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Review

Review

How microbial glycosyl hydrolase activity in the gut mucosa initiates microbial cross-feeding

Maryse D Berkhout[2], Caroline M Plugge[2], and Clara Belzer[1,2]

[2]Laboratory of Microbiology, Wageningen University and Research, Stippeneng 4, Wageningen 6708WE, The Netherlands

[1]To whom correspondence should be addressed: e-mail: clara.belzer@wur.nl

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Abstract

The intestinal epithelium is protected from direct contact with gut microbes by a mucus layer. This mucus layer consists of secreted mucin glycoproteins. The outer mucus layer in the large intestine forms a niche that attracts specific gut microbiota members of which several gut commensals can degrade mucin. Mucin glycan degradation is a complex process that requires a broad range of glycan degrading enzymes, as mucin glycans are intricate and diverse molecules. Consequently, it is hypothesized that microbial mucin breakdown requires concerted action of various enzymes in a network of multiple resident microbes in the gut mucosa. This review investigates the evolutionary relationships of microbial carbohydrate-active enzymes that are potentially involved in mucin glycan degradation and focuses on the role that microbial enzymes play in the degradation of gut mucin glycans in microbial cross-feeding and syntrophic interactions.

Key words: CAZymes, glycosidases, gut microbiota, mucin, syntrophic interactions

Introduction

A subset of the gut microbiota is able to degrade host-derived mucin glycans. Secreted mucin glycoproteins are components of the mucus layer that covers epithelia in harsh bodily environments, such as the respiratory system, the gastrointestinal tract and epithelial surfaces of several organs (Figure 1A) (Linden et al. 2008). In the intestine, the mucus layer forms a physical barrier between the host epithelium and the intestinal lumen. The mucus layer covering the colon epithelium entails two layers. The inner layer consists of sheets of firmly attached mucin networks and is thus virtually impenetrable to gut bacteria. On the other hand, the outer layer is loosely attached and provides a niche for a portion of the gut microbiota (Figure 1B) (Johansson et al. 2008; Paone and Cani 2020). Furthermore, fecal pellets are coated by a mucus layer, encapsulating fecal material and associated microbiota (Bergstrom et al. 2020).

Some members of the commensal gut microbiota hydrolyze mucin glycan moieties and utilize them as a source of carbon, nitrogen, sulfate and energy and metabolize them to short-chain fatty acids (SCFAs). This ability could explain their presence in the outer mucus layer, which forms a distinct niche within the gut (Li et al. 2015). Mucin glycans are complex molecules that are O-linked to the mucin protein backbone.

The synthesis of an *O*-linked mucin glycan starts with the transfer of *N*-acetylgalactosamine (GalNAc) to a serine or threonine residue on the mucin protein backbone, establishing the base of one of the eight known glycan core structures (Brockhausen et al. 2009). In the colon, core 3 is the predominant core structure of secreted mucin, but other cores occur as well (Figure 1C) (Capon et al. 2001; Robbe et al. 2004; Brockhausen et al. 2009; Xia 2010). Subsequently, the core structures can be considerably extended with galactose (Gal) or *N*-acetylglucosamine (GlcNAc) units and can be terminated with GalNAc, sulfate, fucose or sialic acid (Neu5Ac) moieties (Figure 1D) (Podolsky 1985; Brockhausen et al. 2009; Tailford, Owen, et al. 2015). The variety of possible structures strongly contributes to the large glycan diversity and complexity (Corfield 2015; Tailford, Crost, et al. 2015). Therefore, the digestion of mucin glycans is a complex process, which requires an extensive array of carbohydrate-active enzymes (CAZymes) encoded within the microbiome. To achieve

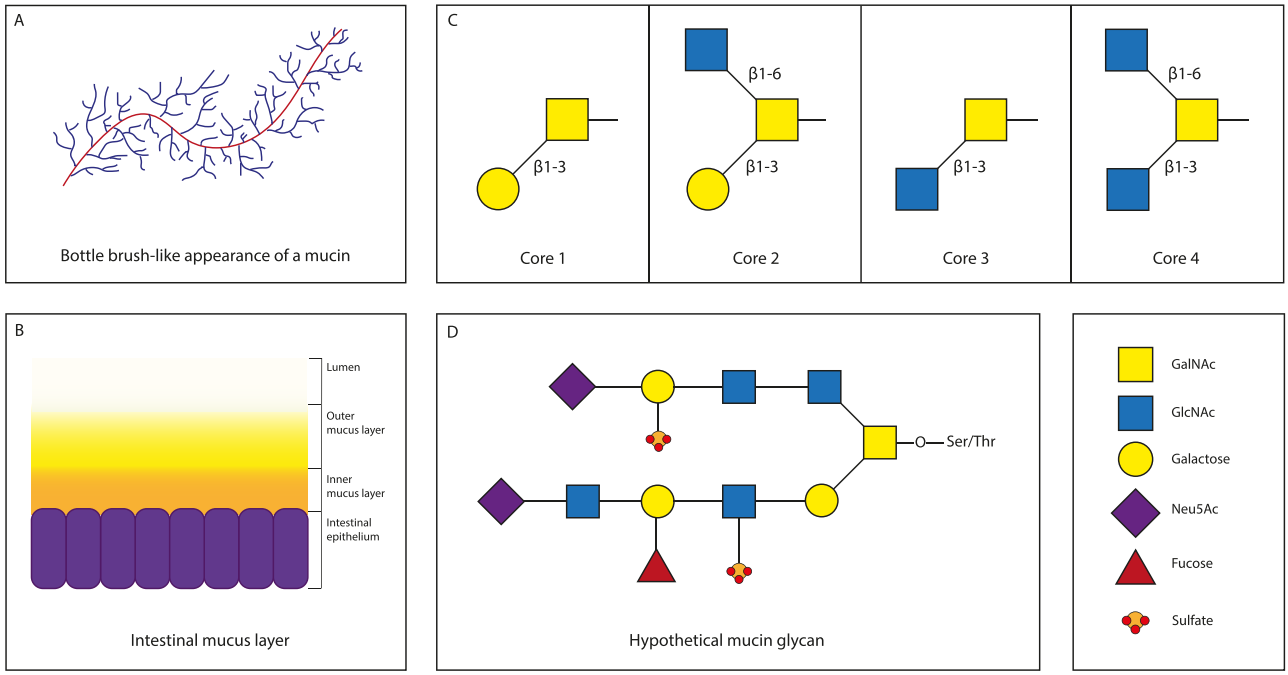

Fig. 1. The predominant mucin glycan core structures found in the human gut and a hypothetical mucin glycan. (**A**) The bottle brush-like structure of a mucin glycoprotein: A secreted MUC2 mucin consists of a protein core (red) and branched, complex glycan extensions (blue). (**B**) The host intestinal epithelial cells secrete mucin glycans that form a mucus layer to avoid direct contact with bacteria. The mucus consists of an inner layer, which is virtually impenetrable to bacteria, and an outer layer, which forms a niche for specific microbiota. (**C**) The predominant core structures in the gut. Core 1 consists of galactose that is β1–3-linked to GalNAc. Core 2 contains an additional GlcNAc that is β1–6-linked to GalNAc. Core 3 consists of GlcNAc β1–3 linked to GalNAc. Core 3 can subsequently be extended to core 4 through the β1–6 linkage of another GlcNAc to the GalNAc. (**D**) A hypothetical mucin glycan that consists of core 2, is extended by several GlcNAc and galactose subunits, and is terminated by sialic acid (Neu5Ac), sulfate (SO_3) and fucose. Mucin glycans are always O-linked to a serine or threonine residue on the protein backbone.

degradation, CAZymes from various members of the gut microbiota need to act sequentially and concertedly (El Kaoutari et al. 2013; Tailford, Crost, et al. 2015; Belzer 2021).

Mucin glycan degrading bacteria initiate microbial interactions in the gut. Gut bacteria seldom produce a complete set of glycosidases that are required for the degradation of mucin glycans into their constituent oligo- and monosaccharides and their downstream metabolism. Therefore, metabolizing mucin glycans in the gut relies on the cooperative action of several microbial species. Cross-feeding within a microbial community involves the exchange of compounds between species and results in increased growth of the community (D'Souza et al. 2018). Gut microbiota that cannot hydrolyze mucin glycans directly benefit from the metabolites of mucin glycan degradation by other microbiota. This can arise through metabolite cross-feeding or substrate cross-feeding. During metabolite cross-feeding, a partner organism uses the end products of the mucin glycan degrader's metabolism. For example, several residents of the gut mucosa convert acetate produced by mucin glycan degraders to butyrate (Mahowald et al. 2009; Wrzosek et al. 2013; Belzer et al. 2017; Bunesova et al. 2018). During substrate cross-feeding, one organism releases extracellular enzymes to hydrolyze mucin glycans to mono-, di- and oligosaccharides that can be taken up into the cell not only by the organism itself but also by other gut symbionts (Ríos-Covián et al. 2016; Smith et al. 2019b). For example, *Bifidobacterium breve* can use sialic acid that is released from mucin glycans by *Bifidobacterium bifidum* (Nishiyama et al. 2018).

Mucin glycan-derived oligo- and monosaccharides can be fermented to SCFAs. The main products of carbohydrate fermentation in the intestine are the beneficial SCFAs, including butyrate, propionate and acetate and gases, which include hydrogen (H_2), methane (CH_4), hydrogen sulfide (H_2S) and carbon dioxide (CO_2) (Flint et al. 2012; El Kaoutari et al. 2013; Morrison and Preston 2016; Hylemon et al. 2018). SCFAs are fatty acids containing six or less carbon atoms (Tan et al. 2014). SCFAs confer a broad range of beneficial effects to the host. For example, butyrate is the main energy source of colonocytes that maintain the integrity of the epithelial lining of the gut (Koropatkin et al. 2014; Morrison and Preston 2016). Therefore, production of butyrate near the epithelium by mucin layer colonizers is favorable (Ouwerkerk et al. 2013). Additionally, acetate and propionate play a role in the host cell lipid metabolism and glucose homeostasis (Koropatkin et al. 2014; Morrison and Preston 2016). Furthermore, SCFAs have anti-inflammatory and anticarcinogenic effects and show antimicrobial activity against intestinal pathogens (Tan et al. 2014; Lamas et al. 2019). Thus, mucin degradation by bacterial communities can enable growth of bacteria that deliver beneficial metabolites to the host.

This review discusses the degradation of gut mucin glycans as a complex process that occurs in an ecological network of commensal gut microbiota that together produce a wide variety of CAZymes. First, an overview of primary mucin glycan degrading commensals is provided. Subsequently, the CAZymes that are potentially involved in mucin glycan degradation in a consortium of primary mucin degraders (PMDs) are analyzed and the evolutionary relationships between mucin degrading CAZymes are investigated. Finally, the cross-feeding interactions between mucin glycan degraders and their mucosal partners are reviewed.

Mucin glycan degraders

A subset of the commensal human gut microbiota that inhabits the intestinal mucus layer can degrade mucin glycans. Genome sequenced bacterial species that are capable of mucin degradation and derive from human gut samples are listed in Table I. However, due to the complexity of mucin glycans, only a limited number of species possess the enzymatic capacity to initiate mucin glycan degradation (Tailford, Crost, et al. 2015). Table I provides an overview of commensal gut bacteria that have been reported to degrade mucin glycans through experimental evidence. This list is limited to bacteria that release mono- and polysaccharides from mucin glycans and can use mucin glycans as their sole carbon and energy source. It does not include microorganisms that grow on mono- and polysaccharides that are released from mucin by other bacteria. Furthermore, primary mucin glycan degraders differ in their associations with health and disease states of the gut. Here, we focus on gut mucosal commensals that are not implicated as a pathogen. In this section, the known strategies of gut mucosal commensals to access mucin glycans are discussed.

Primary mucin glycan degraders, which are able to release parts of the mucin glycan molecule and can grow with mucin as sole carbon and energy source in vitro, have been found within the phyla Firmicutes, Bacteroidetes, Actinobacteria and Verrucomicrobia (Table II). They have different characteristics and strategies to access mucin glycans and differ in their preference for mucin glycans or other glycans that are available in the gut. For example, *Akkermansia muciniphila* that belongs to the phylum Verrucomicrobia is the textbook example of a specialist mucin glycan degrader (Ottman et al. 2017). Specialist species thrive in a more narrow niche as opposed to generalists, which can survive in a broader range of environmental conditions. Specialist mucin glycan degraders are well adapted to the mucus layer and prefer a mucin glycan-degrading lifestyle over the degradation of other carbohydrates. The adaptation to the mucus layer of *A. muciniphila* is exemplified by its tolerance to nanomolar concentrations of oxygen in the mucus layer and by its threonine autotrophy, as threonine is abundant in mucus (Ouwerkerk et al. 2016; Ottman et al. 2017). However, due to the similarity between human milk oligosaccharides (HMOs) and mucin glycan molecules, *A. muciniphila* is also able to degrade HMOs in early life (Kostopoulos et al. 2020). Another primary mucin glycan degrader is *Bacteroides thetaiotaomicron*, which in contrast to *A. muciniphila*, is a true generalist: Its glycan utilization capacity is encoded within 88 putative polysaccharide utilization loci (PULs) (Martens et al. 2008). As generally one PUL encodes all enzymes that degrade and import a specific polysaccharide, the PUL collection of *B. thetaiotaomicron* spans a broad range of dietary and host-derived glycans, allowing rapid adaptation to changes in the dynamic gut environment (Martens et al. 2008; Brown and Koropatkin 2021). Mucin glycans are among the glycans that *B. thetaiotaomicron* can use, but dietary glycans, such as homogalacturonan, levan and chondroitin sulfate, are its preferred substrate (Sonnenburg et al. 2005; Rogers et al. 2013; Pudlo et al. 2015).

CAZymes involved in mucin glycan degradation

The microbiotas residing in the mucus layer collectively express the CAZymes that are required for mucin glycan degradation (El Kaoutari et al. 2013). From Table II, it becomes clear that the exact repertoire of CAZymes that potentially targets mucin glycans differs per PMD species. This is in line with the current hypothesis that mucin glycan degradation occurs in a network of cooperating gut microorganisms (El Kaoutari et al. 2013; Lombard et al. 2014; Arike and Hansson 2016; Corfield 2018).

There are several CAZyme classes involved in glycan digestion. First of all, carbohydrate esterases (CEs) remove ester substituents from glycan chains to enable other CAZyme families to access the carbohydrate chains (El Kaoutari et al. 2013). The second CAZyme enzyme class is the glycosyl hydrolase (GH) family. GHs play a central role in the degradation of carbohydrates by cleaving specific glycosidic bonds through hydrolysis (El Kaoutari et al. 2013). Due to the large size and degree of polymerization of mucin glycans, the first steps of degradation take place extracellularly. Therefore, some GH enzymes are located on the cell surface or are secreted into the environment (Milani et al. 2015). Bacterial GHs play a major role in mucin glycan degradation. The polysaccharide lyase (PL) family forms the third CAZyme enzyme class. Like GHs, PLs are enzymes that cleave glycosidic bonds. However, PLs employ an elimination mechanism to cleave complex carbohydrates (El Kaoutari et al. 2013).

It is assumed that mucin glycan degradation is initiated by the hydrolysis of terminal structures, as terminal structures may prevent access to underlying carbohydrate structures. This includes hydrolysis of sialic acid, fucose and sulfate groups (Tailford, Crost, et al. 2015). For example, GH16 family members are unable to hydrolyze mucin glycan structures when they are decorated with sialic acid (Crouch et al. 2020). Most GHs involved in mucin degradation are hypothesized to be extracellular, as they possess a secretory signal sequence (Supplementary Table SI). The mucin sugars, which are released by extracellular enzymes of mucin degraders, can be utilized by the PMDs themselves or by other bacteria residing in the mucus layer (Arike and Hansson 2016). Sugars of interest can be imported into the cell through specific transporters (Koropatkin et al. 2014). As the wide array of enzymes that is required for the complete degradation of glycans is not encoded within a single bacterial species, members of the microbiota form a collaborative mucin-degrading network (Arike and Hansson 2016; Belzer 2021).

For this review, we assembled a consortium of PMDs in silico in order to provide more insights on cross-feeding actions at the gut mucosa. Members of the PMD consortium are human gut commensals that have been shown to degrade mucin glycans (Table I), and their enzymes are included in the CAZy database (Lombard et al. 2014). The enzymatic capacity of PMD can be found in Table II. The PMD consortium contains *A. muciniphila*, nine *Bacteroides* species, *R. gnavus* and *Ruminococcus torques* and four *Bifidobacterium* species. For each species, the number of genes encoding a GH family member that is implicated in mucin glycan degradation is listed (Table II) (Martens et al. 2008; Tailford, Crost, et al. 2015; Desai et al. 2016; Kosciow and Deppenmeier 2019; Crouch et al. 2020; Pruss et al. 2021).

Figure 2A shows the distribution of these GH family members in the PMD consortium. GH2 enzymes are the most common GH genes, with 229 copies within the PMD consortium, as opposed to GH85 and GH101 hexosaminidases and GH98 galactosidases with each only two representatives in this group of mucin glycan degraders. Approximately, one-third of the GH genes that are putatively involved in mucin glycan degradation in this consortium belong to a family that contains both galactosidases and hexosaminidases (270 genes) (Figure 2B). Genes that belong to a family that exclusively encodes galactosidases (182) or hexosaminidases (225) are also well represented (Figure 2B). Together, the consortium includes 677 genes

Table I. Primary mucin glycan degraders in the human gut; these bacteria have been shown to degrade mucin glycans in vitro

Species	Specialist /generalist	Strain	Type of mucin grown on	Enzymatic activities according to CAZy database	References
Akkermansia muciniphila	Specialist	ATCC BAA-835^T	pPGM and human MUC2	α-Galactosidases (GH27, GH36, GH97 and GH110); β-galactosidase (GH2, GH16 and GH35); α-GlcNAcase (GH89); β-GlcNAcase (GH18, GH20, GH84 and GH85); α-GalNAcase (GH36 and GH109); β-GalNAcase (GH20); fucosidase (GH29 and GH95); sialidase (GH33)	(Derrien et al. 2004) (Derrien 2007) (Png et al. 2010) (Ottman et al. 2017)
Bacteroides caccae	Generalist	VPI-3452A^T (ATCC 43185)	BSM, pPGM	α-Galactosidases (GH27, GH36, GH97 and GH110); β-galactosidase (GH2, GH16 and GH35); α-GlcNAcase (GH89); β-GlcNAcase (GH20 and GH84); α-GalNAcase (GH36 and GH109); β-GalNAcase (GH20); fucosidase (GH29 and GH95); sialidase (GH33)	(Salyers et al. 1977) (Sato et al. 2020)
Bacteroides clarus	Generalist	DSM 22519^T	pPGM	Not in CAZy	(Sato et al. 2020)
Bacteroides coprosuis	Generalist	DSM 18011^T	pPGM	Not in CAZy	(Sato et al. 2020)
Bacteroides faecis	Generalist	DSM 24798^T	pPGM	Not in CAZy	(Sato et al. 2020)
Bacteroides finegoldii	Generalist	DSM 17565^T	pPGM	Not in CAZy	(Sato et al. 2020)
Bacteroides fragilis	Generalist	VPI-2393 ATCC 25285^T (DSM 2151) ATCC 23745	BSM PGM, PCM, purified *O*-glycans from PGM	ATCC 25285: α-galactosidases (GH27, GH36, GH97 and GH110); β-galactosidase (GH2, GH16 and GH35); α-GlcNAcase (GH89); β-GlcNAcase (GH18, GH20 and GH84); α-GalNAcase (GH36 and GH109); β-GalNAcase (GH20); fucosidase (GH29 and GH95); sialidase (GH33) sulfatase	(Salyers et al. 1977) (Roberton and Stanley 1982) (Marcobal et al. 2011) (Sato et al. 2020) (Praharaj et al. 2018)
Bacteroides gallinarum	Generalist	DSM 18171^T	pPGM	Not in CAZy	(Sato et al. 2020)
Bacteroides helcogenes	Generalist	DSM 20613^T	pPGM	α-Galactosidases (GH27, GH36, GH97 and GH110); β-galactosidase (GH2, GH16 and GH35); α-GlcNAcase (GH89); β-GlcNAcase (GH18, GH20 and GH84); α-GalNAcase (GH36 and GH109); β-GalNAcase (GH20); fucosidase (GH29 and GH95); sialidase (GH33)	(Sato et al. 2020)
Bacteroides intestinalis	Generalist	DSM 17393^T	pPGM	α-Galactosidases (GH27, GH36 and GH97); β-galactosidase (GH2, GH16, GH35 and GH42); α-GlcNAcase (GH89); β-GlcNAcase (GH18 and GH20); α-GalNAcase (GH36 and GH109); β-GalNAcase (GH20); fucosidase (GH29 and GH95); sialidase (GH33)	(Sato et al. 2020)

(*Continued*)

Table I. Continued

Species	Specialist /generalist	Strain	Type of mucin grown on	Enzymatic activities according to CAZy database	References
Bacteroides nordii	Generalist	DSM 18764^T	pPGM	Not in CAZy	(Sato et al. 2020)
Bacteroides ovatus	Generalist	DSM 1896^T	pPGM	α-Galactosidases (GH27, GH36 and GH97); β-galactosidase (GH2, GH16, GH35, GH42 and GH98); α-GlcNAcase (GH89); β-GlcNAcase (GH18 and GH20); α-GalNAcase (GH36 and GH109); β-GalNAcase (GH20); fucosidase (GH29 and GH95); sialidase (GH33); sulfatase	(Sato et al. 2020) (Salyers et al. 1977)
Bacteroides stercoris	Generalist	DSM 19555^T	pPGM	Not in CAZy	(Sato et al. 2020)
Bacteroides thetaiotaomicron	Generalist	ATCC 29148^T (DSM 2079)	Glycans from PGM, pPGM	α-Galactosidases (GH27, GH36, GH97 and GH110); β-galactosidase (GH2, GH16, GH35 and GH42); α-GlcNAcase (GH89); β-GlcNAcase (GH18, GH20 and GH84); α-GalNAcase (GH36 and GH109); β-GalNAcase (GH20); fucosidase (GH29 and GH95); sialidase (GH33); sulfatase	(Martens et al. 2008) (Benjdia et al. 2011) (Sato et al. 2020)
Bacteroides uniformis	Generalist	DSM 6597^T	pPGM	Not in CAZy	(Sato et al. 2020)
Bacteroides xylanisolvens	Generalist	DSM 18836^T (XB1A)	pPGM	α-Galactosidase (GH27, GH36 and GH97); β-galactosidase (GH2, GH16, GH35 and GH42); α-GlcNAcase (GH89); β-GlcNAcase (GH18 and GH20); α-GalNAcase (GH36 and GH109); β-GalNAcase (GH20); fucosidase (GH29 and GH95); sialidase (GH33)	(Sato et al. 2020)
Bacteroides massiliensis (*Phocaeicola massiliensis*)	Generalist	DSM 17679^T	Porcine mucin *O*-glycans	Not in CAZy	(Pudlo et al. 2015)
Phocaeicola vulgatus (*Bacteroides vulgatus*)	Generalist	VIII-271F ATCC 8482^T	ppPGM pPGM	α-Galactosidase (GH27, GH36, GH97 and GH110); β-galactosidase (GH2, GH16, GH35 and GH42); α-GlcNAcase (GH89); β-GlcNAcase (GH18, GH20 and GH84); α-GalNAcase (GH36 and GH109); β-GalNAcase (GH20); fucosidase (GH29 and GH95); sialidase (GH33)	(Hoskins et al. 1992) (Png et al. 2010) (Sato et al. 2020)
Barnesiella intestinihominis	Specialist	DSM 21032^T	pPGM	Not in CAZy	(Desai et al. 2016)
Ruminococcus gnavus	Specialist	ATCC 35913	ppPGM and pPGM		(Hoskins et al. 1985) (Corfield et al. 1992) (Crost et al. 2016)

(*Continued*)

Table I. Continued

Species	Specialist /generalist	Strain	Type of mucin grown on	Enzymatic activities according to CAZy database	References
		ATCC 29149^T	pPGM and human MUC2	α-Galactosidase (GH36); β-galactosidase (GH2, GH42 and GH98); β-GlcNAcase (GH18); α-GalNAcase (GH36); fucosidase (GH29 and GH95); sialidase (GH33)	(Png et al. 2010) (Crost et al. 2013)
Ruminococcus torques	Specialist	ATCC 35915	ppPGM		(Hoskins et al. 1985) (Hoskins et al. 1992) (Corfield et al. 1992)
		VIII-239	ppPGM		(Hoskins et al. 1985) (Hoskins et al. 1992) (Corfield et al. 1992)
		ATCC 27756^T	pPGM and human MUC2		(Png et al. 2010)
Bifidobacterium bifidum	Generalist	VIII-210	ppPGM		(Hoskins et al. 1985) (Hoskins et al. 1992) (Corfield et al. 1992)
		24/25 strains	PGM		(Crociani et al. 1994)
		D119 and L22	PGM		(Ruas-Madiedo et al. 2008)
		PRL2010, A8, 324B, 156B, D119 and DSM 20456^T (ATCC 29521), 85B (limited growth on mucin) and L22 (limited growth on mucin)	PGM	DSM 20456^T: α-galactosidase (GH36, GH110); β-galactosidase (GH2, GH16 and GH42); α-GlcNAcase (GH89); β-GlcNAcase (GH20 and GH84); α-GalNAcase (GH36, GH101 and GH129); β-GalNAcase (GH20); fucosidase (GH29 and GH95); Sialidase (GH33)	(Turroni et al. 2010)
Bifidobacterium breve	Generalist	NCIMB8807 (UCC2003)	PGM	α-Galactosidase (GH36); β-galactosidase (GH2, GH35 and GH42); β-GlcNAcase (GH18, GH20 and GH85); α-GalNAcase (GH36 and GH129); β-GalNAcase (GH20); fucosidase (GH95); sialidase (GH33); sulfatase	(Ruas-Madiedo et al. 2008) (Egan et al. 2016)
Bifidobacterium longum subsp. infantis	Generalist	VIII-240	ppPGM		(Hoskins et al. 1985) (Hoskins et al. 1992) (Corfield et al. 1992)
		NCTC11817^T (ATCC 15697)		α-Galactosidase (GH36); β-galactosidase (GH2 and GH42); β-GlcNAcase (GH18 and GH20); α-GalNAcase (GH36 and GH129); β-GalNAcase (GH20); fucosidase (GH29 and GH95); sialidase (GH33)	
B. longum subsp. longum	Generalist	NCIMB8809	PGM, HIM	α-Galactosidase (GH27 and GH36); β-galactosidase (GH2 and GH42); β-GlcNAcase (GH20 and GH85); α-GalNAcase (GH36, GH101 and GH129); β-GalNAcase (GH20)	(Ruas-Madiedo et al. 2008) (Ruiz et al. 2011)

BSM, bovine submaxillary mucin; HIM, human intestinal mucin; PGM, purified pig gastric mucin; pPGM, purified pig gastric mucin; ppPGM, partially purified pig gastric mucin.

Table II. The consortium of PMDs (PMD consortium)

Phylum		Verrucomicrobia	Bacteroidetes								Firmicutes		Actinobacteria				Total
Species		*Akkermansia muciniphila*	*Bacteroides thetaiotaomicron*	*B. fragilis*	*Phocaeicola vulgatus*	*Bacteroides caccae*	*Bacteroides helcogenes*	*Bacteroides intestinalis*	*B. ovatus*	*Bacteroides xylanisolvens*	*Bacteroides uniformis*	*Ruminococcus gnavus*	*Ruminococcus torques*	*Bifidobacterium longum subsp. longum*	*B. longum subsp. infantis*	*B. breve*	*Bifidobacterium bifidum*	
Strain	Activities in this family that are (potentially) involved in mucin glycan degradation (CAZy)	ATCC BAA-835	DSM 2079	DSM 2151	ATCC 8482	ATCC 43185	P 36–108	APC919 174	ATCC 8483	XB1A	NBRC 113350	ATCC 29149	L2-14[a]	NCIM-B8809	NCTC-11817	UCC-2003	ATCC 29521	
GH2	β-Galactosidase and exo-β-glucosaminidase	5	31	2	25	17	11	35	37	23	22	5	2	3	3	5	3	229
GH16	Endo-β1–4-galactosidase	3	3	6	1	7	2	2	4	1	4	0	0	0	0	0	1	34
GH18	Endo-β-*N*-acetylglucosaminidase	1	12	2	2	0	4	3	8	8	3	2	1	0	1	1	0	48
GH20	β-Hexosaminidase, lacto-*N*-biosidase, β-1,6-*N*-acetylglucosaminidase and β-6-SO3-*N*-acetylglucosaminidase	11	14	12	9	12	8	7	13	10	6	0	0	1	3	1	4	111
GH27	α-Galactosidase	1	5	3	1	1	1	3	3	1	0	0	0	1	0	0	0	20
GH29	Fucosidase	4	9	8	8	12	6	4	7	4	3	2	0	0	3	0	1	71
GH33	Sialidase	3	2	4	3	3	3	1	5	4	0	1	0	0	2	1	4	36
GH35	β-Galactosidase	2	3	4	1	4	1	2	2	2	1	0	0	0	0	1	0	23
GH36	α-Galactosidase and α-*N*-acetylgalactosaminidase	3	4	3	3	2	1	4	3	5	2	2	3	2	1	2	1	41
GH42	β-Galactosidase	0	1	0	1	0	0	1	1	1	1	1	0	3	3	2	2	17
GH84	β-*N*-acetylglucosaminidase	1	1	1	1	1	2	0	0	0	1	0	0	0	0	0	2	10
GH85	Endo-β-*N*-acetylglucosaminidases	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	2
GH89	α-*N*-acetylglucosaminidases	2	3	1	1	3	1	2	4	4	2	0	0	0	0	0	1	24
GH95	Fucosidase	2	5	4	4	5	1	7	7	4	1	3	2	0	1	1	1	48
GH97	α-Galactosidase	1	10	4	7	4	2	15	12	6	13	0	0	0	0	0	0	74
GH98	Blood group endo-β1–4-galactosidase	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	2
GH101	Endo-α-*N*-acetylgalactosaminidases	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	2
GH109	α-*N*-acetylgalactosaminidase, β-*N*-acetylhexosaminidase	2	2	2	5	4	2	2	2	1	2	0	0	0	0	0	0	24
GH110	α-Galactosidase	2	2	2	2	2	1	0	0	0	0	0	0	0	0	0	1	12
GH129	α-*N*-acetylgalactosaminidase	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	4
Total		43	107	58	74	77	46	88	109	74	61	17	8	13	18	16	23	

Note: CAZyme families involved in mucin glycan degradation and their distribution over mucin glycan degraders (Martens et al. 2008; Tailford, Crost, et al. 2015; Desai et al. 2016; Kosciow and Deppenmeier 2019; Crouch et al. 2020; Pruss et al. 2021). For each mucin glycan degrader, the number of GHs per family are indicated, as listed in the CAZy database (Lombard et al. 2014). [a]Not the type strain, but only strain included in CAZy, no in vitro proof that this strain degrades mucin glycans.

that encode a galactosidase or hexosaminidase potentially involved in mucin glycan degradation. Additionally, most members of the PMD consortium encode one or several fucosidases (119). Sialidases are the least commonly encoded, with only 36 sequences found in this consortium.

In the remainder of this section, the enzymes that the PMD consortium members potentially employ to degrade mucin glycans are discussed. Supplementary Table SII contains an overview of enzymes from mucin degraders in the PMD consortium that have been previously studied. Furthermore, we investigated the evolutionary relationships between enzymes in this consortium for each GH family by creating phylogenetic trees of each GH family that is potentially involved in mucin glycan degradation. Supplementary Figures S1–S17 represent the phylogenetic trees of these GH families.

Sulfate metabolism

The secreted mucins in the gut are heavily sulfated. The secretion of heavily sulfated mucins is associated with presence of large numbers of microbiota, as is the case in the colon. Therefore, it is hypothesized that sulfation of mucins plays a role in the protection against enzymatic mucin glycan degradation by bacteria (Corfield et al. 1992; Nieuw Amerongen et al. 1998; Tobisawa et al. 2010). Therefore, desulfation may form a bottleneck in mucin glycan degradation (Etienne-Mesmin et al. 2019). Still, some gut bacteria possess the enzyme sulfatase to release sulfate from mucins, which allows access to the underlying glycan chain through hydrolysis of the sulfate esters. In *O*-linked mucin glycans, sulfation occurs as 6*S*-GlcNAc or 3*S*-, 4*S*- or 6*S*-Gal (Luis et al. 2020). In the gut, sulfatases are mainly encoded within the Bacteroidetes phylum (Benjdia et al. 2011). For example, *B. thetaiotaomicron*, *Bacteroides ovatus*, *Bacteroides fragilis* and *Prevotella* strain RS2 and also *B. breve* and *A. muciniphila* possess sulfatases (Salyers et al. 1977; Roberton et al. 1993; Egan et al. 2016; Ottman et al. 2017). Recently, a sulfatase of *B. fragilis* (*Bf*MDS) has been characterized to be a member of the phosphotransferase (APH) family that targets 3*S*-Gal, 6*S*-Gal and 6*S*-GlcNAc (Praharaj et al. 2018). *Bacteroides thetaiotaomicron* encodes sulfatases targeting 3*S*-, 4*S*- and 6*S*-Gal, 3*S*-, 4*S*- and 6*S*-GalNAc and 3*S*-and 6*S*-GlcNAc. This is the first report of a bacterial sulfatase that targets 3*S*-GalNAc, which leads to the hypothesis that this sulfation occurs in host glycans (Luis et al. 2020). In *B. thetaiotaomicron*, one cell-surface located sulfatase (BT1636) that targets 3*S*-Gal plays a crucial role in the growth of this organism on colonic mucin *O*-glycans, as its deletion lead to a similar growth phenotype as was observed for inactivation of all sulfatases (Luis et al. 2020). These findings suggest that most PMDs are capable of desulfating gut mucins, which allows access to the underlying glycan.

Sulfatases require enzymatic conversion of a cysteine or serine residue to Cα-formylglycine to become active. This can occur through two distinct mechanisms, but under anaerobic conditions that prevail in the mucus environment, the anaerobic sulfatase-maturating enzyme (anSME)-mediated activation is thought to occur, as formylglycine-generating enzyme-mediated activation requires molecular oxygen. The sulfatase activity of gut Bacteroidetes through anSME-mediated activation enhances their ability to forage on mucin glycans and thereby increases their fitness (Benjdia et al. 2011; Luis et al. 2020).

After sulfate is released, it can be reduced by sulfate-reducing bacteria (SRB) to produce hydrogen sulfide. Therefore, SRB abundance increases during mucin glycan degradation (Gibson et al. 1988). SRB are considered to be normal members of the gut microbiota but have also been associated with inflammatory bowel disease (IBD) (Carbonero et al. 2012; Dordević et al. 2021). The most common genera of sulfate-reducers in the gut are *Desulfovibrio*, *Desulfobulbus*, *Desulfobacter*, *Desulfomonas* and *Desulfotomaculum* (Gibson et al. 1993). Most gut SRB can use H_2 and lactate as electron donors for the reduction of sulfate, but SCFAs, amino acids, ethanol and organic acids can also function as electron donor, depending on the species (Dordević et al. 2021). The most prominent gut SRB is *Desulfovibrio piger*, which indeed benefits from the liberation of sulfate from mucin by mucin glycan degraders such as *B. thetaiotaomicron* (Rey et al. 2013). Production of hydrogen sulfide is proposed to lead to disruption of sulfur bridges that occur in mucin glycans, increasing the access that mucin glycan degrading bacteria have to the mucus layer (Ijssennagger et al. 2015).

Sialic acid metabolism

Sialic acids are common terminal sugars in mucin. It is thought that termination of sugar chains by sialic acid is a protective mechanism that prevents access of bacterial enzymes to the glycan chain below (Figure 3) (Tailford, Crost, et al. 2015). However, there are several gut bacteria that can use sialic acid as a source of energy, carbon and nitrogen. To release, take up and metabolize sialic acid, an extracellular sialidase, a sialic acid transporter and enzymes for the degradation of sialic acid are required, respectively (Juge et al. 2016).

Sialidases of gut bacteria are grouped within the GH33 family, which comprises extracellular sialidases (Lombard et al. 2014). Within the GH33 family, there are three classes of gut sialidases: hydrolytic α-sialidases, trans-sialidases and intramolecular trans-sialidases (IT-sialidases and anhydrosialidases). Hydrolytic sialidases can cleave sialic acid that is α2–3, α2–6 or α2–8 linked to a carbohydrate. Most sialidases of the human gut bacteria are hydrolytic sialidases, which are encoded across several phyla by mucin glycan degraders such as *B. thetaiotaomicron*, *Phocaeicola vulgatus*, *B. fragilis*, *A. muciniphila* and *B. bifidum* (Table II) (Lipničanová et al. 2020). Trans-sialidases also hydrolyze α2–3-, α2–6- or α2–8-bound sialic acid, but they preferably transfer the released sialic acid to other glycoconjugates. Both hydrolytic sialidases and trans-sialidases belong to enzyme class EC 3.2.1.18 (Juge et al. 2016). IT-sialidases (EC 4.2.2.15) release 2,7-anhydro-α-*N*-acetylneuraminic acid (2,7-anhydro-Neu5Ac) instead of sialic acid, exclusively from α2–3 linked glycans (Tailford, Owen, et al. 2015; Lipničanová et al. 2020). *Ruminococcus gnavus* encodes an IT-sialidase, which may reflect an adaptation to an IBD-affected gut with short mucin glycan chains terminated by sialic acids. Moreover, the release of 2,7-anhydro-Neu5Ac instead of sialic acid may confer a competitive advantage to *R. gnavus*, as other gut organisms may be unable to use this substrate (Tailford, Owen, et al. 2015; Juge et al. 2016). IT-sialidases are encoded within the genomes of other gut bacteria, mainly in the Firmicutes phylum, and are overrepresented in the metagenome of IBD patients (Tailford, Owen, et al. 2015).

Often, the genes that code for sialic acid metabolism are grouped together in the so-called Nan cluster. The Nan cluster contains *nanA*, *nanK* and *nanE*, which encode the enzymes that catalyze the three initial steps of sialic acid degradation from sialic acid to *N*-acetylglucosamine-6-P (GlcNAc-6P). Next, NagA and NagB convert GlcNAc-6P to fructose-6P (Almagro-Moreno and Boyd 2009). Often, the Nan cluster also encodes a transporter that can import sialic acid into the cell (Almagro-Moreno and Boyd 2009). Gut commensals that encode the Nan cluster include *Anaerotruncus colihominis*, *Dorea formicigenerans*, *Dorea longicatena*, *Faecalibacterium praus-*

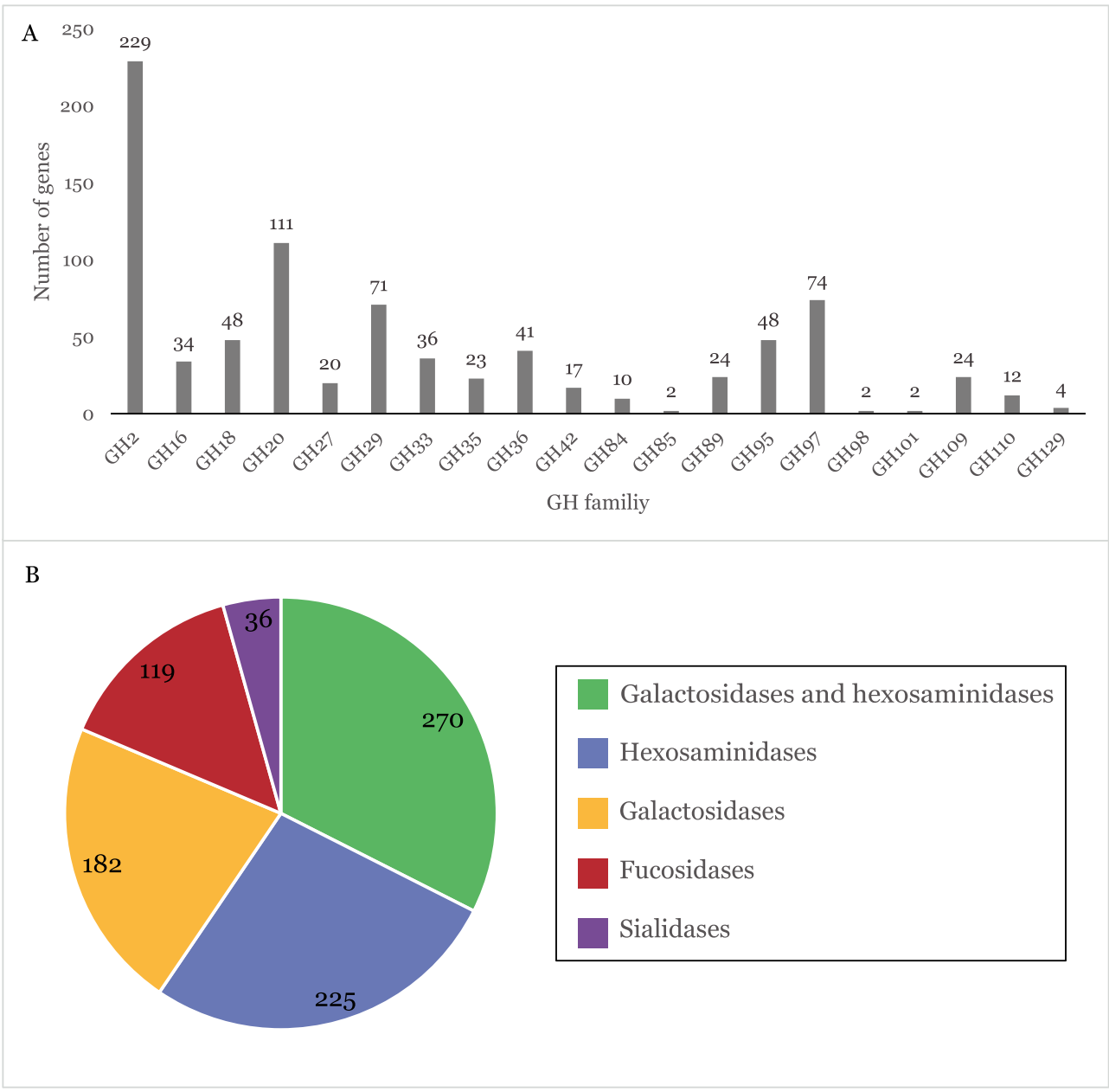

Fig. 2. Functional distribution of GHs implicated in mucin glycan degradation in a consortium of primary mucin glycan degraders (the PMD consortium, Table II). (**A**) Number of genes encoding GH family members that are implicated in mucin glycan degradation in the PMD consortium of mucin glycan degraders that are included in the CAZy database. (**B**) Number of genes within the PMD consortium encoding GH family members grouped by their function. Two families (GH2 and GH36) comprise both galactosidases and hexosaminidases and are therefore shown separately.

nitzii, *Fusobacterium nucleatum*, *R. gnavus*, *Lactobacillus sakei*, *Lactobacillus plantarum*, *Lactobacillus salivarius* and *B. breve* (Almagro-Moreno and Boyd 2009; Egan et al. 2015). Additionally, several intestinal pathogens encode a *nan* cluster (Almagro-Moreno and Boyd 2009). *B. fragilis* utilizes a similar *nanLET* cluster for sialic acid metabolism, which has also been found in the genome of several other *Bacteroides* species (Brigham et al. 2009).

Interestingly, not all gut bacteria encode the complete set of genes required for sialic acid utilization. For example, *B. thetaiotaomicron* and *A. muciniphila* possess sialidases but do not encode a *nan* or *nanLET* cluster for the metabolism of sialic acid (Brigham et al. 2009; van Passel et al. 2011). Conversely, some bacteria encode a *nan* cluster for sialic acid utilization but do not express sialidase activity and therefore depend on other members of the human gut microbiota to release sialic acid for substrate cross-feeding (Tailford, Crost, et al. 2015).

Most of the mucin degraders in the consortium, that is studied here, encode one or multiple GH33 sialidases (Table II). This suggests that they can initiate mucin glycan degradation, as the removal of sialic acid terminating the glycan is proposed to be crucial for access to the underlying glycan. Indeed, two GH33 enzymes from *B. fragilis* (BF4051 and BF1806) and one from *A. muciniphila* (Amuc_1835) were upregulated in response to mucin (Marcobal et al. 2011; Pudlo et al. 2015; Kostopoulos et al. 2020). The four sialidases from *A.*

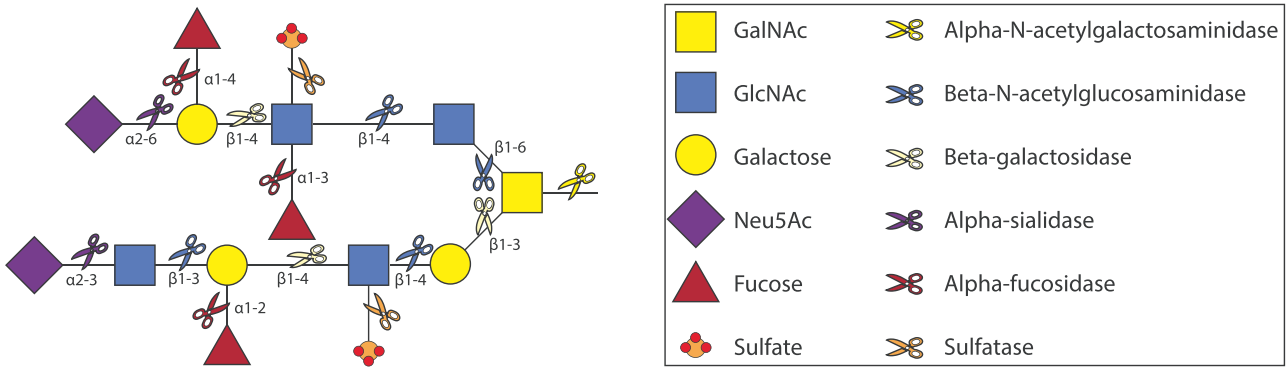

Fig. 3. A hypothetical mucin glycan and the enzyme classes that are required to hydrolyze the bonds between the subunits.

muciniphila (Amuc_0625, Amuc_0623 [listed in CAZy as nonclassified GH], Amuc_1547 and Amuc_1835) all targeted both α2–3- and α2–6-linked sialic acids, which are the sialic acid structures that occur in mucin (Supplementary Table SII) (Huang et al. 2015; Juge et al. 2016).

Phylogenetic analysis of GH33 enzymes encoded by mucin degraders shows several clusters of closely related sialidases from different *Bacteroides* species and a cluster of *B. bifidum* sialidases (Supplementary Figure S1). The sialidase from *R. gnavus* is not closely related to any enzyme that was included in our analysis. Furthermore, several members of the PMD possess multiple sialidases with a large phylogenetic distance. For example, *B. ovatus* encodes five GH33 sialidases that group in five different clusters in our phylogenetic analysis (Supplementary Figure S1). This could indicate functional redundancy.

Fucose metabolism

Fucose is another terminal sugar in mucin glycans, where it is α1–2-, α1–3- or α1–4-linked (Figure 3) (Tailford, Crost, et al. 2015). To access fucose from mucin glycans, it is usually released by the activity of a secreted fucosidase, which makes the liberated fucose also available to other gut microbiota through substrate cross-feeding (Pickard and Chervonsky 2015). Fucose can be metabolized to propionate through the propanediol pathway (Reichardt et al. 2014; Louis and Flint 2017). Additionally, *B. fragilis* has been shown to decorate its surface molecules with fucose that was acquired from the host, providing it with a competitive advantage (Coyne et al. 2005).

Most gut mucin glycan degraders express multiple fucosidases from both families GH29 and GH95 to release fucose from mucin (Table II) (Lombard et al. 2014; Tailford, Crost, et al. 2015). GH95 family members specifically release fucose that is α1–2-linked to galactose through an inverting mechanism, while GH29 family members are less specific and retain the conformation of fucose during hydrolysis (Sakurama et al. 2012; Lombard et al. 2014). The GH29 family is divided in subfamily A and B, where GH29-B comprises fucosidases that are more specific for α1–3 or α1–4 linkages, and GH29-A contains less-specific fucosidases (Sakurama et al. 2012; Liu et al. 2020). Phylogenetic analysis of GH29 fucosidases of mucin degraders (Supplementary Figure S2) revealed several clusters of closely related *Bacteroides* fucosidases from different species, while the GH29 fucosidases of *A. muciniphila*, *R. gnavus* and *B. bifidum* display a larger phylogenetic distance to other enzymes. A recent analysis of *Bifidobacterium* GH29 fucosidases revealed four distinct phylogenetic groups. Interestingly, only one of these groups (GH29-BifA) that consists exclusively of *B. bifidum* fucosidases contains enzymes that are predicted to be membrane-bound. The other three phylogenetic groups are predicted to be intracellular fucosidases (Curiel et al. 2021). Phylogenetic analysis of GH95 fucosidases (Supplementary Figure S3) shows mostly large phylogenetic distances except for some clusters of GH95 fucosidases from several *Bacteroides* species. Similar to *Bifidobacterium* GH29 fucosidases, *Bifidobacterium* GH95 fucosidases form a separate phylogenetic group (GH95-BifA) that contains fucosidases that are predicted to function extracellularly and that contains mostly fucosidases from *B. bifidum* (Curiel et al. 2021).

Gut microbes may possess fucosidases with a range of substrate specificities. As becomes clear from Table II, most mucin glycan degraders encode fucosidases of both family GH29 and GH95. Furthermore, mucin glycan degraders may encode a range of fucose substrate specificities within fucosidase families. For example, *R. gnavus* and *B. thetaiotaomicron* have been shown to encode fucosidases belonging to both subfamilies of GH29, allowing them to access a variety of fucosylated substrates from mucin and HMOs (Sakurama et al. 2012; Wu et al. 2021). Interestingly, *R. gnavus* E1 encodes a fucosidase that can release α1–3-/4-linked fucose from a sialic acid-capped glycan without the need to first remove the sialic acid (Wu et al. 2021). Amuc_0010 from *A. muciniphila* has been shown to cleave fucose that is α1–2-linked to galactose in an HMO structure, but it is postulated that mucin glycans are its preferred substrate over HMOs (Supplementary Table SII) (Kostopoulos et al. 2020).

After release of fucose by an extracellular fucosidase, fucose can be taken up into the cell of gut symbionts, where it is further metabolized to 1,2-propanediol (Scott et al. 2006). 1,2-propanediol can be released into the environment, allowing other gut bacteria such as *Anaerobutyricum hallii* to cross-feed on 1,2-propanediol to produce propionate (Belzer et al. 2017). However, *Roseburia inulinivorans* has been shown to ferment fucose to propionate by itself via the propanediol pathway (Scott et al. 2006; Reichardt et al. 2014). Despite the importance of fucose in regulating bacterial intestinal colonization in adults and infants, only a limited number of fucosidases have been studied at a biochemical level from human gut symbionts (Supplementary Table SII) (Wu et al. 2021).

Galactose metabolism

Galactosidases hydrolyze glycan bonds that contain a galactose subunit. Galactosidases are classified into α-galactosidases and β-galactosidases depending on the conformation of the targeted galactoside bond. As galactoside bonds are common in mucin

glycans (Figure 3), galactosidases are common among mucin glycan degrading bacteria (Table II). β-Galactosidases implicated in mucin glycan degradation have been found in CAZyme families GH2, GH16, GH35, GH42 and GH98 (Table II) (Tailford, Crost, et al. 2015; Kosciow and Deppenmeier 2019; Crouch et al. 2020). α-Galactosidases implicated in mucin glycan degradation have been classified as members of GH27, GH36, GH97 and GH110 (Table II) (Pruss et al. 2021).

β-linked galactoses occur often in mucin glycans. Therefore, β-galactosidases play a central role in the degradation of mucin glycans (Tailford, Crost, et al. 2015). Of the β-galactosidases that are discussed here, families GH2, GH16, GH35 and GH42 contain enzymes that employ a retaining mechanism, and family GH98 contains enzymes that employ an inverting mechanism (Lombard et al. 2014).

GH16 family members hydrolyze a broad range of glycans containing β1–4 or β1–3 glycosidic bonds (Eklöf et al. 2019). During mucin glycan degradation, members of this family can express β1–4-galactosidase activity (Lombard et al. 2014). GH16 enzymes involved in mucin glycan degradation belong to the diverse GH16 subfamily 3 (Viborg et al. 2019). GHs can be classified as exo- or endo-acting depending on where they cleave the glycan chain. Exo-acting GHs cleave their substrate at the end of a glycan chain, whereas endo-acting GHs cleave their substrate in the middle of a glycan chain (Withers and Spencer 2017). Recently, β-galactosidases involved in mucin glycan degradation of the GH16 family have been characterized to be endo-acting cell-surface β1–4-galactosidases that target the Galβ1–4GlcNAc bond in poly-LacNAc chains that often occur in mucin glycans (Crouch et al. 2020). Poly-LacNAc is a chain of repeated LacNAc (*N*-acetyllactosamine and Galβ1–4GlcNAc) disaccharides that are β1–3-linked to each other. This finding challenges the currently accepted model of mucin glycan degradation called exo-trimming, which involves sequential release of subunits by exo-acting extracellular enzymes from the mucin glycan chain (Marcobal et al. 2013; Crouch et al. 2020). After removal of sialic acid from the terminal positions of the mucin glycan, endo-acting cell-surface GH16 enzymes produce oligosaccharides that can be imported into the cell for further degradation (Crouch et al. 2020). In the consortium of mucin glycan degraders, GH16 sequences are mainly encoded by *A. muciniphila* and *Bacteroides* spp. (Table II). Two *B. fragilis* GH16 enzymes, a periplasmic endo-β1–4-galactosidase (BF4060) and a surface endo-β1–4-galactosidase (BF4058), were shown to be involved in mucin glycan degradation (Pudlo et al. 2015; Crouch et al. 2020). Furthermore, three *A. muciniphila* GH16 family members (Amuc_2108, Amuc_0924 and Amuc_0875) showed endo-β1–4-galactosidase activity (Crouch et al. 2020). Our phylogenetic analysis of this family shows not only a large sequential diversity but also some clusters of more closely related enzymes (Supplementary Figure S4). These clusters consist of enzymes from different *Bacteroides* spp. For example, one cluster contains enzymes from *B. thetaiotaomicron*, *B. ovatus* and *Bacteroides caccae*. By contrast, three out of four *Bacteroides uniformis* GH16 enzymes are phylogenetically very different from the other GH16 enzymes in the PMD consortium.

GH2 is the largest enzyme family in this consortium of mucin glycan degrading enzymes (226 sequences). All mucin glycan degraders studied here encode GH2 enzymes. Two of the six GH2 β-galactosidases of *A. muciniphila* have been characterized. Amuc_082 targets core structure Galβ1–3GalNAc. By contrast, Amuc_1666 prefers β1–4-linked galactose as a substrate and mainly targets Galβ1–4GlcNAc (LacNAc) (Kosciow and Deppenmeier 2020). A phylogenetic analysis of bifidobacterial GH2s revealed that they can be divided in nine distinct groups, which often consist of enzymes from the same species (Ambrogi et al. 2019). However, it is unknown whether any of these bifidobacterial GH2 clusters target galactose in mucin. Our own phylogenetic analysis (Supplementary Figure S5) exhibits numerous clusters of GH2 enzymes from *Bacteroides* spp., a cluster of bifidobacterial GH2 enzymes and a cluster containing a bifidobacterial enzyme and *Ruminococcus* spp. enzymes.

Another family of β-galactosidases that are implicated in mucin glycan degradation is GH35. *A. muciniphila* possesses two GH35 β-galactosidases: Amuc_1686 and Amuc_0771 (Supplementary Table SII). Amuc_1686 targets the Galβ1–3GalNAc bond that is present in mucin glycan core structures 1 and 2 (Kosciow and Deppenmeier 2019). Amuc_0771 preferentially targets Galβ1–3GlcNAc and is also able to hydrolyze Galβ1–3GalNAc bonds in the core structures (Guo et al. 2018; Kosciow and Deppenmeier 2020; Kostopoulos et al. 2020). Furthermore, one *B. fragilis* GH35 (BF4061) was upregulated when grown on mucin compared to on glucose (Pudlo et al. 2015). Our phylogenetic analysis of GH35 enzymes of mucin degraders reveals some very closely related enzymes from *Bacteroides* (Supplementary Figure S6). One large cluster of *Bacteroides* GH35 contains enzymes from *Bacteroides intestinalis*, *B. uniformis*, *B. thetaiotaomicron*, *P. vulgatus*, *B. fragilis*, *Bacteroides xylanisolvens*, *B. ovatus* and *B. caccae*. Additionally, there are several smaller clusters of *Bacteroides* enzymes. The PMD consortium contains only one GH35 that is not from a *Bacteroides* spp.: Bbr_1833 from *B. breve*.

GH98 exclusively contains endo-β-galactosidases that target the core galactose in blood group A and B glycoconjugates (Galβ1–4GlcNAc) (Shaikh and Boraston 2012). In the PMD consortium of mucin degraders, only *B. ovatus* and *R. gnavus* encode a GH98 galactosidase (Table II).

GH42 β-galactosidases are encoded by the *Bifidobacterium* spp., several *Bacteroides* spp. and *R. gnavus* in the PMD consortium of mucin degraders (Table II). Phylogenetic analysis of bifidobacterial GH42 enzymes revealed five distinct clusters (Ambrogi et al. 2019). Our phylogenetic analysis shows that *Bifidobacterium* spp. GH42 family members form separate clusters from the *Bacteroides* spp. and *R. gnavus* enzymes (Supplementary Figure S7). Bbr_0529 from *B. breve* and B8809_0415 from *Bifidobacterium longum subsp. longum* cluster display a close evolutionary relationship and were allocated in the same cluster (Ambrogi et al. 2019). Five of the *Bacteroides* GH42 enzymes are in a closely related cluster that is very distant from the other enzymes. By contrast, the GH42 of *B. uniformis* exhibits a large phylogenetic distance to this cluster (Supplementary Figure S10).

α-Linked galactose occurs in the blood group epitopes that can decorate mucin glycans (Lindén et al. 2008). Mucin glycan degrading bacteria express α-galactosidases of the families GH27 (20), GH36 (40), GH97 (74) and GH110 (12) (Table II). GH27 and GH36 contain enzymes that employ a retaining mechanism, while GH110 family members employ an inverting mechanism (Lombard et al. 2014). Interestingly, the GH97 family contains both inverting and retaining enzymes (Gloster et al. 2008). The GH110 family exclusively consists of α-galactosidases that exhibit high specificity to the galactose in the blood group B antigen structures (Galα1–3(Fucα1–2)Galβ1-R) that are present in mucin glycan structures of B secretor hosts (Henrissat 2011; Wakinaka et al. 2013). Our phylogenetic analysis of the GH27 enzymes from the PMD consortium resulted in several clusters of GH27 from *Bacteroides* spp. GH27 enzymes from *B. ovatus*, *A. muciniphila* and *B. longum subsp. longum* were more distantly related (Supplementary Figure S8). Family GH36 contains α-*N*-acetylgalactosaminidases in addition to α-galactosidases

(Lombard et al. 2014). Phylogenetic analysis of the GH36 enzymes in the PMD consortium revealed that enzymes from *Bifidobacterium* spp. cluster together. One cluster is formed by BBBF_1801 (*B. bifidum*), Bbr_1869 (*B. breve*) and B8809_1814 (*B. longum subsp. longum*), and the other cluster consists of Bbr_1856 (*B. breve*) and B8809_1801 (*B. longum subsp. longum*). Furthermore, *R. torques* and *R. gnavus* enzymes are more closely related to each other than to other GH36. Again, several clusters of *Bacteroides* spp. enzymes are apparent (Supplementary Figure S9). In the PMD consortium, only *Bacteroides* spp. and *A. muciniphila* possess GH97. Our phylogenetic analysis of this family reveals some clusters of *Bacteroides* spp. enzymes, but some enzymes, such as DXK01_004960 (*B. intestinalis*) and FE838_05645 (*B. thetaiotaomicron*) are very distantly related to the others. The one GH97 from *A. muciniphila* neither displays a close evolutionary relationship with other enzymes of the PMD consortium. Interestingly, three enzymes from *B. uniformis* form a separate cluster (Supplementary Figure S10). Phylogenetic analysis of GH110 unveils a large phylogenetic distance of the two *A. muciniphila* GH110 to the *Bacteroides* spp. GH110 in the PMD consortium. There are several closely related *Bacteroides* GH110, but some are more distant (Supplementary Figure S11).

GlcNAc and GalNAc metabolism

N-acetylglucosaminidases (GlcNAcases) and *N*-acetylgalactosaminidases (GalNAcases) remove GlcNAc and GalNAc subunits from mucin glycans, respectively. Mucin glycan degraders widely express these hexosaminidases (Table II). GlcNAcases that can act during mucin glycan degradation are found in CAZyme families GH18, GH20, GH84, GH85 and GH89. Families GH18, GH20, GH84 and GH85 contain β-GlcNAcases, while family GH89 contains α-GlcNAcases (Martens et al. 2008; Tailford, Crost, et al. 2015; Desai et al. 2016). α-GalNAcases that possibly contribute to mucin glycan degradation include members of families GH36, GH101, GH109 and GH129 (Tailford, Crost, et al. 2015; Desai et al. 2016; Pruss et al. 2021).

β-GlcNAcases are encoded within families GH18, GH20, GH84 and GH85. Some members of GH18 can be involved in mucin degradation, as BF1312 and BF3414 from *B. fragilis* were upregulated when grown on mucin compared to during growth on glucose, and Amuc_2164 from *A. muciniphila* contains a module that is thought to bind to mucin (van Passel et al. 2011; Pudlo et al. 2015). Our phylogenetic analysis of this family is presented in Supplementary Figure S12. In addition to several clusters of *Bacteroides* spp. GH18, RTO_11235 (*R. torques*) and RGna_11235 (*R. gnavus*) are closely related. Conversely, Amuc_2164 (*A. muciniphila*) and Bbr_0627 (*B. breve*) are more distantly related to the other GH18 enzymes in the PMD consortium. While GH18 is common among this consortium of mucin degraders (47), GH85 is encoded only by two *Bifidobacterium* spp. (Table II) and GH84 is encoded 10 times within the present consortium (Table II, Supplementary Figure S13). Most *Bacteroides* spp. of the PMD consortium encode one similar GH84. The two GH84 from *B. bifidum* are most closely related to each other but with a relatively large phylogenetic distance between them. Additionally, the *A. muciniphila* GH84 does not share a close evolutionary relationship with other enzymes in the PMD consortium (Supplementary Figure S13).

The GH20 family contains hexosaminidases. GH20 family members are well represented among mucin degraders, with 108 GH20 enzymes in this consortium (Table II). Three *A. muciniphila* GH20 family members have been shown to be more abundant in mucin conditions compared to HMO conditions (Amuc_0369, Amuc_1815 and Amuc_1924) (Kostopoulos et al. 2020). Furthermore, Amuc_0369 and Amuc_2136 can hydrolyze terminal β1–3-linked GlcNAc from lacto-*N*-triose (LNT2) (GlcNAcβ1–3Galβ1–4Glc), but not the β1–3-linked GlcNAc in lacto-*N*-tetraose (LNT) (Galβ1–3GlcNAcβ1–3Galβ1–4Glc) (Kostopoulos et al. 2020). Similarly, Bbr_1556 from *B. breve* can hydrolyze the GlcNAcβ1–3Gal bond from LNT2 after removal of the terminal galactose (James et al. 2016). Interestingly, a GH20 family member that was found in *Prevotella* strain RS2 can release terminal 6-SO_3-GlcNAc, omitting the need for a specific desulfating enzyme to access mucin glycans (Rho et al. 2005). Our phylogenetic analysis of this family unveils multiple *Bacteroides* spp. clusters. Furthermore, Amuc_2018 and Amuc_2019 (*A. muciniphila*) are closely related, and B8809_1264 (*B. longum subsp. longum*), Bbr_1556 (*B. breve*) and BBBF_0021 (*B. bifidum*) form a separate group (Supplementary Figure S14).

GH89 family members are α-GlcNAcases. For example, AgnB is a membrane-bound extracellular enzyme from *B. bifidum* that targets the GlcNAcα1–4Gal bond that exists in gastroduodenal mucin glycans (Hanisch et al. 2014; Shimada et al. 2015). Furthermore, Amuc_1220 from *A. muciniphila* was upregulated when grown on pig gastric mucin (PGM) compared to growth on HMOs (Kostopoulos et al. 2020). However, it is not known whether GH89 plays a role in the degradation of intestinal MUC2 as well. Our phylogenetic analysis of this family is presented in Supplementary Figure S15. From this figure, it becomes clear that some *Bacteroides* GH89 form clusters. Most notably, three pairs of *B. ovatus* and *B. xylanisolvens* GH89 have a very close evolutionary relationship (BXY_19450 and Bovatus_02880, BXY_19410 and Bovatus_02876, and BXY_30240 and Bovatus_04030). However, enzymes from *A. muciniphila* and *B. bifidum* and one enzyme from *B. caccae* display larger phylogenetic distance to other GH89 from the PMD consortium (Supplementary Figure S15).

α-GalNAcases can cleave the core GalNAc that is α-linked to a serine or threonine (Tailford, Owen, et al. 2015). GH101 and GH129 are only encoded by two and three *Bifidobacterium* species in the PMD consortium (Table II). Conversely, GH109 is encoded by *A. muciniphila* and *Bacteroides* spp. and is present 24 times in the consortium. Our analysis shows that the enzymes from the *Bacteroides* spp. mostly group in three phylogenetic clusters except for one enzyme from *Bacteroides helcogenes* (Bache_0935) (Supplementary Figure S16). From Supplementary Figure S17, it becomes clear that Bbr_0940 (*B. breve*), BBBF_1685 (*B. bifidum*) and B8809_0763 (*B. longum subsp. longum*) share a close evolutionary relationship.

Altogether, our phylogenetic analysis of the GH families present in the PMD shows that there is a lot of functional redundancy in GH families throughout all PMD members. This may lead to functional stability of the mucosal microbiome but could also contribute to competition for substrates between mucin glycan degrading bacteria.

Microbial interactions in the gut mucosa

In the gut mucus layer, cross-feeding between mucin glycan degraders and partner organisms occurs, which results in the production of metabolites such as SCFAs. The proximity to the host gut epithelium increases the chance that these metabolites will be sensed or absorbed by the host cells. In this section, in vitro and in vivo studies of the cross-feeding interactions at the gut mucosa and their changed metabolic pool are discussed. Cooperation between a mucin glycan degrader and a partner organism increases the total metabolic capacity, which may lead to a more complete and rapid degradation

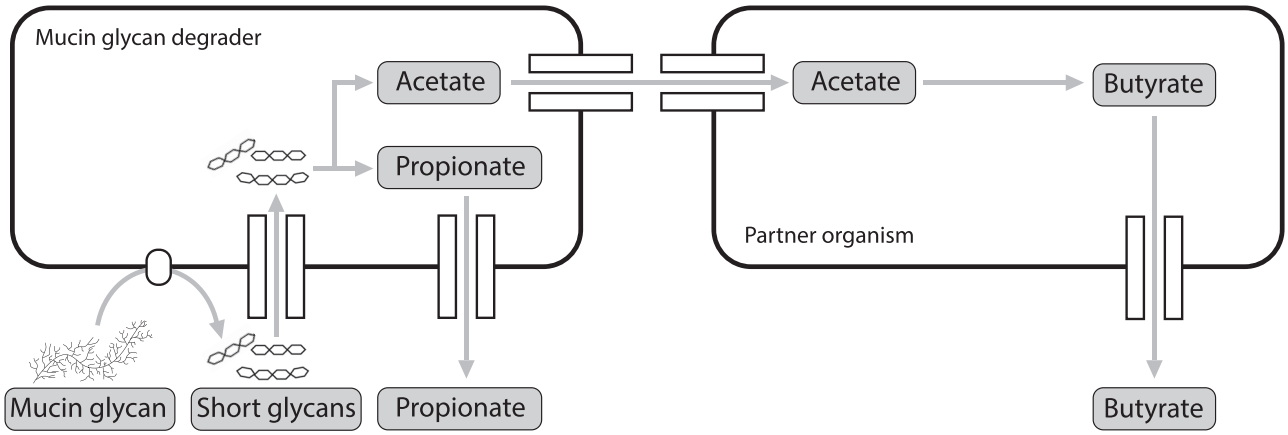

Fig. 4. Cooperative mucin glycan degradation of a mucin glycan degrader and a butyrate-producing partner organism results in the production of SCFAs. Extracellular CAZymes of the mucin glycan degrader release shorter glycans from mucin. These glycans are imported into the cell, where further degradation takes place. This results in the production of SCFAs propionate and acetate. Subsequently, acetate can be taken up by a partner organism, which produces butyrate from acetate. Butyrate is secreted and becomes available to the host.

of the mucin glycan and to a more diverse metabolite pool. These studies involve cocolonization of a germ-free (GF) animal model or cocultures on a mucin medium. A summary of microbial cross-feeding interactions in the intestinal mucosa is provided in Table III.

Metabolite cross-feeding

Metabolite cross-feeding in the intestinal mucus layer occurs when a partner organism feeds on the end products of the metabolism of a mucin glycan degrader. Often, a mucin glycan degrader produces acetate and propionate from mucin glycans, which are then secreted into the environment. Acetate can be converted to butyrate by certain bacteria (Figure 4). For example, cocolonization of the ceca of GF mice with generalist glycan degrader *B. thetaiotaomicron* and butyrate producer *Eubacterium rectale* showed that *E. rectale* consumes acetate produced by *B. thetaiotaomicron* to convert it to butyrate (Mahowald et al. 2009). Similarly, cocolonization of GF rats with *B. thetaiotaomicron* and *F. prausnitzii* demonstrated that *F. prausnitzii* uses acetate produced by *B. thetaiotaomicron* to produce butyrate (Wrzosek et al. 2013) (Figure 4).

The specialist mucin glycan degrader *A. muciniphila* can initiate mucin glycan-driven metabolite cross-feeding interactions in mucin cocultures as well. When grown on PGM, *A. muciniphila* liberates mucin sugars and produces 1,2-propanediol, propionate and acetate. This permits growth of, and butyrate production by *Anaerostipes caccae*, *F. prausnitzii* and *A. hallii* in two-species cocultures. Moreover, there exists mutual cross-feeding between *A. muciniphila* and *A. hallii*. While *A. hallii* benefits from the metabolites produced by *A. muciniphila*, *A. hallii* produces pseudovitamin B12. In turn, *A. muciniphila* uses pseudovitamin B12 as a cofactor for propionate production from succinate (Belzer et al. 2017). However, it should be noted that some *A. muciniphila* strains possess the genes that are required for vitamin B12 synthesis and can therefore produce propionate independent of other organisms (Kirmiz et al. 2020).

Metabolite cross-feeding on a mucin medium has been demonstrated within the *Bifidobacterium* genus as well. Bifidobacteria are dominant members of the breast-fed infant gut, where they degrade HMOs (Hidalgo-Cantabrana et al. 2017). For example, infant *Bifidobacterium* spp. can use fucosyllactose to produce lactate and acetate from the lactose moiety and can use 1,2-propanediol from the fucose moiety of HMOs. Subsequently, *A. hallii* uses the lactate and acetate to produce butyrate and uses the 1,2-propanediol to produce propionate (Schwab et al. 2017). A three-species mucin coculture of *B. bifidum*, *Bifidobacterium infantis* or *B. breve* and *A. hallii* produces propionate and butyrate (Figure 5) (Bunesova et al. 2018).

Hydrogen is produced during fermentation of mucin glycans in the gut, but its accumulation slows fermentation. Nevertheless, hydrogenotrophic microbiota can cross-feed on hydrogen produced during mucin glycan degradation. Acetogens consume hydrogen and carbon dioxide to produce acetate, methanogens utilize hydrogen and carbon dioxide to produce methane (CH_4) and SRB use hydrogen for the reduction of sulfate to hydrogen sulfide (H_2S) (Nava et al. 2012; Smith et al. 2019a; Djemai et al. 2021). Cocolonization of mice with *B. thetaiotaomicron* and methanogen *Methanobrevibacter smithii* revealed that *B. thetaiotaomicron* produced more acetate from dietary glycans than during monoassociation, while *M. smithii* benefitted from formate produced by *B. thetaiotaomicron*. This cocolonization led to higher population densities than cocolonization by *B. thetaiotaomicron* and SRB *D. piger* (Samuel and Gordon 2006). Similarly, mice cocolonization of *B. thetaiotaomicron* and acetogen *Blautia hydrogenotrophica* increased acetate production through bacterial fermentation of dietary glycans compared to *B. thetaiotaomicron* monoassociation (Rey et al. 2010). The hypothesis is that these hydrogen cross-feeding interactions also occur during mucin glycan degradation.

Substrate cross-feeding

Substrate cross-feeding in the gut mucosa occurs when a primary mucin glycan degrader liberates a smaller molecule from a mucin glycan by secreting extracellular enzymes. Next, these smaller molecules become available in the environment, where they can be taken up not only by the mucin degrader itself but also by other residents of the gut mucosa. For example, *A. muciniphila* can sustain both *Roseburia hominis* and *R. inulinivorans* in a mucin-fed three-species culture (Pichler et al. 2020). *A. muciniphila* liberates galacto-*N*-biose (GNB) from mucin. Subsequently, *R. hominis* and *R. inulinivorans* take up GNB, which they can hydrolyze to galactose and GalNAc. Additionally, *R. inulinivorans* hydrolyzes blood group oligomers from mucin through extracellular enzymes. Moreover, it utilizes fucose released by *A. muciniphila*, which leads to the production of lacto-

Table III. Cocultivations that cooperatively break down mucin glycans

Mucin degrader	Partner(s)	Method	Metabolites and gases exchanged	Reference
Bacteroides fragilis	*Desulfovibrio desulfuricans*	Coculture on PGM	*B. fragilis* releases sulfate from mucin, which can be reduced by *D. desulfuricans*	(Willis et al. 1996)
Bacteroides thetaiotaomicron VPI-5482	*Eubacterium rectale* ATCC 33656	Cocolonization of GF mice	*E. rectale* uses acetate produced by *B. thetaiotaomicron* to produce butyrate	(Mahowald et al. 2009)
B. thetaiotaomicron VPI-5482	*Faecalibacterium* A2–165	Cocolonization of GF rats	*Faecalibacterium prausnitzii* uses acetate produced by *B. thetaiotaomicron* to produce butyrate	(Wrzosek et al. 2013)
B. thetaiotaomicron VPI-5482	*Desulfovibrio piger* GOR1	Coculture on chondroitin sulfate and cocolonization of GF mice	*B. thetaiotaomicron* liberates sulfate, which *D. piger* can reduce	(Rey et al. 2013)
Bifidobacterium bifidum PRL2010	*Bifidobacterium breve* UCC2003	Coculture on PGM	*B. breve* can benefit from the carbohydrates released from mucin by *B. bifidum*	(Egan et al. 2014)
Akkermansia muciniphila ATTC BAA-835	*Anaerostipes caccae* L1–92 *Faecalibacterium prausnitzii* A2–165 *Anaerobutyricum hallii* L2–7	Coculture on PGM	*A. muciniphila* produces 1,2-propanediol, propionate and acetate and liberates mucin sugars. Sugars and acetate from *A. muciniphila* support growth of and butyrate production by *A. caccae*, *F. prausnitzii* and *A. hallii*. *A. muciniphila* supplies *A. hallii* with 1,2-propanediol, and *A. hallii* produces pseudovitamin B12 which can be used by *A. muciniphila* for propionate production	(Belzer et al. 2017)
B. bifidum BSM281	*Bifidobacterium infantis* DSM20088 *B. breve* DSM 20213 *Anaerobutyricum hallii* DSM 3353	Coculture on PGM	Cocultivation of *B. bifidum* and *B. infantis* produced more acetate than monoculture of *B. bifidum* and coculture of *B. bifidum* and *A. hallii*. Coculture of *B. bifidum* and *A. hallii* lead to butyrate production. Lactate was produced by *B. bifidum* and *B. infantis* coculture, and formate was produced by coculture of *B. bifidum* and *B. infantis* and *B. bifidum* and *A. hallii*. Three-strain fermentations: *B. bifidum* and *A. hallii* with *B. infantis* or *B. breve*: also propionate production, which was not detected in two-strain cocultures	(Bunesova et al. 2018)
Ruminococcus gnavus ATCC 29149	*Ruminococcus bromii* L2–63	Coculture on PGM	*R. bromii* could not benefit from mucin degradation by *R. gnavus* in coculture	(Crost et al. 2018)
B. bifidum ATCC 15696	*B. breve* JCM 7019	Coculture on PCM	*B. bifidum* releases sialic acid, which can be used by *B. breve*	(Nishiyama et al. 2018)
A. muciniphila DSM 22959	*Roseburia hominis* DSM 16839 *Roseburia inulinivorans* DSM 16841	Coculture on a mucin mixture	*A. muciniphila* supports growth of both *Roseburia* strains. *R. inulinivorans* is likely to utilize blood group A and B oligomers, fucose and sialic acid released by *A. muciniphila*	(Pichler et al. 2020)

N-biose (LNB). LNB can be hydrolyzed to galactose and GlcNAc. Furthermore, *R. inulinivorans* can cross-feed on sialic acid that is released from mucin by *A. muciniphila*. Cross-feeding in this three-species coculture results in butyrate production (Pichler et al. 2020).

In general, specialist HMO-utilizing *Bifidobacterium* spp. are unable to degrade mucin glycans and initiate substrate cross-feeding, potentially because most of their glycan-degrading enzymes are intracellular (Zúñiga et al. 2018). However, *B. bifidum* can degrade mucin glycans (Crociani et al. 1994). A coculture on a mucin medium of mucin degrader *B. bifidum* with nonmucin degrading *B. breve* greatly enhances *B. breve*'s growth (Egan et al. 2014; Bunesova et al. 2018). Therefore, it was hypothesized that *B. breve* benefits from

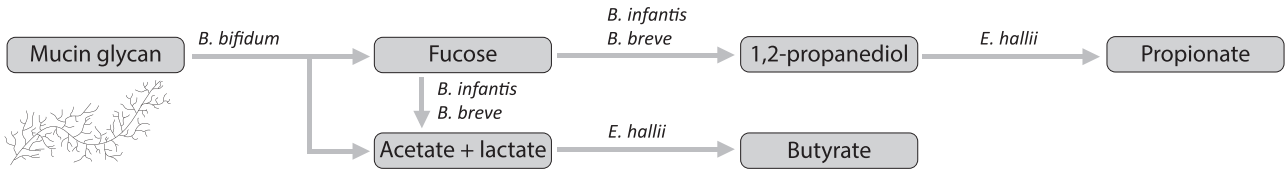

Fig. 5. Cross-feeding of *Bifidobacterium spp.* with butyrate producer *A. hallii*: A three-species mucin coculture of *B. bifidum, B. breve* or *B. infantis* and *A. hallii* produces SCFAs propionate and butyrate. Mucin glycan degradation by *B. bifidum* releases fucose, acetate and lactate. *B. infantis* or *B. breve* can use fucose to produce acetate, lactate and 1,2-propanediol. *A. hallii* uses 1,2-propanediol to produce propionate and uses acetate and lactate to produce butyrate (Bunesova et al. 2018).

fucose, galactose and sialic acid released from mucin by *B. bifidum* (Egan et al. 2014). Later, it was confirmed that *B. bifidum* produces extracellular sialidases that release sialic acid from pig colonic mucin (PCM). In a coculture with *B. bifidum*, *B. breve* can use the free sialic acid to sustain itself (Nishiyama et al. 2018).

Additionally, some mucin glycan degraders can release sulfate from the mucin glycoprotein by expressing extracellular enzymes. This initiates substrate cross-feeding by SRB, which reduces sulfate to produce hydrogen sulfide. It has been shown that *B. fragilis* can sustain *Desulfovibrio desulfuricans* through the release of sulfate when grown on mucin and that *D. piger* can obtain sulfate that is liberated by *B. thetaiotaomicron* in vitro and in vivo (Gibson et al. 1988; Willis et al. 1996; Rey et al. 2013).

However, not all gut commensals are able to benefit from mucin degradation by others. *Ruminococcus gnavus* is able to grow on mucin by releasing fucose and sialic acid (Crost et al. 2013). By contrast, the starch utilizer *Ruminococcus bromii* does not grow with mucin as a sole carbon source. In coculture, *R. bromii* is unable to benefit from mucin glycan degradation by *R. gnavus* (Crost et al. 2018).

Mucosal interactions at community level

Bacterial cross-feeding on mucin glycans can also be studied at community level, both in vitro and in vivo. For example, fiber deprivation of gnotobiotic mice inoculated with a defined bacterial community lead to enhanced abundance of mucin glycan degraders *A. muciniphila* and *B. caccae* and increased expression of CAZymes targeting mucin glycans. This study proposes that a fiber-free diet triggers mucin degraders to feed on the gut mucosal layer. However, no significant differences in SCFA production between a fiber-free and fiber-rich diet were found except for increased succinate concentrations in the fiber-free diet (Desai et al. 2016).

In mucosal simulator of human intestinal microbial ecosystem (M-SHIME) models of the human gut, which include simulation of the mucus layer, inoculated with human stool, revealed that the mucosal layer is dominated by Firmicutes, which mainly includes species from *Clostridium* cluster XIVa that convert lactate and/or acetate to butyrate (Van Den Abbeele et al. 2013). The dominance of these bacteria in the mucus layer may be explained by cross-feeding on products from primary mucin glycan degradation. *Roseburia intestinalis* and *E. rectale* were the most specific mucus colonizers, as opposed to *A. caccae*, which specifically colonized the lumen (Van Den Abbeele et al. 2013).

Overall, microbial cross-feeding initiated by mucin glycan degradation includes cross-feeding on metabolic end products of primary mucin glycan degraders (metabolite cross-feeding) and cross-feeding on molecules that are liberated from mucin glycans by extracellular enzymes of primary mucin glycan degraders (substrate cross-feeding). These interactions can be studied, in vitro and in vivo, in cocultures or in community-level studies. Further research could explore additional defined synthetic microbial communities to elucidate the composition and function of the mucin-glycan driven cross-feeding network that exists in the gut (Vrancken et al. 2019).

Conclusion and outlook

Bacterial degradation of the mucin glycans that cover the intestinal epithelium is a complex process. To achieve complete degradation, a broad range of enzymes encoded within the mucosal microbiome is required. Here, we summarize the evidence that mucin glycan degradation can take place in a network of cross-feeding commensals. First, several studies have shown in vitro or in vivo cross-feeding on mucin between different combinations of mucin glycan degraders and partner organisms, which leads to the production of the beneficial SCFAs. Second, the mucin glycan degraders possess different palettes of GHs that are potentially involved in mucin glycan degradation, suggesting that they could complement each other. Third, a large portion of the enzymes involved in mucin glycan degradation is extracellular, which implies that the products of these enzymatic conversions are released into the environment and may be (partially) available to other organisms.

We described that mucin glycan degraders encode multiple GH families that release the same subunit. In the PMD consortium that was defined for our overview, five GH families that are potentially involved in mucin glycan degradation encode β-galactosidases. This finding suggests that the different GH families have different specificities and therefore play different roles and complement each other. For example, GH98 β-galactosidases exclusively target the galactose in blood group glycoconjugates (Shaikh and Boraston 2012).

Most mucin glycan degraders encode multiple GHs of the same family. Therefore, it is possible that the specificity of enzymes differs not only between but also within GH families. For example, *A. muciniphila* encodes two GH35 β-galactosidases. One of these enzymes specifically targets GNB (Galβ1–3GalNAc), while the other targets LNB (Galβ1–3GlcNAc) but is also able to hydrolyze GNB with lower efficiency (Kosciow and Deppenmeier 2019; Kosciow and Deppenmeier 2020). Possessing different strategies for accessing mucin glycans may provide bacteria with the ability to adapt to available substrates and escape competition. For example, the sialidase from *R. gnavus* releases 2,7-anhydro-Neu5Ac instead of sialic acid, which is possibly not used by other microbiota (Tailford, Owen, et al. 2015). Furthermore, not all the enzymes that are included in Table II will be involved in mucin glycan degradation, as some may target other carbohydrates in the gut. For example, *B. thetaiotaomicron* prefers utilizing dietary polysaccharides but is able to shift to mucin glycan degradation in the absence of these substrates (Sonnenburg et al. 2005; Rogers et al. 2013). This is reflected in the total number of GHs potentially involved in mucin degradation when comparing

generalist glycan degrader *B. thetaiotaomicron* (107 sequences) to specialist mucin degrader *A. muciniphila* (43 sequences). However, it is also possible that one enzyme targets multiple gut carbohydrates, as some enzymes discussed here have been shown to target both HMOs and mucin glycans (Marcobal et al. 2011). By contrast, the mucin glycan degraders may encode multiple genes of the same GH family to achieve functional redundancy (Belzer 2021). Functional redundancy within the mucosal microbiome could increase stability and resilience. Furthermore, a mucin glycan degrader may encode multiple genes with the same function as a form of regulation in the absence of strict transcriptional and translational regulation.

Phylogenetic analyses of the GH families that are potentially involved in mucin glycan degradation provides evidence for both highly specific enzymes and functional redundancy. Some GH sequences do not cluster together with others from the same family, which could possibly mean that these sequences have a different specificity. However, the organisms included in this review are limited to known mucin glycan degraders that are included in the CAZy database, so organisms that were not included here may encode similar enzymes. Other GH sequences have close relatives in mucin glycan degraders within the PMD consortium. This finding provides evidence for functional redundancy in the gut mucosal microbiome. However, it could be that these bacteria compete and fulfill a similar function in the gut microbiome of different individuals but do not co-occur.

Mucosal residents may encode additional enzymes that can contribute to the degradation of mucin glycans, which are not yet included in the CAZy database. On top of that, there are mucin glycan degraders that are still unknown, or have not yet been sequenced. Therefore, the actual diversity of mucin glycan degrading enzymes is even greater than presented in this PMD consortium.

Considerably, more studies need to be done to determine the exact palette of GHs that target mucin glycans. Culturing and sequencing of currently unknown mucin glycan degraders will lead to a completer view of the enzymatic capacity of these bacteria. Transcriptomic studies can elucidate which GHs are active during growth on mucin, and metabolomic studies can provide insights into the substrate specificities of these GHs. Together, these studies will provide more evidence for mucin glycan degradation as a concerted process that involves cooperation of multiple gut microorganisms with different palettes of GHs.

Supplementary data

Supplementary data are available at *Glycobiology* online.

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Conflict of interest statement

None declared.

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Abbreviations

2,7-anhydro-Neu5Ac, 2,7-anhydro-α-*N*-acetylneuraminic acid; BSM, bovine submaxillary mucin; CAZyme, carbohydrate-active enzyme; CE, carbohydrate esterase; Gal, galactose; GalNAc, *N*-acetylgalactosamine; GF, germ-free; GH, glycosyl hydrolase; GlcNAc, *N*-acetylglucosamine; GlcNAc-6P, *N*-acetylglucosamine-6-phosphate; GNB, galacto-*N*-biose; HIM, human intestinal mucin; HMO, human milk oligosaccharide; IBD, inflammatory bowel disease; IT-sialidase, intramolecular trans-sialidase; LacNAc, *N*-acetyllactosamine; LNB, lacto-*N*-biose; LNT, lacto-*N*-tetraose; LNT2, lacto-*N*-triose; Neu5Ac, *N*-acetylneuraminic acid (sialic acid); PCM, pig colonic mucin; PGM, pig gastric mucin; pPGM, purified pig gastric mucin; ppPGM, partially purified pig gastric mucin; PL, polysaccharide lyase; PMD, primary mucin degraders; PUL, polysaccharide utilization locus; SCFA, short-chain fatty acid; SRB, sulfate-reducing bacteria

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