Characterizing the mucin-degrading capacity of the human gut microbiota

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Mucin-degrading microbes are known to harbor glycosyl hydrolases (GHs) which cleave specific glycan linkages. Although several microbial species have been identified as mucin degraders, there are likely many other members of the healthy gut community with the capacity to degrade mucins. The aim of the present study was to systematically examine the CAZyme mucin-degrading profiles of the human gut microbiota. Within the Verrucomicrobia phylum, all Akkermansia glycaniphila and muciniphila genomes harbored multiple gene copies of mucin-degrading GHs. The only representative of the Lentisphaerae phylum, Victivallales, harbored a GH profile that closely mirrored Akkermansia.

In the Actinobacteria phylum, we found several Actinomadura, Actinomyces, Bifidobacterium, Streptacidiphilus and Streptomyces species with mucin-degrading GHs. Within the Bacteroidetes phylum, Alistipes, Alloprevotella, Bacteroides, Fermentomonas Parabacteroides, Prevotella and Phocaeicola species had mucin degrading GHs. Firmicutes contained Abiotrophia, Blautia, Enterococcus, Paenibacillus, Ruminococcus, Streptococcus, and Viridibacillus species with mucin-degrading GHs. Interestingly, far fewer mucin-degrading GHs were observed in the Proteobacteria phylum and were found in Klebsiella, Mixta, Serratia and Enterobacter species. We confirmed the mucin-degrading capability of 23 representative gut microbes using a chemically defined media lacking glucose supplemented with porcine intestinal mucus. These data greatly expand our knowledge of microbial-mediated mucin degradation within the human gut microbiota.

The intestinal mucus layer is a major component of the boundary region separating the luminal contents from the gut mucosa. Mucus functions as a barrier, a lubricant, an immune cell signal, a reservoir of signaling peptides, and a habitat for indigenous enteric bacteria1–6. The intestinal mucus layer is produced by specialized cells known as goblet cells. In the mammalian intestine, goblet cells synthesize and secrete the mucin protein MUC2. The MUC2 protein is decorated with O-glycans, which have core structures of α- and β-linked N-acetyl-glucosamine, N-acetyl-galactosamine, and galactose. These core structures are elongated and commonly modified by α-linked fucose and sialic acid residues3. These structurally complex mucin glycans make up approximately 80% of mucin mass. As glycoproteins, mucus can serve as a nutrient source for the resident gut microbes. Bacteria that harbor specific glycosyl hydrolases (GHs) are capable of enzymatically degrading mucin glycans. These released glycan oligosaccharides can then be used as a primary carbohydrate source for the mucus-associated microbiota, providing a sustainable and consistent nutrient supply2. It has been speculated that the ability to cleave and metabolize mucin O-linked glycans may be an important factor in determining which bacterial species colonize the outer mucus layer.

The degradation of mucin glycans requires the cooperative action of several glycosyl hydrolases encoded by the genomes of mucin-degrading bacteria2,3,9. To access mucin glycans, intestinal microbes must express the GH33 sialidases (also known as neuraminidases), which cleave terminal sialic acid residues. Microbes may also produce GH29 or GH95 to remove fucose residues. Following the removal of terminal sugars, bacteria can harbor N-acetyl-glucosaminidases (GH184, GH185, GH89, GH20), N-acetyl-galactosaminidases (GH101, GH129), and galactosidases (GH2, GH35, GH42, GH98). There are also endo-acting O-glycanases (GH16) which can cleave large glycan structures. Known mucin-degrading bacterial strains include Akkermansia muciniphila, Bacteroides spp., Bifidobacterium spp., Ruminococcus spp., Clostridium spp., Paraclostridium spp. and Prevotella spp.3,8–24. The best studied mucin degrading microbes are Akkermansia muciniphila and Bacteroides spp. Akkermansia muciniphila is considered to be a mucin-specialist, as it can employ several enzyme combinations to hydrolyze up to 85% of mucin structures9. Bacteroides spp. are general glycan degraders and certain Bacteroides spp. are able

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to switch from dietary glycans to mucin glycans due to their extensive arrays of carbohydrate-active enzymes. For example, *B. thetaiotaomicron* can extensively degrade mucin glycans and forage in the mucus layer when plant polysaccharides are absent from the diet. Despite the growing number of bacterial genome sequences available, our knowledge of the mucin-degrading capacity of other microbes, particularly commensal human gut microbes, remains fragmented. The aim of the present study was to systematically examine the CAZyme mucin-degrading profiles of the human gut microbiota.

### Methods

#### Bacterial culturing. The following strains were grown anaerobically at 37 °C in brain-heart-infusion (BHI) supplemented with 2% yeast extract and 0.2% cysteine: *Bacteroides vulgatus* ATCC 8482, *Bacteroides thetaiotaomicron* ATCC 29148, *Bacteroides fragilis* MHG 10513, *Blautia coccosphaerae* ATCC 29236, *Blautia producta* ATCC 27340D, *Parabacteroides distasonis* MHG 10511, *Clostridium butyricum* CB, *Clostridium symbiosum* ATCC 14940, *Clostridium innocuum* ATCC 14501, *Clostridium clostridiforme* ATCC 25532, *Clostridium sporogenes* DSMZ 795, and *Prevotella copri* DSMZ 18205. *Akkermansia muciniphila* ATCC BAA-835 was grown in BHI supplemented with 2% yeast extract, 0.2% cysteine, and 0.4% porcine gastric mucin (Sigma). The following strains were grown anaerobically at 37 °C in Man-DeRosa-Sharp (MRS): *Lactobacillus gasseri* ATCC 33323, *Lactobacillus johnsonii* ATCC 33200, *Lactobacillus brevis* ATCC 27305, *Lactobacillus acidophilus* ATCC 4796, *Bifidobacterium dentium* ATCC 27678, *Bifidobacterium longum* subsp. *infantis* ATCC 15697, *Bifidobacterium bifidum* ATCC 29521, *Bifdobacterium longum* ATCC 55813, and *Bifidobacterium angulatum* ATCC 27355. All cultures were grown in an Anaerobe Systems AS-150 anaerobic chamber supplied with a mixture of 10% CO2, 5% H2, and 85% N2. *E. coli* Nissle 1917 was grown aerobically at 37 °C in LB broth.

To assess mucin-degradation, overnight cultures were centrifuged at 6000×g for 5 min to pellet the bacteria and the bacterial pellet was washed 3× to remove traces of the rich media. After the final wash, the bacterial pellet was resuspended in an equal volume of a chemically defined culture medium ZMB1 lacking glucose and sub-cultured to an optical density (OD600nm) of 0.1. The culture conditions included: (1) ZMB1 lacking glucose, (2) ZMB1 with 100 mM Glucose or (3) ZMB1 lacking glucose with 1 mg/mL pig intestinal mucin (MyBioSource cat# MBS2028824 > 90% purity, dialyzed in water with SnakeSkin™ Dialysis Tubing, 10 K MWCO, FisherSci #P168100). All cultures were grown anaerobically at 37 °C and growth was monitored by measuring OD600nm after 20 hours of incubation.

#### Computational analysis. The glycosyl hydrolase (GH) families involved in mucin degradation were downloaded from the Carbohydrate-Active enZYmes (CAZy) database (https://www.cazy.org) and examined as previously described. Gene copy numbers were collected from all annotated genomes. The glycosyl hydrolases known to be involved in mucin degradation (GH33, 16, 29, 95, 20, 2, 35, 42, 98, 101, 129, 89, 85, and 84) were included for analysis. Of the 20,954 genomes available in the CAZy database, we identified 13,156 genomes containing at least one gene copy of GH33, GH16, GH29, GH95, GH20, GH2, GH35, GH84, and GH89, indicating that at least 60% of the genomes contained at least one gene copy of these GH families.

#### Statistics. GraphPad Prism (version 9) software (GraphPad Inc., La Jolla, CA) was used for all statistics. Growth was examined using a one-way analysis of variance (ANOVA). Differences between the groups were considered significant at P < 0.05 (*).
of GH33 (Fig. 2C), indicating that Akkermansia spp. have the capacity to remove sialic acid and initiate mucin-degradation. The GHs with the largest gene copy range (6–13 gene copies) was GH20, a family containing β-N-acetyl-glucosaminidases (Table 1). No Akkermansia genomes contained GH42, 98, 101, 129 or 85, suggesting that Akkermansia is unable to degrade N-acetyl-galactosamine. To confirm the capacity of A. muciniphila to degrade intestinal mucus, we grew A. muciniphila ATCC BAA-835 is a chemically defined media ZMB1 lacking glucose, containing 100 mM glucose or containing 1 mg/mL porcine intestinal mucus (Fig. 2D). As expected, A. muciniphila had limited growth in ZMB1 with or without glucose but exhibited robust growth in media with porcine intestinal mucus. These findings complement our genome analysis of A. muciniphila ATCC BAA-835 (the BAA-835 genome analysis is found in the second column from the right in Fig. 2C). Additionally, various A. muciniphila strains were also examined to showcase the diversity of GH profiles across the genus, which supports the ability of this species to degrade mucus.

Figure 1. Human gut microbes harboring mucin associated glycosyl hydrolases (GHs) are well distributed among the bacterial phyla. (A) Distribution of genera within each bacteria phyla that possess mucin-related GHs. Distribution of genera within (B) Verrucomicrobia, (C) Lentisphaerae, (D) Actinobacteria, (E) Bacteroidetes, (F) Proteobacteria and (G) Firmicutes phyla that harbor at least one mucin-related GH.
Mucin-related glycosyl hydrolase profiles in the Verrucomicrobia and Lentisphaerae phylum. (A) Representative intestinal mucin glycans structures and corresponding microbial GHs. (B) Heat map of the percentage of Akkermansia glycaniphila or Akkermansia muciniphila genomes that have at least one gene copy of mucin-associated GH in each genome. (C) Heat map depicting the gene copy number of mucin-associated GHs in the strains of Akkermansia and A. muciniphila. (D) Growth analysis of A. muciniphila ATCC BAA-835 in a chemically defined media ZMB1 lacking glucose (media control), with glucose (positive control), or lacking glucose and supplemented with 1 mg/mL porcine intestinal MUC2. Growth was measured by examining the optical density at 600 nm (OD600) after overnight incubation. (E) Heat maps showing the percentage of genomes that have at least one gene copy of each mucin-associated GH and depicting the gene copy number of mucin-associated GHs in the one strain of Victivallales bacterium.

Next, we examined Victivallales bacterium in the Lentisphaerae phylum (Fig. 2E). Genome analysis revealed a similar GH profile to Akkermansia, with genes for GH33, GH16, GH29, GH95, GH20, GH2, GH35, and GH89, suggesting that Victivallales bacterium could enzymatically cleave sialic acid, fucose, galactose, and N-acetyl-glucosamine. Interestingly, Victivallales also harbored gene copies of GH42 and GH129, GHs not found in Akkermansia. The presence of GH129 indicates that Victivallales bacterium can release N-acetyl-galactosamine, a glycan which Akkermansia is not able to cleave. Victivallales bacterium possessed 4 genomes copies of GH33 and 19 gene copies of GH2, which contains β-galactosidases. Although little information is available for Victivallales bacterium, the genome analysis reveals that Victivallales bacterium could degrade mucins.

Within the Actinobacterium phylum, we identified several genera harboring mucin-degrading GHs, including Actinomadura, Actinomyces, Bifidobacterium, Streptacidiphilus and Streptomyces species (Fig. 3A). We observed that 3 of the 4 Actinomadura spp. had 1 gene copy of GH33, as well as gene copies of GH16, GH20, GH2, GH35, GH84 and GH89, suggesting the ability of Actinomadura spp. to remove sialic acid, galactose and N-acetyl-glucosamine (Fig. 3B). We found that all the genomes of Actinomycys israelii, A. naeslundii, A. viscosus and A. weissii, as well as 5 of the 7 genomes of undefined Actinomyces spp. contained 2–3 gene copies of GH33. Actinomyces members also contained gene copies for GH16, GH29, GH20, GH2, GH35, GH42, and GH101. This glycosyl hydrolase profile indicates the Actinomyces spp. can potentially cleave all mucin glycans.

In Bifidobacterium (Fig. 3C), we found that all 11 genomes of B. bifidum had 1–3 gene copies of GH33 and all genomes had GH29, GH95, GH20, GH2, GH42, GH101, GH129, GH89 and GH84. Additionally, 8 of the 11 B. bifidum genomes had one gene copy of GH16. These GH profiles are consistent with previous studies which identify B. bifidum as a mucin degrading microbe since it can remove all mucin glycans. Within the 44 R. breve genomes, we found that 41 genomes had one gene copy of GH33 and the majority of strains had GH95, GH20, GH2, GH42, and GH129, covering all mucin glycan structures (Fig. 3C). B. longum had much more variability in terms of mucin-degrading GHs (Fig. 3D). Only 11 of the 54 genomes contained GH33, the majority of which belonged to the B. longum subspecies infantis subgroup. Variable presence for GH29, GH95, GH20, GH2, GH42, GH101, GH129 and GH85 was identified, with genomes harboring 0–5 gene copies. In contrast, B. angulatum only possessed 2 mucin-associated GHs: GH2 and GH42, suggesting that this species is likely unable to extensively degrade mucins. These data indicate that mucin degradation is species dependent in Bifidobacterium.

Within the two genomes of Streptacidiphilus spp. (Fig. 3E), we found that one of the two genomes had one gene copy of GH33 (sialidase), but both genomes had 10 gene copies of GH16 (endo-O-glycanase) as well as the genes for GH20, GH35, and GH42. Commensal Streptomyces lavendulae, S. lividans, and S. pactum genomes contained GH33, GH95, GH20, GH2, GH35, GH42, and variable presence of GH101, 89 and 84. Among the 113 undefined Streptomyces spp. genomes (Fig. 3E,F), we found that 80 genomes had 1–5 gene copies of GH33, and the majority of strains had gene copies for GH16, GH29, GH95, GH20, GH2, GH35, GH42 and GH84. Streptomyces spp. had several copies of GH2, with some strains possessing 10 gene copies. These data suggest that Streptomyces species are well adapted to remove sialic acid, fucose, galactose, and N-acetyl-glucosamine.

To confirm our genome findings, we also examined the growth of key Bifidobacteria in ZMB1 with or without glucose or intestinal mucus (Fig. 3G). Our genome analysis revealed that B. dentium ATCC 27678 and B. angulatum ATCC 27535 did not possess GH33 and had only 2–3 mucin-associated GHs, while B. longum and B. bifidum had several gene copies of GH33 and other mucin-degrading GHs. In our growth analysis, we did not detect growth above the ZMB1 media baseline when intestinal mucus was added, indicating that these species cannot degrade intestinal mucus to use as a carbon source. In contrast, B. longum subs. infantis ATCC 15697, B. longum ATCC 55813, and B. bifidum ATCC 29521 had enhanced growth when mucus was present, indicating that these strains can degrade mucins.

Analysis of genomes within the Bacteroidetes phylum revealed mucin degrading GHs in Alistipes, Alloprevotella, Bacteroides, Fermentimonas, Parabacteroides, Prevotella and Phocaeicola species (Fig. 4A). Only 2 of the 5 Alistipes spp. genomes had one gene copy of GH33, but all genomes had GH20 and GH2 (galactosidase) and most genomes had GH16 (endo-O-glycanase) and GH29 (fucosidase) (Fig. 4B). The one genome of Alloprevotella had GH33, GH16, GH29, GH95, GH20, GH2, GH89, GH85 and GH84, one more GH family than Akkermansia, potentially indicating that this microbe could be a mucin-degrader. The one genome of Fermentimonas caenicola also had gene copies of GH33, GH16, GH29, GH95, GH20, GH2, and GH42. Although there are few reports on this microbe, the GH profiles suggest that this Fermentimonas caenicola could also be a mucin degrader. Consistent with the literature, we found a large repertoire of GHs involved in mucin degradation in the Bacteroides spp. genomes (Fig. 4C). We found that 3 of the 4 B. caccae genomes had 2–3 gene copies of GH33 and all genomes had gene copies of GH16 (endo-O-glycanase), GH95 (fucosidase), GH2 (galactosidase), and 84 (N-acetyl-glucosaminidases). Additionally, 3 of the 4 genomes had gene copies for GH29, GH20, and GH35 families. All of the two B. cellulositylicus genomes harbored GH33, GH16, GH29, GH95, GH20, GH2,
| GH family | Enzyme commission # | Description                                                                 |
|-----------|---------------------|-----------------------------------------------------------------------------|
| 2         | EC 3.2.1.23         | β-galactosidase                                                             |
|           | EC 3.2.1.25         | β-mannosidase                                                               |
|           | EC 3.2.1.31         | β-glucuronidase                                                             |
|           | EC 3.2.1.55         | α-L-arabinofuranosidase                                                     |
|           | EC 3.2.1.152        | Mannosylglycoprotein endo-β-mannosidase                                      |
|           | EC 3.2.1.165        | Exo-β-glucosaminidase                                                       |
|           | EC 3.2.1.4-         | α-L-arabinopyranosidase                                                     |
|           | EC 3.2.1.7          | β-galacturonidase                                                           |
|           | EC 3.2.1.37         | β-xylanidase                                                                |
|           | EC 3.2.1.146        | β-D-galactofuranosidase                                                     |
|           | EC 3.2.1.21         | β-glucosidase                                                               |
| 16        | EC 2.4.1.207        | Xyloglucan:xyloglucosyltransferase                                          |
|           | EC 3.2.1.103        | Keratan-sulfate endo-1,4-β-galactosidase                                    |
|           | EC 3.2.1.39         | Endo-1,3-β-glucanase/laminaranine                                           |
|           | EC 3.2.1.6          | Endo-1,3(4)-β-glucanase                                                    |
|           | EC 3.2.1.73         | Licheninase                                                                 |
|           | EC 3.2.1.81         | β-agarase                                                                   |
|           | EC 3.2.1.83         | α-carrageenase                                                              |
|           | EC 3.2.1.151        | Xyloglucanase                                                               |
|           | EC 3.2.1.181        | Endo-β-1,3-galactanase                                                      |
|           | EC 3.2.1.178        | β-porphyranase                                                              |
|           | EC 3.2.1.35         | Hyaluronidase                                                               |
|           | EC 3.2.1.-          | Endo-β-1,4-galactosidase                                                    |
|           | EC 2.4.1.-          | Chitin β-1,6-glucanoyltransferase                                           |
|           | EC 2.4.1.-          | β-transglycosidase                                                          |
|           | EC 3.2.1.-          | β-glycosidase                                                               |
|           | EC 3.2.1.-          | α-carrageenase                                                              |
| 20        | EC 3.2.1.52         | β-hexosaminidase                                                            |
|           | EC 3.2.1.140        | Lacto-N-biosidase                                                           |
|           | EC 3.2.1.-          | β-1,6-N-acetylglucosaminidase                                               |
|           | EC 3.2.1.-          | β-6-SO3-N-acetylglucosaminidase                                             |
| 29        | EC 3.2.1.51         | α-L-fucosidase                                                              |
|           | EC 3.2.1.111        | α-1,3/1,4-L-fucosidase                                                      |
|           | EC 3.2.1.63         | α-1,2-L-fucosidase                                                          |
| 33        | EC 3.2.1.18         | Sialidase or neuraminidase                                                  |
|           | EC 2.4.1.-          | Trans-sialidase                                                             |
|           | EC 4.2.2.15         | Anhydrosialidase                                                            |
|           | EC 3.2.1.-          | Kdo hydrolase                                                               |
|           | EC 3.2.1.-          | 2-Keto-3-deoxyxynononic acid hydrolase/KDNase                               |
| 35        | EC 3.2.1.23         | β-galactosidase                                                             |
|           | EC 3.2.1.165        | Exo-β-glucosaminidase                                                       |
|           | EC 3.2.1.-          | Exo-β-1,4-galactanase                                                       |
|           | EC 3.2.1.-          | β-1,3-galactosidase                                                         |
| 42        | EC 3.2.1.23         | β-galactosidase                                                             |
|           | EC 3.2.1.-          | α-L-arabinopyranosidase                                                     |
| 84        | EC 3.2.1.52         | N-acetyl β-glucosaminidase                                                  |
|           | EC 3.2.1.35         | Hyaluronidase                                                               |
|           | EC 3.2.1.169        | [Protein]-3-O-(GlcNAc)-l-Ser/Thr β-N-acetylglucosaminidase                  |
| 85        | EC 3.2.1.96         | Endo-β-N-acetylglucosaminidase                                              |
| 89        | EC 3.2.1.50         | α-N-acetylglucosaminidase                                                   |
| 95        | EC 3.2.1.51         | α-L-fucosidase                                                              |
|           | EC 3.2.1.63         | α-1,2-L-fucosidase                                                          |
|           | EC 3.2.1.-          | α-L-galactosidase                                                           |
| 98        | EC 3.2.1.102        | Endo-β-1,4-galactosidase                                                    |
|           | EC 3.2.1.-          | Endo-β-1,4-galactosidase                                                    |
|           | EC 3.2.1.8          | endo-β-1,4-xylanase                                                         |

Continued
with all genomes harboring GH33, GH16, GH29, GH95, GH20, GH2, GH35, GH84 (Fig. 4F). In addition, and N-acetyl-glucosamine. The Parabacteroides genomes mirrored the GH84. These data indicate that most while all the P. melaninogenica genomes we found that 11 of the 12 had GH33 genes, P. melaninogenica P. jejuni many GH95, GH20, GH2, GH45, GH42 and 5 of the 6 genomes containing GH89. These data support the notion that Bacteroides xylanisolvens All
B. thetaiotaomicron Bacteroides GH35, GH89 and GH84. Additionally, 17 of 18 of the genomes also had GH95. We also examined 7 undefined Enterobacter spp., and found gene copies of GH33, GH16, GH29, GH29, GH95, GH20, GH2, and GH48 (Fig. 4D). All B. uniformis members also had 1 gene copy of GH42 and B. vulgatus had 1 gene copy of GH42 and GH89. B. xylanisolvens genomes mirrored the other Bacteroides spp., with all 6 genomes harboring GH33, GH16, GH29, GH95, GH20, GH2, GH45, GH42 and 5 of the 6 genomes containing GH89. These data support the notion that many Bacteroides members are mucin-degraders.

Within the 16 Prevotella genomes (Fig. 4E), we found that the one P. copri and three P. jejuni genomes had GH33 gene copies for GH33, GH16, GH29, GH85, GH20, and GH2. P. jejuni also had 1–3 gene copies of GH85 and GH84. Furthermore, in the 12 P. melaninogenica genomes we found that 11 of the 12 had GH33 genes, while all the P. melaninogenica genomes had 1–4 gene copies of GH16, GH29, GH95, GH20, GH2, GH85, and GH84. These data indicate that most P. melaninogenica are well adapted to cleave sialic acid, fucose, galactose, and N-acetyl-glucosamine. The Parabacteroides genomes mirrored the Prevotella spp. mucin-degrading GHSs, with all genomes harboring GH33, GH16, GH29, GH95, GH20, GH2, GH35 and GH84 (Fig. 4F). In addition, within Bacteroidetes we found that all the 16 Phocaeicola spp. genomes contained genes for GH33, GH16, GH29, GH95, GH20, GH2, GH35, GH89 and GH84. We observed that 15 of the 16 Phocaeicola spp. genomes also had one gene copy of GH42 (Fig. 4G). We observed multiple gene copies of GH2 in Bacteroidetes. B. cellulosilyticus possessed the most GH2 gene copies, with 44 gene copies in total. To confirm the mucin-degrading capacity of B. vulgatus, B. fragilis, and B. thetaiotaomicron, we grew B. vulgatus ATCC 8482, B. fragilis MGH 10513 and B. thetaiotaomicron ATCC 29148 in ZMB1 with or without glucose or intestinal mucus (Fig. 4H). As expected, each of these microbes grew in ZMB1 lacking glucose supplemented with intestinal mucus, consistent with previous literature showing these microbes can degrade mucins. We also grew Prevotella copri DSMZ 18205 and Parabacteroides merdae MGH 10511 to assess the ability of these Prevotella strains to grow in the presence of mucus. P. merdae did not grow in mucus, which was consistent with our analysis which showed no gene copies of GH33. Interestingly, although the one genome of P. copri in our analysis had GH33 expression and other mucin-degrading GHSs, our P. copri DSMZ 18205 strain did not grow in ZMB1 lacking glucose with mucus, suggesting that growth might be strain specific.

Compared to the mucin-degrading microbes identified in other phyla, we observed far fewer mucin-degrading GHSs in the Proteobacteria phylum, with only 3–4 GHS families found in Klebsiella, M. catarrhalis and Enterobacter spp. (Fig. 5A). All 46 of the Klebsiella aerogenes genomes had one gene copy of GH33 (sialidase) and 1–2 gene copies of GH2 (galactosidase). Ten of the 46 K. aerogenes genomes also had expression of GH42 (galactosidase) and 41 of the genomes had 1–2 gene copies of GH20 (galactosidase), suggesting the ability of these strains to remove galactose residues (Fig. 5B). Similarly, all 23 undefined Klebsiella spp. genomes had 1–3 gene copies of GH2, but only 13 of the 23 genomes had GH33, 14 genomes had GH42 genes and 3 of the genomes had GH20 (Fig. 5C). No other mucin-degrading GHS genes were observed. Of the four Mixta spp., which includes M. catarrhalis and M. intestinalis, we found that all three genomes had 1–2 gene copies of GH33, GH20 and GH2, but no other mucin-related GHSs were identified (Fig. 5D). We observed large variation in the 8 Serratia fonticola genomes. Only one of the genomes had GH33, 6 genomes had GH116, 7 genomes had GH20 and all 8 genomes had GH22 gene copies. In the Enterobacter genera, only 15 of the 73 E. cloacae genomes had one gene copy of GH33, although most of the strains had GH20 and GH22 gene copies (Fig. 5E). Similarly, only 5 of the 36 undefined Enterobacter spp. had GH33, while almost all the strains had GH20 and GH2 (Fig. 5F). Growth analysis of E. coli Nissle 1917 in ZMB1 with or without mucus, which was not one of the E. coli with GH33 expression in our genome analysis, confirmed the inability of this species to use mucus as the sole carbon source (Fig. 5G). These data suggest that commensal Proteobacteria are far less adept at degrading mucin than their gut microbiota counterparts.

Finally, we examined the Firmicutes phylum and found that Abiotrophia, Blautia, Enterococcus, Paenibacillus, Ruminococcus, Streptococcus, and Viridibacillus species harbored several mucin-degrading GHSs (Fig. 6A–C). The one genome of Abiotrophia defectiva had one gene copy of GH33 and 1–2 gene copies of GH29, GH20, GH2, GH35, GH101, and GH85. Within Blautia, B. cocoides and B. hansenii had one gene copy of GH33 and genes for GH29, GH95, GH20, GH2, GH101, GH83 and GH84 (Fig. 6D). In contrast, B. obeum, B. producta and undefined Blautia spp. had no gene copies of GH33, but did have variable gene copies (0–20) of GH16, GH29, GH20, GH2, GH35, and GH42, indicating the ability to remove fucose and galactose. Among the Enterococcus strains, only 1 of the 4 E. casseliflavus strains, 1 of the 4 E. durans strains, 2 of the 4 E. gallinarum, and 1 of the 4 undefined Enterococcus spp. possessed GH33. Variable numbers of gene copies were observed in GH29, GH95,
GH20, GH2, GH35, GH42 and GH85. In *Paenibacillus* (Fig. 6E), we observed that *P. barcinonensis* and *P. latus* genomes had one gene copy of GH33 and both genomes harbored GH16, GH29, GH95, GH2, and GH35, while *P. latus* also had gene copies for GH20 and GH85. Of the 29 genomes of undefined *Paenibacillus* spp., we found
that only 4 strains had GH33, but the majority of strains had gene copies of GH16, GH29, GH95, GH20, GH2, GH35 and GH42, suggesting that *Paenibacillus* spp. can remove fucose and galactose. We observed that all three *Ruminococcus gnavus* genomes had one gene copy of GH33, while undefined *Ruminococcus* spp. and *R. torques* did not harbor GH33 (Fig. 6F). Most *Ruminococcus* strains possessed GH29, GH85, GH2 and GH42. Among the streptococci, we found that all 8 *S. intermedius* genomes contained GH33, GH29, GH20, GH2, GH35, and GH85 (Fig. 6G). We also observed that 6 of the 9 *S. mitis* spp. had GH33 and most strains had gene copies of GH29, GH95, GH20, GH2, GH35 and GH85. Only one genome was available for *Viridibacillus* spp. and this genome had GH33 and GH35.

Pathogenic *Clostridium* spp., such as *C. perfringens*, have previously been shown to degrade mucins, but little information exists on mucin degradation by commensal *Clostridium* spp. of the 14 *C. butyricum* genomes, we found that only one of the genomes harbored GH33 and none of the *C. sporogenes* or undefined *Clostridium* spp. possessed GH33 (Fig. 6H). Compared to other species, commensal *Clostridium* spp. had only a few mucin-associated GHS, including GH16, GH95, and GH42. These profiles suggest that commensal *Clostridium* spp. are unlikely to be involved in substantial mucin degradation. Based on our genome analysis, we predicted that commensal *Clostridium* spp. could not degrade intact mucus and use mucus to enhance growth. To address this question, we examined the growth of several *Clostridium* spp., including *C. butyricum* CB, *C. symbiosum* ATCC 14940, *C. inoculum* ATCC 14501, *C. clostridiforme* ATCC 25532, and *C. sporogenes* DSMZ 795 in media with

**Figure 4.** Mucin-related glycosyl hydrolase profiles in the Bacteroidetes phylum. (A) Heat map of the genera within the Bacteroidetes phylum that have at least one gene copy of each mucin-associated GH 33, 16, 29, 95, 20, 2, 35, 42, 98, 101, 129, 89, 85, and 84. Heat map showing the gene copy number of mucin-associated GHS in the strains of (B) *Alistipes*, *Alloprevotella*, and *Fermenitomonas*, (C) *Bacteroides*, specifically *B. caccae*, *B. dorei*, *B. intestinalis*, *B. fragilis* and *B. ovatus*, (D) *Bacteroides*, specifically *Bacteroides* spp., *B. thetaiotaomicron*, *B. uniformis*, and *B. xylanisolvens*, (E) *Prevotella copri*, *P. jejuni*, and *P. melaninogena*, (F) *Parabacteroides* and *P. distasonis*, and (G) *Phocaeicola coprophilus*, *P. dorei*, and *P. vulgatus*. (H) Growth analysis of *Bacteroides vulgatus* ATCC 8482, *B. thetaiotaomicron* ATCC 29148, *B. fragilis* MGH 10513, *Prevotella merdae* MGH 10511, and *Prevotella copri* DSMZ 18205 in a chemically defined media ZMB1 lacking glucose (media control), with glucose (positive control), or lacking glucose and supplemented with 1 mg/mL porcine intestinal MUC2. Growth was measured by examining the optical density at 600 nm (OD600nm) after overnight incubation.
or without mucus (Fig. 6I). Consistent with our analysis, none of the *Clostridium* spp. had enhanced growth with mucus. Our genome analysis indicated that *Blautia coccoides* possessed multiple GHs involved in mucin degradation and we predicted that this strain would be capable of using mucin glycans as the sole carbon source. Similar to our GH profile, we found that *B. coccoides* had statistically significant growth with mucus compared to media without mucus. Finally, we examined *Lactobacillus*, which according to our genome analysis only have 1–4 mucin-associated GHs and do not harbor GH33. We grew *Lactobacillus gasseri* ATCC 33323, *L. johnsonii* ATCC 33200, *L. brevis* ATCC 27305, and *L. acidophilus* ATCC 4796 in media with and without mucus and found that mucus did not enhance the growth of many *Lactobacillus* spp. (Fig. 6J). These data provide a comprehensive analysis of mucin-associated GH profiles within commensal gut microbes and highlight that only specific gut strains have mucin-degrading capacity.

**Discussion**

To survive in the ever-changing environment of the gastrointestinal tract, gut microbes must be adept at foraging for nutrient sources. One way microbes deal with the varying availability of dietary carbohydrates is to forage glycans in the host mucus layer. Mucin glycans are degraded by the sequential action of multiple mucin-associated GHs. Sialic acid residues that terminate mucin glycans have been proposed to limit glycan degradation, thereby
Figure 6. Mucin-related glycosyl hydrolase profiles in the Firmicutes phylum. (A–C) Heat map of the Firmicutes genomes that have at least one gene copy of mucin-associated GHs 33, 16, 29, 95, 20, 2, 35, 42, 98, 101, 129, 89, 85, and 84. Heat map showing the gene copy number of mucin-associated GHs in the strains of (D) Abiotrophia defectiva, Blautia coccoides, B. hansenii, B. obeum, B. producta, Blautia spp., Enterococcus casseliflavus, E. durans, E. gallinarum, and Enterococcus spp., (E) Paenibacillus, specifically Paenibacillus spp., P. barcinonensis and P. lautus, (D) Ruminococcus, including Ruminococcus spp., R. gnavus and R. torques, (F) Streptococcus, including S. australis (Sa), S. intermedius (Si), S. mitis (Sm), Streptococcus spp. and Viridibacillus spp. (G) Clostridium, including C. butyricum (Cb), C. sporogenes (Cs), and Clostridium spp. (H) Growth analysis of Clostridium butyricum CB, Clostridium symbiosum ATCC 14940, Clostridium innocuum ATCC 14501, Clostridium clostridiforme ATCC 25532, and Clostridium sporogenes DSMZ 795 (H), as well as Lactobacillus gasseri ATCC 33323, L. johnsonii ATCC 33200, L. brevis ATCC 27305, L. acidophilus ATCC 4796 (I) in a chemically defined media ZMB1 lacking glucose (media control), with glucose (positive control), or lacking glucose and supplemented with 1 mg/mL porcine intestinal MUC2. Growth was measured by examining the optical density at 600 nm (OD600) after overnight incubation.
protecting the mucin glycan structure. Mucin glycans are also commonly terminated by fucose residues. As a result, mucin-degrading microbes commonly encode siaIidases and fucosidases to remove the terminal glycan structures, which then allow access to the extended core structures. Freed monosaccharides can then be used by the mucin-degrading bacteria themselves or scavenged by other bacteria. Our comparative genomic approach has revealed that many gut microbes found in healthy individuals possess GH33 and other mucin-degrading GHs, indicating that these microbes have the capacity for extensive mucin degradation. Consistent with other findings, we found that Akkermansia harbored 9 different GH families and A. muciniphila ATCC BAA-835 was able to grow in a chemically defined medium with porcine intestinal mucus as the sole carbon source. We also identified several commensal bacteria with mucin-associated GH profiles and GHs numbers (≥ 8 GH families) similar to Akkermansia, including Victivallales bacterium, Bifidobacterium bifidum, Streptomyces lividans, Blautia cocoides, B. hansenii, Bacteroides caccae, B. cellulosilyticus, B. dorei, B. fragilis, B. intestinialis, B. ovatus, B. thetaiotaomicron, B. uniformis, B. vulgatus, B. xylanisolvens, Fermentimonas caenicola, Parabacteroides distasonis, Phocaeicola coprophilus, P. dorei, P. vulgatus, and Paenibacillus lautus. These genomic data suggest that several gut microbes are able to completely degrade intestinal mucin glycans.

Two microbes we identified that appear to possess the ability for extensive mucin degradation are Victivallales bacterium and Fermentimonas caenicola. The family Victivallaceae has only two cultured representatives: Victivallis vadinensis strain CelloT and the uncharacterized strain NML080035. These microbes are Gram-negative and anaerobic. There are also 16S rRNA gene sequences from uncultured Victivallaceae. Culturable V. vadinensis can use galactose as a primary carbon source. In our genome analysis, we found that Victivallales bacterium possessed GH33, GH16, GH29, GH95, GH20, GH2, GH35, GH42, GH89, and GH129. Several of these glycosyl hydrolases are galactosidases that act on the terminal glycan residues to use as a carbon source. E. caenicola was isolated from the stool of a healthy Senegalese child as part of a study aiming at culturing gut microbes. F. caenicola is Gram-negative, facultatively anaerobic bacillus. Beyer et al. found using an API 50 CH strip that F. caenicola also grows with galactose. In our genome analysis, we found that F. caenicola harbors several galactosidases (GH2, GH20, and GH42), suggesting that removal of galactose from glycan core structures could promote F. caenicola growth. Our analysis also identified that the F. caenicola genome had gene copies of GH29, which contains a fucosidase. Interestingly, Beyer et al. found that F. caenicola was unable to grow with d-fucose or L-fucose. Other microbes, like B. bifidum, have been shown to cleave fucose and cross-feed other bacteria, like Eubacterium hallii, which cannot degrade complex glycans. It is possible that fucose release by F. caenicola may promote the growth of other microbes. Although there are no studies examining growth of Victivallales bacterium or F. caenicola with other mucin-related sugars or intact mucus, based on the GH profile, we predict that these microbes could use intact mucus and potentially use other mucin glycan sugars as carbon sources.

Although we focused on microbes with GH profiles indicative of more complete mucin degradation, it is well known that microbes can act in concert to break down glycan structures. In pioneering studies in the 1980s, Hoskins et al. examined fecal bacteria grown in mucin-based medium and found that 1% of the microbiota was able to use mucin as a carbon source, including the genera Bifidobacterium and Ruminococcus. Recent in silico analysis, which is not reliant on culturing techniques, has demonstrated that up to 86% of the human gut microbiota encode genes for cleavage of mucin glycans. We also found that 62% of all microbes and 83% of human gut microbes in the CAZy database encode genes for mucin-degradation. These studies, as well as our own, have found that only specific bacterial species have a sufficient repertoire of enzymes to disassemble complex mucin glycans and that the complete degradation of mucin often requires the action of several bacteria. Our analysis reveals that many bacteria possess multiple gene copies of GHs targeting internal glycans. These findings suggest that mucolytic bacteria with GH33 may initiate glycan break-down and then the less-specialized bacteria with internal glycan GHs can participate in degradation.

Based on our studies, we believe that the core GH-ome for mucin degradation includes GH33, 29, 95, and 20/35. More extensive degradation of internal glycans incorporates GH84/85/89 and 101. Our genome analysis suggests that mucin-degrading microbes possess >4 mucin-associated GHs. Additionally, microbes that extensively degrade mucin, like A. muciniphila, B. bifidum, and B. thetaiotaomicron, possess >9 mucin-associated GHs. Despite the fact that many commensal microbes are not capable of extensive mucin degradation, the GH profile of bacteria such as Clostridium indicates that they could contribute to degradation when paired with another bacteria. For example, if A. muciniphila removes sialic acid, several Clostridium species could remove fucose with GH95. After A. muciniphila removes N-acetyl-glucosamine, almost all Clostridium could remove galactose with GH2 or GH42. These cross-feeding events likely occur in vivo and contribute to the health of the mucus layer. Future studies using mucus cross-feeding will likely shed light into the complex interplay because mucin-degrading microbes.

Since mucin glycan degradation disrupts the first protection of the host mucus layer, host glycan foraging by mucolytic bacteria is commonly considered a initial stage in pathogenesis. While this notion likely only holds true for excessive mucin degradation, many consider mucin-glycan break-down to be a normal process and a key strategy for mucin-associated microbes. Given the continuous turnover of the epithelial cells and mucus in the human gastrointestinal tract, mucin degradation by commensal gut microbes is not likely to contribute to barrier dysfunction. Additionally, the capacity to degrade mucin is particularly important for early colonizers of the gut. Infants are commonly colonized with mucin-degrading Bifidobacterium bifidum, B. longum subsp. infantis, and B. breve. One study in Sweden identified the establishment of mucin-degrading bacteria during the first months of life. We speculate that mucin glycan degradation gives colonizing microbes an advantage after the termination of breast milk and allows them to exert their beneficial influence on gut homeostasis.

One potential limitation of this work is that it is difficult to predict the exact specificity of CAZymes based on family membership. However, substrate categories can be broadly inferred within the CAZy families, even if the precise specificity of each protein in the family is challenging to predict. An advantage of this type of genome
analysis is that it does not require complex culturing, which can be challenging since many intestinal microbes are not able to grow in classical laboratory media. Genomic strategies have now been widely applied and are bringing new information about the diversity and function of human gut microbiota. Our genomic characterization has shed light on commensal gut species with mucin-degrading properties. We believe this work enhances the foundation for examining mucin-degradation within the human intestine.

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References

1. Corfield, A. P., Wagner, S. A., Clamp, J. R., Kriaris, M. S. & Hoskins, L. C. Mucin degradation in the human colon: Production of sialidase, sialate O-acetyleraserase, N-acetylneuraminate lyase, arylesterase, and glycosulfatase activities by strains of fecal bacteria. Infect. Immun. 60, 3971–3978. https://doi.org/10.1128/iai.60.3971-3978.1992 (1992).

2. Bell, A. & Fuge, N. Mucosal glycan degradation of the host by the gut microbiota. Glycobiology 31, 691–696. https://doi.org/10.1093/glycob/cwa097 (2021).

3. Talford, L. E., Crost, E. H., Kavanagh, D. & Fuge, N. Mucin glycan foraging in the human gut microbiome. Front. Genet. 6, 81. https://doi.org/10.3389/fgene.2015.00081 (2015).

4. McGuckin, M. A., Linden, S. K., Sutton, P. & Florin, T. H. Mucin dynamics and enteric pathogens. Nat. Rev. Microbiol. 9, 265–278. https://doi.org/10.1038/nrmicro2588 (2011).

5. McDole, J. R. et al. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. Nature 483, 345–349. https://doi.org/10.1038/nature10863 (2012).

6. Aihara, E., Engevik, K. A. & Montrose, M. H. Trefoil factor peptides and gastrointestinal function. Annu. Rev. Physiol. 79, 357–380. https://doi.org/10.1146/annurev-physiol-021115-105447 (2017).

7. Marcobal, A., Southwick, A. M., Earle, K. A. & Sonnenburg, J. L. A refined palea: Bacterial consumption of host glycans in the gut. Glyobiology 23, 1038–1046. https://doi.org/10.1093/glycob/cw040 (2013).

8. Fang, J. et al. Slimy partners: The mucus barrier and gut microbiota in alleviative colitis. Exp. Mol. Med. 53, 772–787. https://doi.org/10.1038/s41776-021-00617-8 (2021).

9. Crouch, L. I. et al. Prominent members of the human gut microbiota express endo-acting O-glycanases to initiate mucin breakdown. Nat. Commun. 11, 4017. https://doi.org/10.1038/s41467-020-17847-5 (2020).

10. Derrien, M., Vaughan, E. H., Phuget, C. M. & de Vos, W. M. Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. Int. J. Syst. Evol. Microbiol. 54, 1469–1476. https://doi.org/10.1099/ijs.0.08373-0 (2004).

11. Sonnenburg, J. L. et al. Glycan foraging in vivo by an intestine-adapted bacterial symbiont. Science 307, 1955–1959. https://doi.org/10.1126/science.1109051 (2005).

12. Desai, M. S. et al. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. Cell 167, 1339–1353.e1332. https://doi.org/10.1016/j.cell.2016.10.043 (2016).

13. Pudlo, N. A. et al. Symbiotic human gut bacteria with variable metabolic priorities for host mucosal glycans. MBio 6, e01282-e01215 (2015).

14. Martens, E. C., Chiang, H. C. & Gordon, J. I. Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. Cell Host Microbe 4, 447–457. https://doi.org/10.1016/j.chom.2008.09.007 (2008).

15. Comstock, L. E. Importance of glycans to the host-bacteroides mutualism in the mammalian intestine. Cell Host Microbe 5, 522–526. https://doi.org/10.1016/j.chom.2009.05.010 (2009).

16. Png, C. W. et al. Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of mucin by other bacteria. Am. J. Gastroenterol. 105, 2420–2428. https://doi.org/10.1111/aaj.12081 (2010).

17. Kiyohara, M. et al. alpha-N-acetylgalactosaminidase from infant-associated bifidobacteria belonging to novel glycoside hydrolase family 129 is implicated in alternative mucin degradation pathway. J. Biol. Chem. 287, 693–700. https://doi.org/10.1074/jbc.M111. 277384 (2012).

18. Bell, A. et al. Elucidation of a sialic acid metabolism pathway in mucus-foraging Ruminococcus gnarus unravels mechanisms of bacterial adaptation to the gut. Nat. Microbiol. 4, 2393–2404. https://doi.org/10.1038/s41564-019-0590-7 (2019).

19. Crost, E. H. et al. Utilisation of mucin glycans by the human gut symbiont Ruminococcus gnarus is strain-dependent. PLoS ONE 8, e76541. https://doi.org/10.1371/journal.pone.0076541 (2013).

20. Ruiz-Madider, M., Macia, P., Gneimonde, M., Fernandez-Garcia, M., to the suckling period. Proc. Nat. Acad. Sci. USA 107, 19514–19519. https://doi.org/10.1073/pnas.1011100107 (2010).

21. Ravcheev, D. A. & Thiele, I. Comparative genomic analysis of Bifidobacterium bifidum PRL2010 reveals metabolic pathways for host-derived glycan foraging. Proc. Natl. Acad. Sci. USA 107, 19514–19519. https://doi.org/10.1073/pnas.1011100107 (2010).

22. Raimondi, N., Musmeci, E., Candelere, E., Amaretti, A. & Rossi, M. Identification of mucin degraders of the human gut microbiota. Sci. Rep. 11, 11094. https://doi.org/10.1038/s41598-021-9553-4 (2021).

23. Trastoy, B., Naegeli, A., Anso, I., Sjogren, J. & Guerin, M. E. Structural basis of mammalian mucin processing by the human gut symbiont Akkermansia muciniphila. Nat. Commun. 11, 4844. https://doi.org/10.1038/s41467-020-18466-y (2020).

24. Bjure, M. K., Martens, E. C. & Gordon, J. I. Functional genomic and metabolic studies of the adaptations of a prominent adult gut symbiont, Bacteroides thetaiotaomicron, to the suckling period. J. Biol. Chem. 281, 36269–36279. https://doi.org/10.1074/jbc.M605592000 (2006).

25. Zhang, G., Mills, D. A. & Block, D. E. Development of chemically defined media supporting high-cell-density growth of lactococci, enterococci, and streptococci. Appl. Environ. Microbiol. 75, 1080–1087. https://doi.org/10.1128/AEM.01418-08 (2009).

26. Cantarel, B. L. et al. The carbohydrate-active enzymes database (CAZy): An expert resource for glycogenomics. Nucleic Acids Res. 37, D233–238. https://doi.org/10.1093/nar/gkn663 (2009).

27. El Kaoutari, A., Armougom, F., Gordon, J. I., Raoult, D. & Henrissat, B. The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. Nat. Rev. Microbiol. 11, 497–504. https://doi.org/10.1038/nrmicro3050 (2013).

28. Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P. M. & Henrissat, B. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res. 42, D490–495. https://doi.org/10.1093/nar/gkt1178 (2014).

29. Park, B. H., Karpinets, T. V., Syed, M. H., Leuze, M. R. & Uberbacher, E. C. CAZyMines Analysis Toolkit (CAZ): Web service for searching and analyzing carbohydrate-active enzymes in a newly sequenced organism using CAZy database. Glycobiology 20, 1574–1584. https://doi.org/10.1093/glycob/cwq106 (2010).
32. Henrissat, B. & Bairoch, A. New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem. J.* 293(Pt 3), 781–788. https://doi.org/10.1042/bj2930781 (1993).

33. Henrissat, B. A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem. J.* 280(Pt 2), 309–316. https://doi.org/10.1042/bj2800309 (1991).

34. Chen, I. A. et al. The IMG/M data management and analysis system v.6.0: New tools and advanced capabilities. *Nucleic Acids Res.* 49, 751–763. https://doi.org/10.1093/nar/gkaa939 (2021).

35. Low, K. E., Smith, S. P., Abbott, D. W. & Boraston, A. B. The glycoconjugate-degrading enzymes of *Clostridium perfringens*: Tailored catalysts for breaching the intestinal mucus barrier. *Glycobiology* 31, 681–690. https://doi.org/10.1093/glycob/cwaa050 (2021).

36. Plugge, C. M. & Zoetendal, E. G. In *The Prokaryotes: Other Major Lineages of Bacteria and The Archaea* (eds Rosenberg, E. et al.) 1019–1021 (Springer, 2014).

37. Beye, M. et al. Draft genome sequence of *Fermentimonas caenicola* strain SIT8, isolated from the human gut. *Stand Genomic Sci.* 13, 8. https://doi.org/10.1186/s40793-018-0310-6 (2018).

38. Bunesova, V., Lacroix, C. & Schwab, C. Mucin cross-feeding of infant Bifidobacteria and *Eubacterium hallii*. *Microb. Ecol.* 75, 228–238. https://doi.org/10.1007/s00248-017-1037-4 (2018).

39. Miller, R. S. & Hoskins, L. C. Mucin degradation in human colon ecosystems. Fecal population densities of mucin-degrading bacteria estimated by a “most probable number” method. *Gastroenterology* 81, 759–765 (1981).

40. Hoskins, L. C. & Boulding, E. T. Mucin degradation in human colon ecosystems. Evidence for the existence and role of bacterial subpopulations producing glycosidases as extracellular enzymes. *J. Clin. Invest.* 67, 163–172. https://doi.org/10.1172/jci110009 (1981).

41. Lim, E. S. et al. Early life dynamics of the human gut virome and bacterial microbiome in infants. *Nat. Med.* 21, 1228–1234. https://doi.org/10.1038/nm.3950 (2015).

42. Backhed, F. et al. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe* 17, 690–703. https://doi.org/10.1016/j.chom.2015.04.004 (2015).

43. Makino, H. 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* 8, e78331. https://doi.org/10.1371/journal.pone.0078331 (2013).

44. Midtvedt, A. C., Carlstedt-Duke, B. & Midtvedt, T. Establishment of a mucin-degrading intestinal microflora during the first two years of human life. *J. Pediatr. Gastroenterol. Nutr.* 18, 321–326. https://doi.org/10.1097/00005176-199404000-00012 (1994).

45. Cantarel, B. L., Lombard, V. & Henrissat, B. Complex carbohydrate utilization by the healthy human microbiome. *PLoS ONE* 7, e28742. https://doi.org/10.1371/journal.pone.0028742 (2012).

**Author contributions**

Concept and design (J.G., M.A.E.), intellectual contribution (J.G., T.T., M.A.E.), data acquisition (J.G., T.T.), data analysis, statistical analysis, and interpretation (J.G., T.T., M.A.E.), drafting the manuscript (J.G.), editing the manuscript (J.G., T.T., M.A.E.), obtained funding (M.A.E.).

**Competing interests**

The authors declare no competing interests.

**Additional information**

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