breve UCC2003	Increased MUC2 expression	Neonatal conventional mice	[35]
B. breve (probiotic cocktail)	Increased goblet cells per crypt and increased mucus levels	Adult conventional mice	[130]

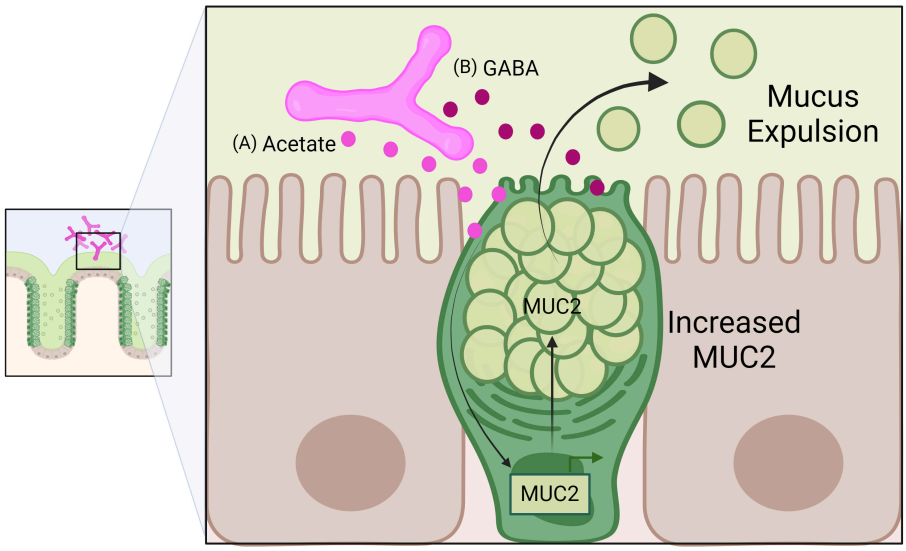

Figure 2. Representative diagram of *Bifidobacterium-goblet* cell interactions. (A) *Bifidobacterium* species can generate acetate, which can elevate MUC2 expression and protein; (B) *Bifidobacterium* species can also generate varying levels of GABA, which can activate autophagy-driven expulsion of mucus. Through these mechanisms, Bifidobacteria are speculated to positively regulate goblet cells. GABA: *B. dentium*-generated gamma-aminobutyric acid.

containing *B. breve* also increased the number of goblet cells per crypt and increased the production of mucus compared with controls[130]. Collectively, these data indicate that *Bifidobacterium* strains influence goblet cell function and mucus production.

Modulation of mucus by Bifidobacteria in inflammation and infectious diseases

There is a wide array of data that demonstrate the substantial benefits of *Bifidobacterium* in the context of disease. Colitis is one of the most frequently investigated intestinal diseases, and a variety of *Bifidobacterium* species have exhibited the ability to alleviate major complications of colitis. In general, *Bifidobacterium* species have been shown to (1) limit inflammation-associated goblet cell and mucus depletion and MUC2 and (2) reduce pro-inflammatory cytokines [Figure 3, Tables 4 and 5].

Table 4. Literature review of strain-specific effects of *Bifidobacterium* species on mucus modulation in the context of inflammation or infectious disease

Bifidobacterium species	Finding	Intestinal site	Experimental model	Ref.
B. bifidum FL-276.1	Increased MUC2, improved mucus, reduce colitis	Colon	DSS-colitis	[166]
B. bifidum FL-228.1	Increased MUC2, improved mucus, reduce colitis	Colon	DSS-colitis	[166]
B. bifidum BGN4	Improved mucus, reduce colitis	Colon	DSS-colitis	[168]
B. longum subsp. *longum* YS108R	Increased MUC2, improved mucus, reduce colitis	Colon	DSS-colitis	[169]
B. longum Bif10	Increased MUC2, improved mucus, reduce colitis	Colon	DSS-colitis	[171]
B. breve Bif11	Increased MUC2, improved mucus, reduce colitis	Colon	DSS-colitis	[171]
B. breve CBT BR3	Improved mucus, reduce colitis	Colon	DSS-colitis	[170]
B. animalis subsp. *lactis* A6	Improved mucus, reduce colitis	Colon	DSS-colitis	[167]
B. infantis GMCC0460.1	Improved mucus, reduce colitis	Colon	DSS-colitis	[172]
B. infantis 2017012	Improved mucus, reduce colitis	Colon	DSS-colitis	[176]
B. infantis unclassified strain	Improved mucus, reduce colitis	Colon	DSS-colitis	[176]
B. breve H4-2	Improved mucus, reduce colitis	Colon	DSS-colitis	[174]
B. breve H9-3	Improved mucus, reduce colitis	Colon	DSS-colitis	[174]
B. lactis BL-99	Improved mucus, reduce colitis	Colon	Zebrafish colitis model	[177]
B. dentium ATCC 27678	Increased MUC2, improved mucus, reduce colitis	Colon	TNBS-colitis	[96]
B. infantis unclassified strain	Improved mucus, reduce colitis	Colon	TNBS-colitis	[178]
B. longum Bar 33	Improved mucus, reduce colitis	Colon	TNBS-colitis	[176]
B. animalis subsp. lactis CNCM-I2494	Improved mucus, reduce colitis	Colon	DNBS-colitis	[179]
B. bifidum E3	Increased MUC2, improved mucus	Small intestine	LPS-induced injury	[180]
B. infantis E4	Increased MUC2, improved mucus	Small intestine	LPS-induced injury	[180]
B. lactis BB12	Increased MUC2, improved mucus	Small intestine	LPS-induced injury	[180]
B. bifidum OLB637	Increased mucin expression	Small intestine	Rat model of NEC	[181]
B. bifidum G9-1	Increased MUC2, improved mucus	Small intestine	Rotavirus mouse model	[185]
B. infantis PCM	Improved mucus	Small intestine	Cronobacter sakazakii mouse model	[186]

Colitis-inducing compounds are known to activate ER stress[131-134], and ER stress has been linked to intestinal inflammation in multiple animal models[135-139]. Goblet cells are particularly sensitive to ER stress since producing and folding MUC2 is a complex process[140,141]. It has been speculated that modulation of goblet cell ER stress by *Bifidobacterium* species may represent a key pathway by which bifidobacteria promote intestinal health. In mucus-producing Caco-2 cells, the application of live *B. breve* YIT 12272 and *B. adolescentis* YIT 4011T alleviated tunicamycin-induced ER stress[142]. In another study using mucus-producing T84 cells, it was shown that *B. dentium* ATCC 27678-secreted metabolites could also suppress tunicamycin- or thapsigarin-induced ER stress[96]. Analysis of the *B. dentium* metabolites revealed that this strain generated substantial levels of γ-glutamylcysteine, a compound that can be converted into the powerful antioxidant glutathione and suppress oxidative and ER stress[131,133,143-147]. *B. dentium* metabolites harboring γ-glutamylcysteine and application of commmerically available γ-glutamylcysteine both elevated glutathione, suppressed inflammatory NF-κB activation, reduced IL-8 secretion, and attenuated the induction of the unfolded protein response (UPR) genes GRP78, CHOP, and sXBP1 in T84 cells and TNBS-treated mice[96]. These data suggest that *Bifidobacterium* species can reduce goblet cell ER stress.

Table 5. Literature review of strain-specific effects of *Bifidobacterium* species on immune modulation in the context of inflammation

***Bifidobacterium* species**	**Finding**	**Body site**	**Experimental model**	**Ref.**
B. infantis	Reduced pro-inflammatory cytokines & increased IL-10	Colon	TNBS colitis	[176]
B. breve CBT BR3	Reduced pro-inflammatory cytokines & increased IL-10	Colon	TNBS colitis	[170]
B. longum and B. animalis (probiotic cocktail)	Reduced pro-inflammatory cytokines & increased IL-10	Colon	TNBS colitis	[178]
B. dentium ATCC 27678	Reduced pro-inflammatory cytokines & increased IL-10	Serum and colon	TNBS colitis	[96]
Bifidobacterium animalis subspecies *lactis CNCM-I2494*	Reduced pro-inflammatory cytokines & increased IL-10	Colon and T cells	DNBS colitis	[179]
B. longum Bif10	Reduced pro-inflammatory cytokines	Serum and colon	DSS colitis	[171]
B. breve Bif11	Reduced pro-inflammatory cytokines	Serum and colon	DSS colitis	[171]
B. longum Bif16	Reduced pro-inflammatory cytokines	Serum and colon	DSS colitis	[171]

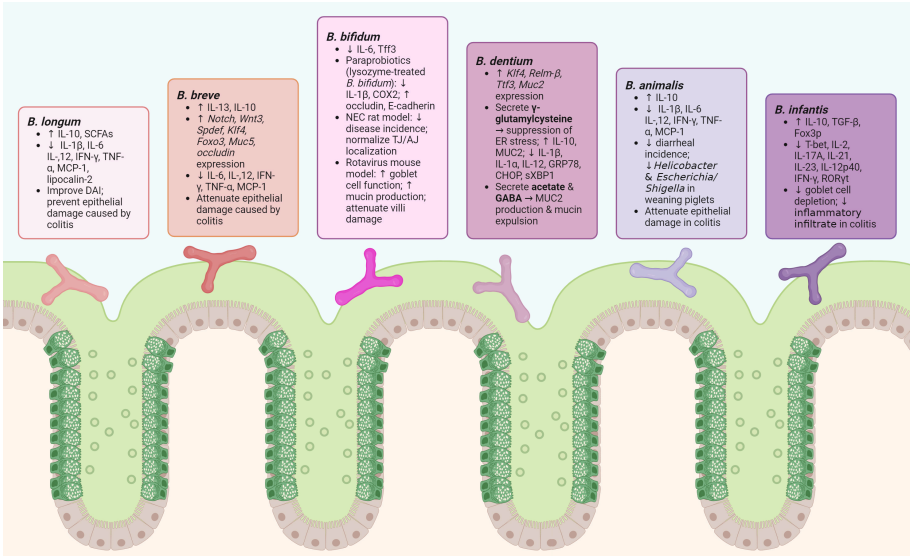

Figure 3. Diagram outlining the major beneficial effects, especially in improving goblet cell function and in reducing inflammation, per *Bifidobacterium* species in disease models. GABA: *B. dentium*-generated gamma-aminobutyric acid; NEC: necrotizing enterocolitis; SCFAs: short-chain fatty acids.

When goblet cells undergo ER stress, they are unable to adequately synthesize and secrete MUC2, leading to a reduction in goblet cell number and a thinning of the intestinal mucus layer. Several animal models have shown that goblet cell ER stress or loss of mucus leads to intestinal inflammation (*Winnie*, MUC2$^{-/-}$, AGR2$^{-/-}$, glycan deficiency, *etc.*)[148-153]. These animal model phenotypes closely resemble the intestinal issues observed in inflammatory bowel disease (IBD) patients, particularly in ulcerative colitis patients[138,154-157]. Ulcerative colitis patients have decreased goblet cell numbers, truncated mucin glycosylation, reduced mucus layer thickness, and limited mucus integrity[137,155-160]. Loss of both the thickness and integrity of the mucus layer is thought to promote bacterial-epithelial interactions and drive inflammation[161-165].

Several studies have found that *Bifidobacterium* species can limit the reduction of goblet cells and improve the mucus barrier in the setting of chemically induced intestinal inflammation [Table 4]. For example, *B. bifidum*, *B. longum*, *B. longum* subsp. *longum*, *B. breve*, and *B. animalis* subsp. *lactis* were shown to increase MUC2, improve the mucus barrier, and ameliorate DSS-induced colitis[166-172]. A probiotic mixture containing *B. infantis* was also shown to enhance the mucus barrier in DSS-treated mice[173]. *B. infantis* and *B. breve* were likewise found to limit the reduction of goblet cells in DSS models[174-176], and *B. lactis* was found to improve goblet cell counts in a zebrafish model of intestinal inflammation[177]. Along the same lines, *B. dentium* was also shown to increase MUC2, limit goblet cell reduction, and improve the mucus layer in a TNBS-induced model of colitis[96]. *B. infantis* and *B. longum* were also found to improve goblet cell numbers in TNBS-induced colitis[176,178], while *B. animalis* subsp. *lactis* restored goblet cell populations in dinitrobenzene sulfonicacid (DNBS)-challenged mice[179]. These studies indicate that *Bifidobacterium* species can reduce goblet cell loss and mucus depletion in the setting of TNBS and DNBS-induced colitis.

Bifidobacterium species also have positive roles in modulating mucus in other inflammatory models. For example, *B. bifidum*, *B. infantis*, and *B. lactis* increased MUC2 in the small intestine during LPS-induced injury[180]. In a rat model of necrotizing enterocolitis (NEC), *B. bifidum* was shown to increase mucin and TFF3 expression and decrease the disease severity[181]. *B. longum* EVC001 and *B. infantis* BB-02 also decreased NEC occurrence in animals[182,183]. Even more promising is a double-blind, randomized, controlled study of very-low-birth-weight preterm infants, in which a combination of *B. breve* strain Yakult and *L. casei* strain Shirota completely prevented the occurrence of NEC in the intervention group, whereas 3.5% of the cases developed NEC in control without probiotics[184]. The mechanism by which *Bifidobacterium* confers its benefits in NEC is not fully understood but may be similar to colitis involving the mucus layer, intestinal permeability, and inflammation.

Rotavirus gastroenteritis is another disease where *Bifidobacterium* species have been shown to beneficially modulate the mucus layer. *B. bifidum* G9-1 was shown to increase MUC2, normalize mucin-positive goblet cells in the small intestine, and reduce the incidence, diarrheal scores, and intestinal damage in the supplemented group with rotavirus compared to the control group with rotavirus alone[185]. *B. infantis* PCM has also been shown to maintain goblet cells and reduce epithelial damage in the small intestine of mice infected with the pathogen *Cronobacter sakazakii*[186]. These data demonstrate that goblet cells and mucin production are also beneficially influenced by bifidobacteria in the small and large intestines in multiple inflammatory models.

Pro-inflammatory cytokines have been shown to negatively regulate goblet cells, while anti-inflammatory compounds such as IL-10 are known to alleviate ER stress and enhance goblet cell function. Another pathway by which *Bifidobacterium* species positively modulate goblet cells is through the modulation of intestinal cytokines [Table 5]. In TNBS-induced colitis mouse models, supplementation of *B. infantis*, *B. breve*, and probiotic cocktail mixes that included *B. longum* Bar 33 and *B. animalis* subsp. *lactis* Bar 30 resulted in reduced levels of several pro-inflammatory cytokines, e.g., IL-2, IL-1β, IL-13, IL-12p40, IL-17A, IL-21, IL-23, IFN-γ, TNF-α, and MCP-1, relative to the untreated TNBS groups[170,176,178]. These strains additionally led to rises in the anti-inflammatory cytokine IL-10[170,176,178]. Similarly, *B. dentium* reduced serum IFN-γ, IL-1α, IL-1β, IL-12, and TNF-α with a concomitant increase in IL-10 in comparison to the TNBS control mice[96]. Another study using DSS revealed that *B. breve* and *B. longum* lowered both systemic and colonic levels of TNF-α, IL-1β, and IL-6[171]. These studies suggest that in addition to directly modulating goblet cells through metabolites and suppression of ER stress, *Bifidobacterium* strains may be indirectly modulating goblet cell function via immune regulation.

OVERALL EFFECTS OF BIFIDOBACTERIUM-MUCUS INTERACTIONS ON THE HOST

The literature suggests that the intestinal mucus layer plays a crucial role in the interaction of *Bifidobacterium* species with the host. It appears that the majority of *Bifidobacterium* species bind to intestinal mucus and establish a unique niche that affords them an advantageous position for their beneficial activities. Within the mucus layer, some *Bifidobacterium* species can degrade mucus, while others must rely on other nutrient sources. In the mucus layer, *Bifidobacterium* species likely perform the following functions: (1) exclude pathogens; (2) cross-fed commensal bacteria; (3) limit excessive mucus degradation; (4) secrete compounds such as acetate, which elevate MUC2 expression and increase mucus production; (5) reduce goblet cell ER stress; (6) limit inflammation- and infection-driven goblet cell loss; (7) suppress pro-inflammatory cytokines; and (8) increase anti-inflammatory pro-goblet cell IL-10.

The literature points to the capacity for *Bifidobacterium* species to beneficially modulate goblet cell number and function, thereby regulating the mucus layer and intestinal barrier function. This modulation of the goblet cells by *Bifidobacterium* is likely even more important during the setting of infection and inflammation. Through these interactions, *Bifidobacterium* species facilitate a dynamic interplay that contributes to gut homeostasis and overall host health.

LIMITATIONS AND GAPS IN THE FIELD

While these findings are compelling, there are still several gaps in knowledge. First, it is unclear which *Bifidobacterium* strains are the most effective at positively regulating goblet cell function. Very few studies have performed head-to-head comparisons of different *Bifidobacterium* strains and studies vary in terms of mouse strain (C57B6/J, BALBc, Swiss Webster, *etc.*), colonization status (mono-association, gnobotioic with defined communities, conventional, *etc.*), and challenge (TNBS, DSS, DNBS, LPS, infection *etc.*). These variables make it difficult to tease out the nuances between strains and effects. Second, the metabolites that drive goblet cell-specific attributes of *Bifidobacterium* are not well characterized. It is well documented that *Bifidobacterium* species can generate acetate and this SCFA can elevate MUC2 levels, but it is likely that other metabolites also stimulate MUC2. In addition to modulating MUC2 levels, *Bifidobacterium* species can influence goblet cells in other ways, such as suppressing ER stress, promoting autophagy, and stimulating mucus expulsion. Likewise, it is not clear how bifidobacteria members regulate IL-10 production, which could indirectly affect goblet cell homeostasis. These pathways need to be explored with multiple *Bifidobacterium* strains.

The advent of intestinal organoids is a promising new technology to address *Bifidobacteri*um-goblet cell interactions. This model maintains segment specificity, is not immortalized, and is not cancer-derived. Importantly, intestinal organoids harbor MUC2-positive goblet cells and have been previously used to examine bacterial-host interactions, including *Bifidobacterium*[95,187-189]. We anticipate that many future studies will employ this model to define the mechanisms by which *Bifidobacterium* species regulate goblet cells and interact with intestinal mucus.

Although there are still large gaps in the field, the wealth of literature allows us to make some key observations on conserved bifidobacteria functions, such as mucus binding, suppression of inflammation-driven goblet cell depletion, and elevation of MUC2. Understanding the interaction between *Bifidobacterium* and the intestinal mucus layer is imperative for unraveling the mechanisms underlying their beneficial effects. With this knowledge, there is immense potential for developing targeted therapeutic interventions.

DECLARATIONS

Authors' contributions

Drafted manuscript: Gutierrez A, Puckett B
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Provided funding: Engevik MA

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

All work was carried out in accordance with the WHO Code of Ethics (Helsinki Declaration) on experiments with humans.

Consent for publication

Not applicable.

REFERENCES

1. Song C, Chai Z, Chen S, Zhang H, Zhang X, Zhou Y. Intestinal mucus components and secretion mechanisms: what we do and do not know. *Exp Mol Med* 2023;55:681-91. DOI PubMed PMC
2. Corfield AP, Wagner SA, Clamp JR, Kriaris MS, Hoskins LC. Mucin degradation in the human colon: production of sialidase, sialate O-acetylesterase, N-acetylneuraminate lyase, arylesterase, and glycosulfatase activities by strains of fecal bacteria. *Infect Immun* 1992;60:3971-8. DOI PubMed PMC
3. Bell A, Juge N. Mucosal glycan degradation of the host by the gut microbiota. *Glycobiology* 2021;31:691-6. DOI PubMed PMC
4. Tailford LE, Crost EH, Kavanaugh D, Juge N. Mucin glycan foraging in the human gut microbiome. *Front Genet* 2015;6:81. DOI PubMed PMC
5. McGuckin MA, Lindén SK, Sutton P, Florin TH. Mucin dynamics and enteric pathogens. *Nat Rev Microbiol* 2011;9:265-78. DOI PubMed
6. McDole JR, Wheeler LW, McDonald KG, et al. Goblet cells deliver luminal antigen to $CD103^+$ dendritic cells in the small intestine. *Nature* 2012;483:345-9. DOI PubMed PMC
7. Aihara E, Engevik KA, Montrose MH. Trefoil factor peptides and gastrointestinal function. *Annu Rev Physiol* 2017;79:357-80. DOI PubMed PMC
8. Croucher SC, Houston AP, Bayliss CE, Turner RJ. Bacterial populations associated with different regions of the human colon wall. *Appl Environ Microbiol* 1983;45:1025-33. DOI PubMed PMC
9. Vasapolli R, Schütte K, Schulz C, et al. Analysis of transcriptionally active bacteria throughout the gastrointestinal tract of healthy individuals. *Gastroenterology* 2019;157:1081-92.e3. DOI
10. Turroni F, Marchesi JR, Foroni E, et al. Microbiomic analysis of the bifidobacterial population in the human distal gut. *ISME J* 2009;3:745-51. DOI
11. Collado MC, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. Imbalances in faecal and duodenal *Bifidobacterium* species composition in active and non-active coeliac disease. *BMC Microbiol* 2008;8:232. DOI PubMed PMC
12. von Wright A, Vilpponen-salmela T, Llopis MP, et al. The survival and colonic adhesion of *Bifidobacterium infantis* in patients with ulcerative colitis. *Int Dairy J* 2002;12:197-200. DOI
13. Ahmed S, Macfarlane GT, Fite A, McBain AJ, Gilbert P, Macfarlane S. Mucosa-associated bacterial diversity in relation to human terminal ileum and colonic biopsy samples. *Appl Environ Microbiol* 2007;73:7435-42. DOI PubMed PMC
14. Chassaing B, Gewirtz AT. Identification of inner mucus-associated bacteria by laser capture microdissection. *Cell Mol Gastroenterol*

Hepatol 2019;7:157-60. DOI PubMed PMC
15. O'Callaghan A, van Sinderen D. Bifidobacteria and their role as members of the human gut microbiota. *Front Microbiol* 2016;7:925. DOI PubMed PMC
16. O'Neill I, Schofield Z, Hall LJ. Exploring the role of the microbiota member *Bifidobacterium* in modulating immune-linked diseases. *Emerg Top Life Sci* 2017;1:333-49. DOI PubMed PMC
17. Grimm V, Westermann C, Riedel CU. Bifidobacteria-host interactions - an update on colonisation factors. *Biomed Res Int* 2014;2014:960826. DOI PubMed PMC
18. Engevik MA, Luk B, Chang-Graham AL, et al. *Bifidobacterium dentium* fortifies the intestinal mucus layer via autophagy and calcium signaling pathways. *mBio* 2019;10:e01087-19. DOI PubMed PMC
19. Ruas-Madiedo P, Gueimonde M, Fernández-García M, de los Reyes-Gavilán CG, Margolles A. Mucin degradation by *Bifidobacterium* strains isolated from the human intestinal microbiota. *Appl Environ Microbiol* 2008;74:1936-40. DOI PubMed PMC
20. Png CW, Lindén SK, Gilshenan KS, et al. Mucolytic bacteria with increased prevalence in IBD mucosa augment *in vitro* utilization of mucin by other bacteria. *Am J Gastroenterol* 2010;105:2420-8. DOI
21. Lugli GA, Milani C, Turroni F, et al. Comparative genomic and phylogenomic analyses of the bifidobacteriaceae family. *BMC Genomics* 2017;18:568. DOI PubMed PMC
22. Lugli GA, Milani C, Turroni F, et al. Investigation of the evolutionary development of the genus *Bifidobacterium* by comparative genomics. *Appl Environ Microbiol* 2014;80:6383-94. DOI PubMed PMC
23. Milani C, Turroni F, Duranti S, et al. Genomics of the genus *Bifidobacterium* reveals species-specific adaptation to the glycan-rich gut environment. *Appl Environ Microbiol* 2016;82:980-91. DOI PubMed PMC
24. Nuriel-Ohayon M, Neuman H, Koren O. Microbial changes during pregnancy, birth, and infancy. *Front Microbiol* 2016;7:1031. DOI PubMed PMC
25. Makino H, Martin R, Ishikawa E, et al. Multilocus sequence typing of bifidobacterial strains from infant's faeces and human milk: are bifidobacteria being sustainably shared during breastfeeding? *Benef Microbes* 2015;6:563-72. DOI
26. Newman J. How breast milk protects newborns. *Sci Am* 1995;273:76-9. DOI PubMed
27. Arboleya S, Salazar N, Solís G, et al. Assessment of intestinal microbiota modulation ability of *Bifidobacterium* strains in *in vitro* fecal batch cultures from preterm neonates. *Anaerobe* 2013;19:9-16. DOI
28. Bäckhed F, Roswall J, Peng Y, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe* 2015;17:690-703. DOI PubMed
29. Lim ES, Zhou Y, Zhao G, et al. Early life dynamics of the human gut virome and bacterial microbiome in infants. *Nat Med* 2015;21:1228-34. DOI PubMed PMC
30. Makino H, Kushiro A, Ishikawa E, et al. Mother-to-infant transmission of intestinal bifidobacterial strains has an impact on the early development of vaginally delivered infant's microbiota. *PLoS One* 2013;8:e78331. DOI PubMed PMC
31. Turroni F, Peano C, Pass DA, et al. Diversity of bifidobacteria within the infant gut microbiota. *PLoS One* 2012;7:e36957. DOI PubMed PMC
32. Khonsari S, Suganthy M, Burczynska B, Dang V, Choudhury M, Pachenari A. A comparative study of bifidobacteria in human babies and adults. *Biosci Microb Food H* 2016;35:97-103. DOI PubMed PMC
33. Luk B, Veeraragavan S, Engevik M, et al. Postnatal colonization with human "infant-type" *Bifidobacterium* species alters behavior of adult gnotobiotic mice. *PLoS One* 2018;13:e0196510. DOI PubMed PMC
34. Luck B, Engevik MA, Ganesh BP, et al. Bifidobacteria shape host neural circuits during postnatal development by promoting synapse formation and microglial function. *Sci Rep* 2020;10:7737. DOI PubMed PMC
35. Kiu R, Treveil A, Harnisch LC, et al. Bifidobacterium breve UCC2003 induces a distinct global transcriptomic program in neonatal murine intestinal epithelial cells. *iScience* 2020;23:101336. DOI PubMed PMC
36. Sudo N, Chida Y, Aiba Y, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol* 2004;558:263-75. DOI PubMed PMC
37. Turroni F, Milani C, Ventura M, van Sinderen D. The human gut microbiota during the initial stages of life: insights from bifidobacteria. *Curr Opin Biotechnol* 2022;73:81-7. DOI PubMed
38. Taft DH, Liu J, Maldonado-Gomez MX, et al. Bifidobacterial dominance of the gut in early life and acquisition of antimicrobial resistance. *mSphere* 2018;3:e00441-18. DOI PubMed PMC
39. Lin C, Lin Y, Zhang H, et al. Intestinal 'infant-type' Bifidobacteria mediate immune system development in the first 1000 days of life. *Nutrients* 2022;14:1498. DOI PubMed PMC
40. Mitsuoka T. Taxonomy and ecology of Bifidobacteria. *Bifidobacteria Microflora* 1984;3:11-28. DOI
41. Ouwehand AC, Isolauri E, Kirjavainen PV, Salminen SJ. Adhesion of four Bifidobacterium strains to human intestinal mucus from subjects in different age groups. *FEMS Microbiol Lett* 1999;172:61-4. DOI PubMed
42. Arboleya S, Watkins C, Stanton C, Ross RP. Gut Bifidobacteria populations in human health and aging. *Front Microbiol* 2016;7:1204. DOI PubMed PMC
43. Sovran B, Hugenholtz F, Elderman M, et al. Age-associated impairment of the mucus barrier function is associated with profound changes in microbiota and immunity. *Sci Rep* 2019;9:1437. DOI PubMed PMC
44. Elderman M, Sovran B, Hugenholtz F, et al. The effect of age on the intestinal mucus thickness, microbiota composition and

immunity in relation to sex in mice. *PLoS One* 2017;12:e0184274. DOI PubMed PMC
45. Saffrey MJ. Aging of the mammalian gastrointestinal tract: a complex organ system. *Age* 2014;36:9603. DOI PubMed PMC
46. He F, Ouwehan AC, Hashimoto H, Isolauri E, Benno Y, Salminen S. Adhesion of *Bifidobacterium* spp. to human intestinal mucus. *Microbiol Immunol* 2001;45:259-62. DOI PubMed
47. Kirjavainen PV, Ouwehand AC, Isolauri E, Salminen SJ. The ability of probiotic bacteria to bind to human intestinal mucus. *FEMS Microbiol Lett* 1998;167:185-9. DOI PubMed
48. Harata G, Yoda K, Wang R, et al. Species- and age/generation-dependent adherence of *Bifidobacterium bifidum* to human intestinal mucus in vitro. *Microorganisms* 2021;9:542. DOI PubMed PMC
49. Mantziari A, Tölkkö S, Ouwehand AC, et al. The effect of donor human milk fortification on the adhesion of probiotics in vitro. *Nutrients* 2020;12:182. DOI PubMed PMC
50. Juntunen M, Kirjavainen PV, Ouwehand AC, Salminen SJ, Isolauri E. Adherence of probiotic bacteria to human intestinal mucus in healthy infants and during rotavirus infection. *Clin Diagn Lab Immunol* 2001;8:293-6. DOI PubMed PMC
51. Ouwehand AC, Niemi P, Salminen SJ. The normal faecal microflora does not affect the adhesion of probiotic bacteria in vitro. *FEMS Microbiol Lett* 1999;177:35-8. DOI
52. Collado MC, Gueimonde M, Sanz Y, Salminen S. Adhesion properties and competitive pathogen exclusion ability of bifidobacteria with acquired acid resistance. *J Food Prot* 2006;69:1675-9. DOI PubMed
53. Kainulainen V, Reunanen J, Hiippala K, et al. BopA does not have a major role in the adhesion of Bifidobacterium bifidum to intestinal epithelial cells, extracellular matrix proteins, and mucus. *Appl Environ Microbiol* 2013;79:6989-97. DOI PubMed PMC
54. Collado MC, Jalonen L, Meriluoto J, Salminen S. Protection mechanism of probiotic combination against human pathogens: in vitro adhesion to human intestinal mucus. *Asia Pac J Clin Nutr* 2006;15:570-5. PubMed
55. Collado MC, Meriluoto J, Salminen S. Development of new probiotics by strain combinations: is it possible to improve the adhesion to intestinal mucus? *J Dairy Sci* 2007;90:2710-6. DOI PubMed
56. Gueimonde M, Margolles A, de los Reyes-Gavilán CG, Salminen S. Competitive exclusion of enteropathogens from human intestinal mucus by Bifidobacterium strains with acquired resistance to bile - a preliminary study. *Int J Food Microbiol* 2007;113:228-32. DOI PubMed
57. Collado MC, Gueimonde M, Hernández M, Sanz Y, Salminen S. Adhesion of selected *Bifidobacterium* strains to human intestinal mucus and the role of adhesion in enteropathogen exclusion. *J Food Prot* 2005;68:2672-8. DOI PubMed
58. Gueimonde M, Noriega L, Margolles A, de los Reyes-Gavilan CG, Salminen S. Ability of *Bifidobacterium* strains with acquired resistance to bile to adhere to human intestinal mucus. *Int J Food Microbiol* 2005;101:341-6. DOI PubMed
59. Engevik MA, Danhof HA, Hall A, et al. The metabolic profile of *Bifidobacterium dentium* reflects its status as a human gut commensal. *BMC Microbiol* 2021;21:154. DOI PubMed PMC
60. los Reyes-Gavilán CG, Suárez A, Fernández-García M, Margolles A, Gueimonde M, Ruas-Madiedo P. Adhesion of bile-adapted *Bifidobacterium* strains to the HT29-MTX cell line is modified after sequential gastrointestinal challenge simulated in vitro using human gastric and duodenal juices. *Res Microbiol* 2011;162:514-9. DOI
61. Kadlec R, Jakubec M. The effect of prebiotics on adherence of probiotics. *J Dairy Sci* 2014;97:1983-90. DOI PubMed
62. Bernet MF, Brassart D, Neeser JR, Servin AL. Adhesion of human bifidobacterial strains to cultured human intestinal epithelial cells and inhibition of enteropathogen-cell interactions. *Appl Environ Microbiol* 1993;59:4121-8. DOI PubMed PMC
63. Gandhi A, Shah NP. Effect of salt stress on morphology and membrane composition of *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum*, and their adhesion to human intestinal epithelial-like Caco-2 cells. *J Dairy Sci* 2016;99:2594-605. DOI
64. Laparra JM, Sanz Y. Comparison of in vitro models to study bacterial adhesion to the intestinal epithelium. *Lett Appl Microbiol* 2009;49:695-701. DOI PubMed
65. Aissi EA, Lecocq M, Brassart C, Bouquelet S. Adhesion of some *Bifidobacterial* strains to human enterocyte-like cells and binding to mucosal glycoproteins. *Microb Ecol Health Dis* 2001;13:32-9. DOI
66. Zhang G, Zhao J, Wen R, Zhu X, Liu L, Li C. 2'-Fucosyllactose promotes *Bifidobacterium bifidum* DNG6 adhesion to Caco-2 cells. *J Dairy Sci* 2020;103:9825-34. DOI PubMed
67. Riedel CU, Foata F, Goldstein DR, Blum S, Eikmanns BJ. Interaction of bifidobacteria with Caco-2 cells-adhesion and impact on expression profiles. *Int J Food Microbiol* 2006;110:62-8. DOI PubMed
68. Morita H, He F, Fuse T, et al. Adhesion of lactic acid bacteria to caco-2 cells and their effect on cytokine secretion. *Microbiol Immunol* 2002;46:293-7. DOI
69. Serafini F, Strati F, Ruas-Madiedo P, et al. Evaluation of adhesion properties and antibacterial activities of the infant gut commensal *Bifidobacterium bifidum* PRL2010. *Anaerobe* 2013;21:9-17. DOI
70. Izquierdo E, Medina M, Ennahar S, Marchioni E, Sanz Y. Resistance to simulated gastrointestinal conditions and adhesion to mucus as probiotic criteria for *Bifidobacterium longum* strains. *Curr Microbiol* 2008;56:613-8. DOI PubMed
71. Collado MC, Grześkowiak Ł, Salminen S. Probiotic strains and their combination inhibit in vitro adhesion of pathogens to pig intestinal mucosa. *Curr Microbiol* 2007;55:260-5. DOI PubMed
72. Macfarlane S, Woodmansey EJ, Macfarlane GT. Colonization of mucin by human intestinal bacteria and establishment of biofilm communities in a two-stage continuous culture system. *Appl Environ Microbiol* 2005;71:7483-92. DOI PubMed PMC
73. Klijn A, Mercenier A, Arigoni F. Lessons from the genomes of bifidobacteria. *FEMS Microbiol Rev* 2005;29:491-509. DOI

PubMed

74. Westermann C, Gleinser M, Corr SC, Riedel CU. A critical evaluation of bifidobacterial adhesion to the host tissue. *Front Microbiol* 2016;7:1220. DOI PubMed PMC
75. Xiong Y, Zhai Z, Lei Y, Xiao B, Hao Y. A novel major pilin subunit protein fimm is involved in adhesion of *Bifidobacterium longum* BBMN68 to intestinal epithelial cells. *Front Microbiol* 2020;11:590435. DOI PubMed PMC
76. Turroni F, Serafini F, Foroni E, et al. Role of sortase-dependent pili of *Bifidobacterium bifidum* PRL2010 in modulating bacterium-host interactions. *Proc Natl Acad Sci U S A* 2013;110:11151-6. DOI PubMed PMC
77. Nishiyama K, Yamamoto Y, Sugiyama M, et al. *Bifidobacterium bifidum* extracellular sialidase enhances adhesion to the mucosal surface and supports carbohydrate assimilation. *mBio* 2017;8:e00928-17. DOI PubMed PMC
78. González-Rodríguez I, Sánchez B, Ruiz L, et al. Role of extracellular transaldolase from *Bifidobacterium bifidum* in mucin adhesion and aggregation. *Appl Environ Microbiol* 2012;78:3992-8. DOI PubMed PMC
79. Yuan J, Wang B, Sun Z, et al. Analysis of host-inducing proteome changes in *Bifidobacterium longum* NCC2705 grown in vivo. *J Proteome Res* 2008;7:375-85. DOI
80. Schell MA, Karmirantzou M, Snel B, et al. The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. *Proc Natl Acad Sci U S A* 2002;99:14422-7. DOI PubMed PMC
81. Wei X, Yan X, Chen X, et al. Proteomic analysis of the interaction of *Bifidobacterium longum* NCC2705 with the intestine cells Caco-2 and identification of plasminogen receptors. *J Proteomics* 2014;108:89-98. DOI
82. Candela M, Biagi E, Centanni M, et al. Bifidobacterial enolase, a cell surface receptor for human plasminogen involved in the interaction with the host. *Microbiology* 2009;155:3294-303. DOI
83. Sun Y, Zhu DQ, Zhang QX, et al. The expression of GroEL protein amplified from *Bifidobacterium animalis* subsp. *lactis* KLDS 2. 0603 and its role in competitive adhesion to Caco-2. *Food Biotechnol* 2016;30:292-305. DOI
84. Shkoporov AN, Khokhlova EV, Kafarskaia LI, et al. Search for protein adhesin gene in *Bifidobacterium longum* genome using surface phage display technology. *Bull Exp Biol Med* 2008;146:782-5. DOI
85. Suzuki R, Katayama T, Kitaoka M, et al. Crystallographic and mutational analyses of substrate recognition of endo-alpha-N-acetylgalactosaminidase from *Bifidobacterium longum*. *J Biochem* 2009;146:389-98. DOI PubMed
86. Garrido D, Kim JH, German JB, Raybould HE, Mills DA. Oligosaccharide binding proteins from *Bifidobacterium longum* subsp. *infantis* reveal a preference for host glycans. *PLoS One* 2011;6:e17315. DOI PubMed PMC
87. Tam R, Saier MH Jr. Structural, functional, and evolutionary relationships among extracellular solute-binding receptors of bacteria. *Microbiol Rev* 1993;57:320-46. DOI PubMed PMC
88. O'Connell Motherway M, Zomer A, Leahy SC, et al. Functional genome analysis of *Bifidobacterium breve* UCC2003 reveals type IVb tight adherence (Tad) pili as an essential and conserved host-colonization factor. *Proc Natl Acad Sci U S A* 2011;108:11217-22. DOI PubMed PMC
89. Nishiyama K, Takaki T, Sugiyama M, et al. Extracellular vesicles produced by *Bifidobacterium longum* export mucin-binding proteins. *Appl Environ Microbiol* 2020;86:e01464-20. DOI PubMed PMC
90. Mantziari A, Mannila E, Collado MC, Salminen S, Gómez-Gallego C. Exogenous polyamines influence in vitro microbial adhesion to human mucus according to the age of mucus donor. *Microorganisms* 2021;9:1239. DOI PubMed PMC
91. Tuo Y, Song X, Song Y, et al. Screening probiotics from Lactobacillus strains according to their abilities to inhibit pathogen adhesion and induction of pro-inflammatory cytokine IL-8. *J Dairy Sci* 2018;101:4822-9. DOI
92. Monteagudo-Mera A, Rastall RA, Gibson GR, Charalampopoulos D, Chatzifragkou A. Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. *Appl Microbiol Biotechnol* 2019;103:6463-72. DOI PubMed PMC
93. Klaenhammer TR, Kullen MJ. Selection and design of probiotics. *Int J Food Microbiol* 1999;50:45-57. DOI PubMed
94. Tuomola E, Crittenden R, Playne M, Isolauri E, Salminen S. Quality assurance criteria for probiotic bacteria. *Am J Clin Nutr* 2001;73:393S-8S. DOI PubMed
95. Engevik MA, Luck B, Visuthranukul C, et al. Human-derived *Bifidobacterium dentium* modulates the mammalian serotonergic system and gut-brain axis. *Cell Mol Gastroenterol Hepatol* 2021;11:221-48. DOI PubMed PMC
96. Engevik MA, Herrmann B, Ruan W, et al. *Bifidobacterium dentium*-derived y-glutamylcysteine suppresses ER-mediated goblet cell stress and reduces TNBS-driven colonic inflammation. *Gut Microbes* 2021;13:1-21. DOI PubMed PMC
97. Fukuda S, Toh H, Hase K, et al. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* 2011;469:543-7. DOI
98. Yu JY, He XL, Puthiyakunnon S, et al. Mucin2 is required for probiotic agents-mediated blocking effects on meningitic e. coli-induced pathogenicities. *J Microbiol Biotechnol* 2015;25:1751-60. DOI
99. Rivière A, Gagnon M, Weckx S, Roy D, De Vuyst L. Mutual cross-feeding interactions between *Bifidobacterium longum* subsp. *longum* NCC2705 and *eubacterium rectale* ATCC 33656 explain the bifidogenic and butyrogenic effects of arabinoxylan oligosaccharides. *Appl Environ Microbiol* 2015;81:7767-81. DOI PubMed PMC
100. Bunesova V, Lacroix C, Schwab C. Mucin cross-feeding of infant bifidobacteria and eubacterium hallii. *Microb Ecol* 2018;75:228-38. DOI PubMed
101. Schwab C, Ruscheweyh HJ, Bunesova V, Pham VT, Beerenwinkel N, Lacroix C. Trophic interactions of infant Bifidobacteria and eubacterium hallii during L-Fucose and fucosyllactose degradation. *Front Microbiol* 2017;8:95. DOI PubMed PMC
102. Rios-Covian D, Gueimonde M, Duncan SH, Flint HJ, de los Reyes-Gavilan CG. Enhanced butyrate formation by cross-feeding

between *Faecalibacterium prausnitzii* and *Bifidobacterium adolescentis*. *FEMS Microbiol Lett* 2015;362:fnv176. DOI PubMed

103. Glover JS, Ticer TD, Engevik MA. Characterizing the mucin-degrading capacity of the human gut microbiota. *Sci Rep* 2022;12:8456. DOI PubMed PMC
104. Fang J, Wang H, Zhou Y, Zhang H, Zhou H, Zhang X. Slimy partners: the mucus barrier and gut microbiome in ulcerative colitis. *Exp Mol Med* 2021;53:772-87. DOI PubMed PMC
105. Crouch LI, Liberato MV, Urbanowicz PA, et al. Prominent members of the human gut microbiota express endo-acting O-glycanases to initiate mucin breakdown. *Nat Commun* 2020;11:4017. DOI
106. Raba G, Luis AS. Mucin utilization by gut microbiota: recent advances on characterization of key enzymes. *Essays Biochem* 2023;67:345-53. DOI PubMed PMC
107. Marcobal A, Southwick AM, Earle KA, Sonnenburg JL. A refined palate: bacterial consumption of host glycans in the gut. *Glycobiology* 2013;23:1038-46. DOI PubMed PMC
108. Turroni F, Bottacini F, Foroni E, et al. Genome analysis of *Bifidobacterium bifidum* PRL2010 reveals metabolic pathways for host-derived glycan foraging. *Proc Natl Acad Sci U S A* 2010;107:19514-9. DOI PubMed PMC
109. Ruiz L, Gueimonde M, Couté Y, et al. Evaluation of the ability of *Bifidobacterium longum* to metabolize human intestinal mucus. *FEMS Microbiol Lett* 2011;314:125-30. DOI
110. Katayama T, Sakuma A, Kimura T, et al. Molecular cloning and characterization of *Bifidobacterium bifidum* 1,2-alpha-L-fucosidase (AfcA), a novel inverting glycosidase (glycoside hydrolase family 95). *J Bacteriol* 2004;186:4885-93. DOI PubMed PMC
111. Egan M, Motherway MO, Kilcoyne M, et al. Cross-feeding by *Bifidobacterium breve* UCC2003 during co-cultivation with *Bifidobacterium bifidum* PRL2010 in a mucin-based medium. *BMC Microbiol* 2014;14:282. DOI PubMed PMC
112. Takada H, Katoh T, Sakanaka M, Odamaki T, Katayama T. GH20 and GH84 β-*N*-acetylglucosaminidases with different linkage specificities underpin mucin O-glycan breakdown capability of *Bifidobacterium bifidum*. *J Biol Chem* 2023;299:104781. DOI PubMed PMC
113. Gibson GR, Beatty ER, Wang X, Cummings JH. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* 1995;108:975-82. DOI PubMed
114. Zhou JS, Gopal PK, Gill HS. Potential probiotic lactic acid bacteria *Lactobacillus rhamnosus* (HN001), *Lactobacillus acidophilus* (HN017) and *Bifidobacterium lactis* (HN019) do not degrade gastric mucin in vitro. *Int J Food Microbiol* 2001;63:81-90. DOI
115. Subramani DB, Johansson ME, Dahlén G, Hansson GC. *Lactobacillus* and *Bifidobacterium* species do not secrete protease that cleaves the MUC2 mucin which organises the colon mucus. *Benef Microbes* 2010;1:343-50. DOI
116. Derrien M, van Passel MW, van de Bovenkamp JH, Schipper RG, de Vos WM, Dekker J. Mucin-bacterial interactions in the human oral cavity and digestive tract. *Gut Microbes* 2010;1:254-68. DOI PubMed PMC
117. Norin KE, Gustafsson BE, Lindblad BS, Midtvedt T. The establishment of some microflora associated biochemical characteristics in feces from children during the first years of life. *Acta Paediatr Scand* 1985;74:207-12. DOI PubMed
118. Midtvedt AC, Carlstedt-Duke B, Midtvedt T. Establishment of a mucin-degrading intestinal microflora during the first two years of human life. *J Pediatr Gastroenterol Nutr* 1994;18:321-6. DOI
119. Karav S, Casaburi G, Frese SA. Reduced colonic mucin degradation in breastfed infants colonized by *Bifidobacterium longum* subsp. *infantis* EVC001. *FEBS Open Bio* 2018;8:1649-57. DOI PubMed PMC
120. Klaassens ES, Boesten RJ, Haarman M, et al. Mixed-species genomic microarray analysis of fecal samples reveals differential transcriptional responses of bifidobacteria in breast- and formula-fed infants. *Appl Environ Microbiol* 2009;75:2668-76. DOI PubMed PMC
121. Belzer C. Nutritional strategies for mucosal health: the interplay between microbes and mucin glycans. *Trends Microbiol* 2022;30:13-21. DOI PubMed
122. Desai MS, Seekatz AM, Koropatkin NM, et al. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* 2016;167:1339-53.e21. DOI PubMed PMC
123. Schroeder BO, Birchenough GMH, Ståhlman M, et al. Bifidobacteria or fiber protects against diet-induced microbiota-mediated colonic mucus deterioration. *Cell Host Microbe* 2018;23:27-40.e7. DOI PubMed PMC
124. Yoshihara T, Oikawa Y, Kato T, et al. The protective effect of *Bifidobacterium bifidum* G9-1 against mucus degradation by *Akkermansia muciniphila* following small intestine injury caused by a proton pump inhibitor and aspirin. *Gut Microbes* 2020;11:1385-404. DOI PubMed PMC
125. Mangin I, Dossou-Yovo F, Lévêque C, et al. Oral administration of viable *Bifidobacterium pseudolongum* strain patronus modified colonic microbiota and increased mucus layer thickness in rat. *FEMS Microbiol Ecol* 2018;94:fiy177. DOI
126. 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. *Am J Physiol Gastrointest Liver Physiol* 2007;292:G315-22. DOI PubMed
127. Burger-van Paassen N, Vincent A, Puiman PJ, et al. The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: implications for epithelial protection. *Biochem J* 2009;420:211-9. DOI
128. Romond MB, Bezirtzoglou E, Romond C. Colonization of the Murine Gut by *Bifidobacterium bifidum* and *Clostridium perfringens* during ageing. *Microb Ecol Health Dis* 1998;10:91-4. DOI
129. Romond MB, Haddou Z, Mielcareck C, Romond C. Bifidobacteria and human health: regulatory effect of indigenous bifidobacteria on Escherichia coli intestinal colonization. *Anaerobe* 1997;3:131-6. DOI PubMed
130. Toumi R, Abdelouhab K, Rafa H, et al. Beneficial role of the probiotic mixture ultrabiotique on maintaining the integrity of intestinal

mucosal barrier in DSS-induced experimental colitis. *Immunopharmacol Immunotoxicol* 2013;35:403-9. DOI

131. Crespo I, San-Miguel B, Prause C, et al. Glutamine treatment attenuates endoplasmic reticulum stress and apoptosis in TNBS-induced colitis. *PLoS One* 2012;7:e50407. DOI PubMed PMC
132. Takagi T, Homma T, Fujii J, et al. Elevated ER stress exacerbates dextran sulfate sodium-induced colitis in PRDX4-knockout mice. *Free Radic Biol Med* 2019;134:153-64. DOI
133. Ardite E, Sans M, Panés J, Romero FJ, Piqué JM, Fernández-Checa JC. Replenishment of glutathione levels improves mucosal function in experimental acute colitis. *Lab Invest* 2000;80:735-44. DOI PubMed
134. Grisham MB, Volkmer C, Tso P, Yamada T. Metabolism of trinitrobenzene sulfonic acid by the rat colon produces reactive oxygen species. *Gastroenterology* 1991;101:540-7. DOI PubMed
135. Brandl K, Rutschmann S, Li X, et al. Enhanced sensitivity to DSS colitis caused by a hypomorphic *Mbtps1* mutation disrupting the ATF6-driven unfolded protein response. *Proc Natl Acad Sci U S A* 2009;106:3300-5. DOI PubMed PMC
136. Kaser A, Lee AH, Franke A, et al. XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. *Cell* 2008;134:743-56. DOI PubMed PMC
137. Heazlewood CK, Cook MC, Eri R, et al. Aberrant mucin assembly in mice causes endoplasmic reticulum stress and spontaneous inflammation resembling ulcerative colitis. *PLoS Med* 2008;5:e54. DOI PubMed PMC
138. Zhao F, Edwards R, Dizon D, et al. Disruption of Paneth and goblet cell homeostasis and increased endoplasmic reticulum stress in *Agr2*-/- mice. *Dev Biol* 2010;338:270-9. DOI PubMed PMC
139. Bertolotti A, Wang XZ, Novoa I, et al. Increased sensitivity to dextran sodium sulfate colitis in IRE1β-deficient mice. *J Clin Invest* 2001;107:585-93. DOI PubMed PMC
140. Cao SS. Epithelial ER stress in Crohn's disease and ulcerative colitis. *Inflamm Bowel Dis* 2016;22:984-93. DOI PubMed
141. Cao SS, Zimmermann EM, Chuang BM, et al. The unfolded protein response and chemical chaperones reduce protein misfolding and colitis in mice. *Gastroenterology* 2013;144:989-1000.e6. DOI PubMed PMC
142. Akiyama T, Oishi K, Wullaert A. Bifidobacteria prevent tunicamycin-induced endoplasmic reticulum stress and subsequent barrier disruption in human intestinal epithelial Caco-2 monolayers. *PLoS One* 2016;11:e0162448. DOI PubMed PMC
143. Allen J, Bradley RD. Effects of oral glutathione supplementation on systemic oxidative stress biomarkers in human volunteers. *J Altern Complement Med* 2011;17:827-33. DOI PubMed PMC
144. Chakravarthi S, Jessop CE, Bulleid NJ. The role of glutathione in disulphide bond formation and endoplasmic-reticulum-generated oxidative stress. *EMBO Rep* 2006;7:271-5. DOI PubMed PMC
145. Holmes EW, Yong SL, Eiznhamer D, Keshavarzian A. Glutathione content of colonic mucosa: evidence for oxidative damage in active ulcerative colitis. *Dig Dis Sci* 1998;43:1088-95. DOI PubMed
146. Hwang C, Sinskey AJ, Lodish HF. Oxidized redox state of glutathione in the endoplasmic reticulum. *Science* 1992;257:1496-502. DOI PubMed
147. Quintana-Cabrera R, Fernandez-Fernandez S, Bobo-Jimenez V, et al. γ-Glutamylcysteine detoxifies reactive oxygen species by acting as glutathione peroxidase-1 cofactor. *Nat Commun* 2012;3:718. DOI PubMed PMC
148. Hasnain SZ, Tauro S, Das I, et al. IL-10 promotes production of intestinal mucus by suppressing protein misfolding and endoplasmic reticulum stress in goblet cells. *Gastroenterology* 2013;144:357-68.e9. DOI
149. Van der Sluis M, De Koning BA, De Bruijn AC, et al. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. *Gastroenterology* 2006;131:117-29. DOI
150. Tadesse S, Corner G, Dhima E, et al. MUC2 mucin deficiency alters inflammatory and metabolic pathways in the mouse intestinal mucosa. *Oncotarget* 2017;8:71456-70. DOI PubMed PMC
151. Bergstrom K, Fu J, Johansson MEV, et al. Core 1- and 3-derived O-glycans collectively maintain the colonic mucus barrier and protect against spontaneous colitis in mice. *Mucosal Immunol* 2017;10:91-103. DOI PubMed PMC
152. Maurel M, Obacz J, Avril T, et al. Control of anterior GRadient 2 (AGR2) dimerization links endoplasmic reticulum proteostasis to inflammation. *EMBO Mol Med* 2019;11:e10120. DOI PubMed PMC
153. Park SW, Zhen G, Verhaeghe C, et al. The protein disulfide isomerase AGR2 is essential for production of intestinal mucus. *Proc Natl Acad Sci U S A* 2009;106:6950-5. DOI PubMed PMC
154. Trabucchi E, Mukenge S, Baratti C, Colombo R, Fregoni F, Montorsi W. Differential diagnosis of Crohn's disease of the colon from ulcerative colitis: ultrastructure study with the scanning electron microscope. *Int J Tissue React* 1986;8:79-84. PubMed
155. Hanski C, Born M, Foss HD, et al. Defective post-transcriptional processing of MUC2 mucin in ulcerative colitis and in Crohn's disease increases detectability of the MUC2 protein core. *J Pathol* 1999;188:304-11. PubMed
156. Wenzel UA, Magnusson MK, Rydström A, et al. Spontaneous colitis in Muc2-deficient mice reflects clinical and cellular features of active ulcerative colitis. *PLoS One* 2014;9:e100217. DOI PubMed PMC
157. Tytgat KM, van der Wal JW, Einerhand AW, Büller HA, Dekker J. Quantitative analysis of MUC2 synthesis in ulcerative colitis. *Biochem Biophys Res Commun* 1996;224:397-405. DOI PubMed
158. Pullan RD, Thomas GA, Rhodes M, et al. Thickness of adherent mucus gel on colonic mucosa in humans and its relevance to colitis. *Gut* 1994;35:353-9. DOI PubMed PMC
159. Raouf AH, Tsai HH, Parker N, Hoffman J, Walker RJ, Rhodes JM. Sulphation of colonic and rectal mucin in inflammatory bowel disease: reduced sulphation of rectal mucus in ulcerative colitis. *Clin Sci* 1992;83:623-6. DOI PubMed
160. Larsson JM, Karlsson H, Crespo JG, et al. Altered O-glycosylation profile of MUC2 mucin occurs in active ulcerative colitis and is

associated with increased inflammation. *Inflamm Bowel Dis* 2011;17:2299-307. DOI

161. Antoni L, Nuding S, Wehkamp J, Stange EF. Intestinal barrier in inflammatory bowel disease. *World J Gastroenterol* 2014;20:1165-79. DOI PubMed PMC
162. Johansson ME, Gustafsson JK, Holmén-Larsson J, et al. Bacteria penetrate the normally impenetrable inner colon mucus layer in both murine colitis models and patients with ulcerative colitis. *Gut* 2014;63:281-91. DOI PubMed PMC
163. Zhang K, Kaufman RJ. From endoplasmic-reticulum stress to the inflammatory response. *Nature* 2008;454:455-62. DOI PubMed PMC
164. Shkoda A, Ruiz PA, Daniel H, et al. Interleukin-10 blocked endoplasmic reticulum stress in intestinal epithelial cells: impact on chronic inflammation. *Gastroenterology* 2007;132:190-207. DOI
165. Eri RD, Adams RJ, Tran TV, et al. An intestinal epithelial defect conferring ER stress results in inflammation involving both innate and adaptive immunity. *Mucosal Immunol* 2011;4:354-64. DOI PubMed PMC
166. Cui QY, Tian XY, Liang X, et al. *Bifidobacterium bifidum* relieved DSS-induced colitis in mice potentially by activating the aryl hydrocarbon receptor. *Food Funct* 2022;13:5115-23. DOI
167. Wang H, Fan C, Zhao Z, Zhai Z, Hao Y. Anti-inflammatory effect of Bifidobacterium animalis subsp. lactis A6 on DSS-induced colitis in mice. *J Appl Microbiol* 2022;133:2063-73. DOI
168. Lee SY, Lee BH, Park JH, Park MS, Ji GE, Sung MK. Bifidobacterium bifidum BGN4 paraprobiotic supplementation alleviates experimental colitis by maintaining gut barrier and suppressing nuclear factor kappa B activation signaling molecules. *J Med Food* 2022;25:146-57. DOI PubMed
169. Yan S, Yang B, Ross RP, et al. *Bifidobacterium longum* subsp. *longum* YS108R fermented milk alleviates DSS induced colitis via anti-inflammation, mucosal barrier maintenance and gut microbiota modulation. *J Funct Foods* 2020;73:104153. DOI
170. Park IS, Kim JH, Yu J, et al. *Bifidobacterium breve* CBT BR3 is effective at relieving intestinal inflammation by augmenting goblet cell regeneration. *J Gastroenterol Hepatol* 2023;38:1346-54. DOI
171. Singh S, Bhatia R, Khare P, et al. Anti-inflammatory *Bifidobacterium strains* prevent dextran sodium sulfate induced colitis and associated gut microbial dysbiosis in mice. *Sci Rep* 2020;10:18597. DOI
172. Chen Y, Jin Y, Stanton C, et al. Alleviation effects of *Bifidobacterium breve* on DSS-induced colitis depends on intestinal tract barrier maintenance and gut microbiota modulation. *Eur J Nutr* 2021;60:369-87. DOI
173. Chen Y, Zhang L, Hong G, et al. Probiotic mixtures with aerobic constituent promoted the recovery of multi-barriers in DSS-induced chronic colitis. *Life Sci* 2020;240:117089. DOI
174. Niu MM, Guo HX, Cai JW, Meng XC. *Bifidobacterium breve* alleviates DSS-induced colitis in mice by maintaining the mucosal and epithelial barriers and modulating gut microbes. *Nutrients* 2022;14:3671. DOI
175. Zhou L, Liu D, Xie Y, Yao X, Li Y. *Bifidobacterium infantis* induces protective colonic PD-L1 and foxp3 regulatory T cells in an acute murine experimental model of inflammatory bowel disease. *Gut Liver* 2019;13:430-9. DOI
176. Zuo L, Yuan KT, Yu L, Meng QH, Chung PC, Yang DH. *Bifidobacterium infantis* attenuates colitis by regulating T cell subset responses. *World J Gastroenterol* 2014;20:18316-29. DOI
177. Chen M, Liu C, Dai M, Wang Q, Li C, Hung W. *Bifidobacterium lactis* BL-99 modulates intestinal inflammation and functions in zebrafish models. *PLoS One* 2022;17:e0262942. DOI
178. Roselli M, Finamore A, Nuccitelli S, et al. Prevention of TNBS-induced colitis by different Lactobacillus and Bifidobacterium strains is associated with an expansion of gammadeltaT and regulatory T cells of intestinal intraepithelial lymphocytes. *Inflamm Bowel Dis* 2009;15:1526-36. DOI PubMed
179. Martín R, Laval L, Chain F, et al. Bifidobacterium animalis ssp. lactis CNCM-I2494 restores gut barrier permeability in chronically low-grade inflamed mice. *Front Microbiol* 2016;7:608. DOI PubMed PMC
180. Yue Y, Wang Y, Xie Q, et al. *Bifidobacterium bifidum* E3 combined with *Bifidobacterium longum subsp. infantis* E4 improves LPS-induced intestinal injury by inhibiting the TLR4/NF-κB and MAPK signaling pathways *in vivo*. *J Agric Food Chem* 2023;71:8915-30. DOI PubMed
181. Khailova L, Dvorak K, Arganbright KM, et al. Bifidobacterium bifidum improves intestinal integrity in a rat model of necrotizing enterocolitis. *Am J Physiol Gastrointest Liver Physiol* 2009;297:G940-9. DOI PubMed PMC
182. Lueschow SR, Boly TJ, Frese SA, et al. *Bifidobacterium longum* subspecies *infantis* strain EVC001 decreases neonatal murine necrotizing enterocolitis. *Nutrients* 2022;14:495. DOI PubMed PMC
183. Bergmann KR, Liu SX, Tian R, et al. Bifidobacteria stabilize claudins at tight junctions and prevent intestinal barrier dysfunction in mouse necrotizing enterocolitis. *Am J Pathol* 2013;182:1595-606. DOI
184. Braga TD, da Silva GAP, de Lira PIC, de Carvalho Lima M. Efficacy of Bifidobacterium breve and Lactobacillus casei oral supplementation on necrotizing enterocolitis in very-low-birth-weight preterm infants: a double-blind, randomized, controlled trial. *Am J Clin Nutr* 2011;93:81-6. DOI PubMed
185. Kawahara T, Makizaki Y, Oikawa Y, et al. Oral administration of Bifidobacterium bifidum G9-1 alleviates rotavirus gastroenteritis through regulation of intestinal homeostasis by inducing mucosal protective factors. *PLoS One* 2017;12:e0173979. DOI
186. Weng M, Ganguli K, Zhu W, Shi HN, Walker WA. Conditioned medium from Bifidobacteria infantis protects against Cronobacter sakazakii-induced intestinal inflammation in newborn mice. *Am J Physiol Gastrointest Liver Physiol* 2014;306:G779-87. DOI
187. Engevik KA, Matthis AL, Montrose MH, Aihara E. Organoids as a model to study infectious disease. In: Medina C, López-baena FJ, editors. Host-Pathogen Interactions. New York: Springer; 2018. p. 71-81. DOI

188. Engevik MA, Banks LD, Engevik KA, et al. Rotavirus infection induces glycan availability to promote ileum-specific changes in the microbiome aiding rotavirus virulence. *Gut Microbes* 2020;11:1324-47. DOI

189. Engevik MA, Danhof HA, Chang-Graham AL, et al. Human intestinal enteroids as a model of *Clostridioides difficile*-induced enteritis. *Am J Physiol Gastrointest Liver Physiol* 2020;318:G870-88. DOI