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Karav, Sercan; Casaburi, Giorgio; and Frese, Steven A., "Reduced colonic mucin degradation in breastfed infants colonized by *Bifidobacterium longum* subsp. *infantis* EVC001" (2018). *Faculty Publications in Food Science and Technology*. 272.  
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# Reduced colonic mucin degradation in breastfed infants colonized by *Bifidobacterium longum* subsp. *infantis* EVC001

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## Keywords

*Bacteroides*; *Bifidobacterium infantis*; glycome; mucin; mucinlike glycans

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(Received 23 April 2018, revised 31 July 2018, accepted 3 August 2018)

doi:10.1002/2211-5463.12516

Mucin glycoproteins play an important role in protecting the gut epithelium by keeping gut microbes from direct contact with the gut epithelium while allowing for diffusion of small molecules from the lumen to the epithelium. The mucin glycocalyx can be degraded by gut bacteria such as *Bacteroides* and *Akkermansia*, but other bacteria, such as *Bifidobacterium longum* subsp. *infantis*, cannot consume mucin glycans. Untargeted mass spectrometry profiles were compared to microbiome profiles to assess how different gut microbiomes affect colonic mucin degradation. Samples obtained from nine infants colonized by *Bifidobacterium infantis* EVC001 and from 10 infants colonized by higher levels of mucolytic taxa (controls), including *Bacteroides*, were compared. Previously performed untargeted nano-high-performance liquid chromatography-chip/time-of-flight mass spectrometry was used to detect and quantify glycans originating from colonic mucin. Colonic mucin-derived *O*-glycans from control infants composed 37.68% ( $\pm$  3.14% SD) of the total glycan structure pool, whereas colonic mucin-derived *O*-glycans made up of only 1.78% ( $\pm$  0.038% SD) of the total in *B. infantis* EVC001 samples. The relative abundance of these colonic mucin-derived *O*-glycans in the total glycan pool was higher among control, 26.98% ( $\pm$  8.48% SD), relative to *B. infantis*-colonized infants, 1.68% ( $\pm$  1.12% SD). Key taxa, such as *Bacteroidaceae*, were significantly and positively correlated with the abundance of these structures, while *Bifidobacteriaceae* were significantly and negatively associated with these structures. These results suggest that colonization of infants by *B. infantis* may diminish colonic glycan degradation and help maintain barrier function in the gastrointestinal tract of infants.

Mucins are highly glycosylated *O*-linked glycoproteins secreted by exocrine glands and mucosa. They contain a wide variety of glycan structures, which make up 80% of the glycoprotein mass. These glycans attach to the polypeptide backbone via serine or threonine

residues with *N*-acetylgalactosamine. Eight *O*-glycan cores have been identified [1]. Among these cores, core 1-, 2-, 3-, and 4-derived glycans are predominantly found in intestinal mucins. Specifically, core 1 (Gal $\beta$ 1-3GalNAc $\alpha$ 1-Ser/Thr) and core 2 (Gal $\beta$ 1,3

## Abbreviations

ACN, acetonitrile; FA, formic acid; FDR, false discovery rate; GH, glycosyl hydrolase; HMO, human milk oligosaccharide; HPLC, high-performance liquid chromatography; OS, oligosaccharide; OTU, operational taxonomic unit; PCoA, principal coordinate analysis; TOF, time of flight.

(GlcNAc $\beta$ 1,6)GalNAc $\alpha$ 1-Ser/Thr structures are found in gastric and duodenal mucins, whereas colonic mucins contain predominantly core 3, 4 (GlcNAc $\beta$ 1,6 (GlcNAc $\beta$ 1,3) GalNAc $\alpha$ Ser/Thr) elongated structures [2,3]. Mucin degradation by mucolytic taxa like *Bacteroides* is achieved with a wide variety of enzymes, such as proteases, sulfatases, fucosidases, neuraminidases,  $\beta$ -galactosidases,  $\alpha$ -N-acetylgalactosaminidases,  $\alpha$ -N-acetylglucosaminidases, and exo/endo- $\beta$ -N-acetylglucosaminidases [4]. Although the presence of mucin-consuming taxa is associated with increased mucin production, it is unclear whether this is a beneficial trait as the breakdown of the mucin barrier is often associated with negative impacts to the underlying gut epithelium [5], and its loss can be a pathway to infection [6], loss of gut epithelial barrier function [7], or spontaneous inflammation resembling colitis [8]. Further, mucin degradation provides a niche that fosters taxa whose cytotoxic products can lead to colon cancer [9].

Human milk contains structurally analogous carbohydrates in the form of N-linked and O-linked glycoproteins and glycolipids [10] as well as free glycans, known as human milk oligosaccharides (HMOs) [11]. Several gut bacteria found in breastfed infants are able to release and consume mucin glycans (e.g., *Bacteroides*), and while some species of *Bifidobacterium* (e.g., *B. bifidum*) are able to grow on mucin as a sole carbon source, others can not (e.g., *Bifidobacterium infantis*) [12]. Interestingly, the taxa that consume both mucin glycans and HMOs appear to use the same glycolytic capacities and regulatory networks to consume these structures, whereas specialized taxa consume HMOs in unique pathways, and appear to only express these genes in response to a limited number of carbohydrates [13–15]. Thus, adaptation to these two structurally similar carbon sources appears to be highly specific among different infant gut-associated bifidobacteria. If this is true, then the role of mucolytic taxa in the infant gut is unclear. Further, to what extent is mucus degraded in the infant gut and which taxa are responsible? To begin to address these questions, a library of known colonic mucin-derived O-glycans was compiled and used to query untargeted mass spectra of fecal samples from infants from a previous study [16,17]. We hypothesized that the previously demonstrated modification of the gut microbiome resulted in the modulation of mucin degradation by gut microbes. A second part of the hypothesis was that colonization with *B. infantis*, which does not degrade mucin, and the subsequent reduction in mucolytic taxa would diminish mucin degradation, as measured by the

abundance of freed colonic mucin-derived O-glycans in the infant's stool.

## Materials and methods

### Fecal sample collection and analysis

To examine the effect of *Bifidobacterium longum* subsp. *infantis* (*B. infantis*) EVC001 colonization on gut mucin degradation at day 29 postnatal, fecal samples were collected from healthy, breastfed infants fed  $1.8 \times 10^{10}$  CFU per day *B. longum* subsp. *infantis* EVC001 from Day 7 postnatal to Day 29 postnatal ( $n = 9$ ) and healthy breastfed infants who were not fed *B. longum* subsp. *infantis* EVC001 ( $n = 10$ ). These samples were randomly selected from the larger study population for additional analysis using untargeted mass spectrometry data that had been collected initially for the study of human milk glycans found in the infant's feces. The initial clinical study was a partially randomized study, and subject populations, as well as ethical approval for sample collection, were previously noted [16]. Demographics describing the randomly selected subset of the total population examined in this analysis is presented in Table S1.

The microbiome composition of these samples was also previously determined. These data are publicly deposited in the NCBI SRA (PRJNA390646) and were analyzed using QIIME 1.9.1 [18] as previously described [17]. Briefly, paired-end sequencing of the V4 region of the 16S rRNA gene was performed on an Illumina MiSeq at the University of California Davis Genome Center (Davis, CA). Open-reference operational taxonomic unit (OTU) picking was completed using UCLUST at 97% identity, and low-abundance OTUs were removed as recommended [19]. Across the full data set, there was a mean of 9216 reads ( $SD \pm 4505$  reads) per sample and samples with at least than 2779 reads were included for analysis. Here, 19 samples ( $n = 9, 10$ ) matching paired mass spectrometry data were selected from this overall population and analyzed in the context of the untargeted spectra as described below.

### Analysis of spectra obtained with Nano-high-performance liquid chromatography-chip/time-of-flight mass spectrometry (nano-LC-MS)

Oligosaccharide (OS) isolation and purification from these fecal samples were performed previously and reported by Frese et al. [17]. The structures of human colonic mucin O-glycans were characterized by analysis on a nano-HPLC-Chip-TOF mass spectrometer using the methods described by Davis et al. [20], and this approach was previously reported for these samples where the concentration of structurally similar HMOs was determined [17]. Briefly, the high-performance liquid chromatography

(HPLC) system used was an Agilent 1200 series unit with a microfluidic chip, which was coupled to an Agilent 6220 series time of flight (TOF) mass spectrometer via chip cube interface. The capillary pump on the chromatography unit loaded the sample onto the 40-nL enrichment column at a flow rate of  $4.0 \mu\text{L}\cdot\text{min}^{-1}$  with a  $1 \mu\text{L}$  injection volume. A nano pump was used for analyte separation on the analytical column, which was  $75 \times 43 \text{ mm}$  and packed with porous graphitized carbon. Separation was accomplished using a binary gradient of aqueous solvent A [3% acetonitrile (ACN)/water (v/v) in 0.1% formic acid (FA)] and organic solvent B [90% ACN/water (v/v) in 0.1% FA] using a method developed for HMO separation (7, 8). The sample was introduced into the TOF mass spectrometer via electrospray ionization, which was tuned and calibrated using a dual nebulizer electrospray source with calibrant ions ranging from  $m/z$  118.086 to 2721.895, and data were collected in the positive mode. These untargeted spectra were analyzed in the present study as described below.

### Glycan data analysis

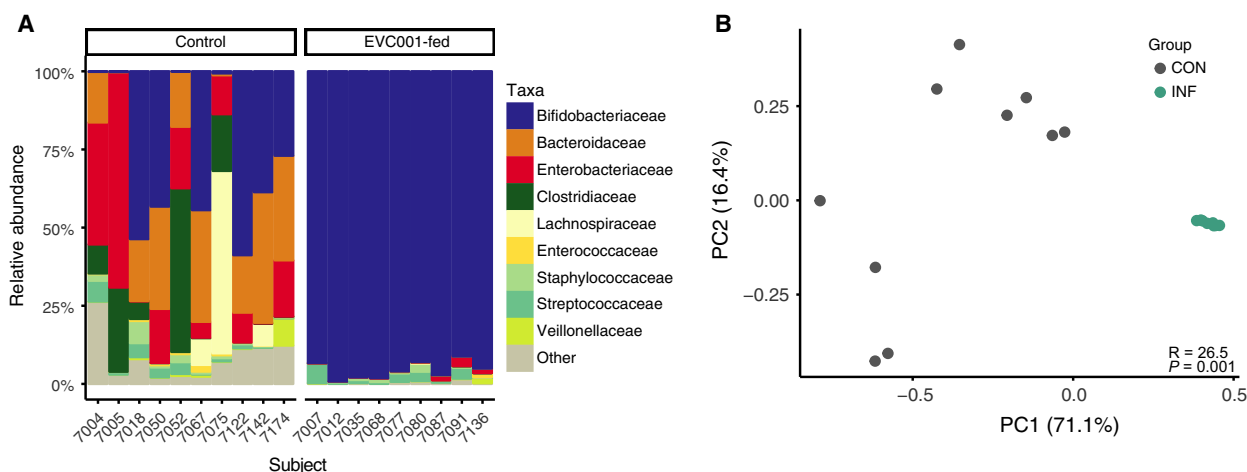
The untargeted mass spectra were collected (as above) and analyzed using Agilent MassHunter Workstation Data Acquisition version B.02.01 on the nano-HPLC-Chip-TOF. The ‘Find Compounds by Molecular Feature’ function of the software was used to identify mucin glycan species within 20 ppm of theoretical masses. Compound abundances were expressed as volume in ion counts that corresponded to absolute abundances of the compounds in each sample. 1HexNAc-1NeuAc, 1HexNAc-1Hex-NeuAc, 2HexNAc-1NeuAc, 2HexNAc-1Hex-1Fuc, 2HexNAc-1Hex-1NeuAc, 2HexNAc-1Hex-2Fuc, 3HexNAc-1Hex-1Fuc, 2He

xNAc-1Hex-1Fuc-1NeuAc, 2HexNAc-1Hex-1Fuc-2NeuAc, 3HexNAc-1Hex-2NeuAc and 3HexNAc-1Hex-2Fuc-1NeuAc were monitored as they are discriminatively human colonic mucin *O*-glycans [21].

### Statistical analysis

Multiple *t*-tests, corrected using the Holm–Sidak method for multiple comparisons, were carried out in Graph Pad Prism 7 (GRAPHPAD Software, La Jolla, CA, USA). Wilcoxon rank-sum test was used for single comparisons. *P* values, or adjusted *P* values, of 0.05 or less in comparisons were considered significantly different. Differences in bacterial community composition and colonic mucin-derived *O*-glycans were calculated in several, complementary ways. First, a weighted UniFrac distance matrix [22] was used to visualize differences in community composition according to treatment group using principal coordinate analysis (PCoA). Second, a Bray–Curtis dissimilarity index between all colonic mucin-derived *O*-glycan species was visualized via PCoA. To evaluate the effect size of EVC001 colonization, both weighted UniFrac and Bray–Curtis dissimilarity matrices were tested via PerMANOVA multivariate comparisons with 999 permutations and FDR-corrected *P*-values. Mantel tests were used to assess significant relationships between the phylogenetic distance of the bacterial communities and the colonic mucin-derived *O*-glycan abundance. Colonic mucin-derived *O*-glycan abundance was transformed to dissimilarity matrices using Euclidean distance, while phylogenetic distance was obtained via the weighted UniFrac algorithm. Tests were performed using Pearson’s product-moment correlation coefficient (*r*) with 999 permutations and a two-tailed test.

To compute specific correlations between observations of bacterial taxa and colonic mucin-derived *O*-glycan



**Fig. 1.** (A) The relative abundance of the taxa, as reported at the family level by Frese *et al.* 2017, of each fecal sample in this analysis. (B) PCoA of the gut microbiome at the family level; control (CON) samples are shown as gray points, and EVC001-fed infant samples are shown as teal points. 87.5% of total variation was described in the first two principal components (PC1 and PC2). PERMANOVA comparisons identified a significant difference between the two treatment groups by composition ( $R = 26.5$ ,  $P = 0.001$ ).

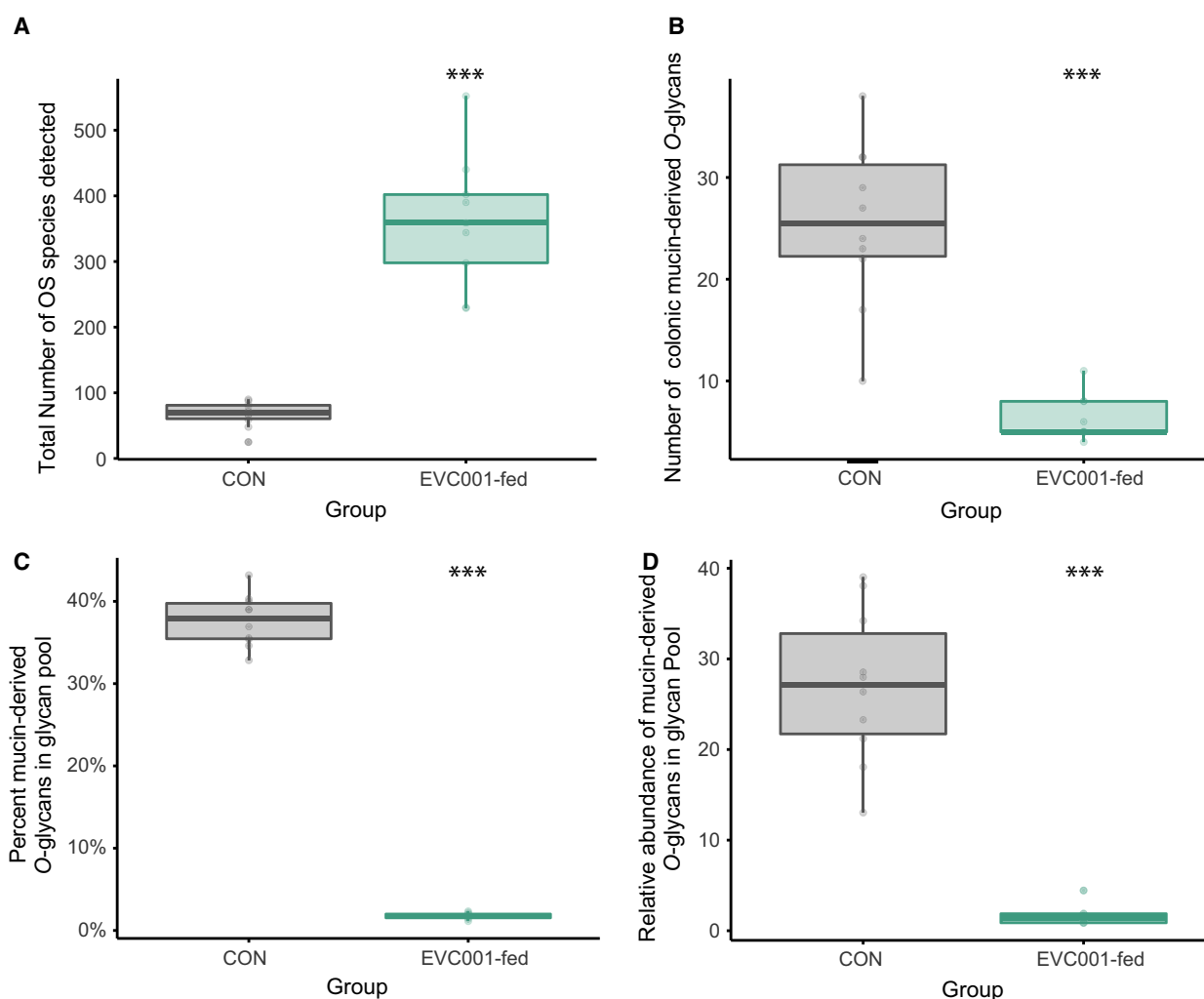
structures, a Spearman’s  $\rho$  test was used. Raw correlation statistics were tested for likelihood using Fisher’s  $Z$  transformation and  $P$ -values corrected via Benjamini–Hochberg false discovery rate (FDR) procedure.

## Results and Discussion

### Comparisons of the gut microbiome among a subselected cohort

The gut microbiome of samples analyzed by mass spectrometry from infants profiled by Frese *et al.* is shown in Fig. 1A. Infants were fed  $1.8 \times 10^{10}$  CFU per day of *B. infantis* EVC001 for 21 days from Day 7 to Day 29 postnatal. Infants fed EVC001 had

significantly high levels of *Bifidobacterium* and significantly lower levels of *Bacteroides* than control infants not fed *B. infantis* EVC001. *Bacteroides* was the predominant mucolytic taxon identified in the samples [23]. Notably, *B. infantis* fails to grow on colonic mucin as a sole carbon source [15], and the relative abundance of *Bacteroidaceae* was negatively correlated with the abundance of *Bifidobacteriaceae* (Spearman’s  $\rho = -0.65$ ;  $P = 0.0029$ ). Among the 19 samples profiled by nano-HPLC-Chip-TOF here, the gut microbiome profiles for nine infants fed *B. infantis* EVC001 were significantly different as tested by PERMANOVA ( $R = 26.5$ ,  $P = 0.001$ ) from that of the 10 control infants (Fig. 1B). Even when all *Bifidobacterium*-matched reads were filtered from the



**Fig. 2.** Comparison of fecal glycome and colonic mucin-derived *O*-glycans of control and EVC001-fed infant feces. (A) Total number of OS detected across treatment groups. (B) Number of colonic mucin-derived *O*-glycans across treatment groups. (C) Relative abundance of the total number of colonic mucin-derived *O*-glycans in the total OS pool across treatment groups. (D) Percent of the OS assigned to colonic mucin-derived *O*-glycans in the total OS abundance across treatment groups.



samples, the residual microbial communities were still significantly different according to treatment group when compared by PERMANOVA ( $R = 13.8$ ,  $P = 0.001$ ).

### Fecal glycomics indicates differences among release of colonic mucin-derived *O*-glycans from glycoproteins *in vivo*

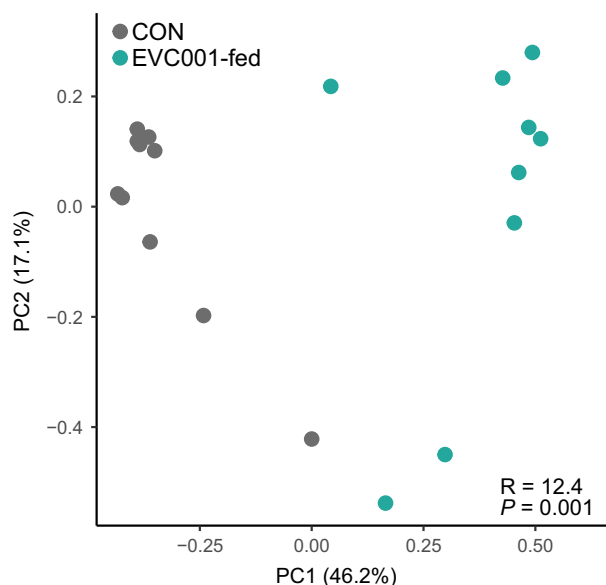
The total OS compositions of the samples were determined by the untargeted approach of nano-HPLC-Chip-TOF. The compounds were characterized using previously published libraries [21,24–26]. Among these compositions, free HMOs and free colonic mucin-derived *O*-glycans were found in the infant fecal glycome. The main focus of this study was to understand the degradation of human colonic mucin glycans by different gut microbiome profiles. This was determined as the difference between a gut microbiome from infants colonized with *B. infantis* EVC001 and microbiomes with a greater abundance of mucolytic taxa, such as *Bacteroidaceae*. As target molecules, 1HexNAc-1NeuAc, 1HexNAc-1Hex-NeuAc, 2HexNAc-1NeuAc, 2HexNAc-1Hex-1Fuc, 2HexNAc-1Hex-1NeuAc, 2HexNAc-1Hex-2Fuc, 3HexNAc-1Hex-

1Fuc, 2HexNAc-1Hex-1Fuc-1NeuAc, 2HexNAc-1Hex-1Fuc-2NeuAc, 3HexNAc-1Hex-2NeuAc and 3HexNAc-1Hex-2Fuc-1NeuAc were selected as typical human colonic mucin glycans, as shown by Robbe *et al.* [21]. The mass spectrometry monitoring these structures showed that the number of total OS structures (including isomers and anomers) in samples from control and EVC001-fed infants ranged from  $67.4 \pm 19.81$  and  $360.44 \pm 102.52$ , respectively ( $P < 0.001$ ; Fig. 2A). Although the control samples contained fewer total OS structures, the number of freed human colonic mucin-derived *O*-glycans of the total OS was significantly higher  $25.4 (\pm 8.09)$ , whereas only  $6.33 (\pm 2.24)$  structures were colonic mucin-derived *O*-glycans in samples from EVC001-fed infants ( $P < 0.001$ , Wilcoxon test; Fig. 2B). As a proportion, the relative abundance of colonic mucin-derived *O*-glycans was significantly higher in control samples than in samples from EVC001-fed infants in terms of both the number of structures ( $37.68\% \pm 3.14\%$  and  $1.78\% \pm 0.385\%$ , respectively; Fig. 2C,  $P < 0.001$ , Wilcoxon test) and their proportion of the total OS profile ( $26.98\% \pm 8.48\%$  and  $1.68 \pm 1.12\%$ , respectively; Fig. 2D,  $P < 0.001$ , Wilcoxon test).

### Associations between colonic mucin degradation and the fecal microbiome

Overall, freed colonic mucin *O*-glycan composition differed significantly between EVC001-colonized and control infants when tested via PERMANOVA ( $R = 12.4$ ,  $P = 0.001$ ; Fig. 3). However, to compare the composition of the gut microbiome with the abundance of both total colonic mucin-derived *O*-glycans and the specific structures monitored here, a Mantel test was used to correlate these structures with the overall microbiome composition. Broadly, the total colonic mucin-derived *O*-glycan abundance was significantly correlated with the microbiome composition (Mantel's  $R = 0.39$ ,  $P = 0.01$ ). Of these, only 1\_0\_0\_1 (Mantel's  $R = 0.22$ ,  $P = 0.05$ ), 1\_1\_0\_1 (Mantel's  $R = 0.3$ ,  $P = 0.027$ ), 2\_1\_1\_0 (Mantel's  $R = 0.46$ ,  $P = 0.003$ ), 2\_1\_1\_1 (Mantel's  $R = 0.42$ ,  $P = 0.003$ ), 2\_1\_2\_0 (Mantel's  $R = 0.69$ ,  $P = 0.001$ ), 3\_1\_1\_0 (Mantel's  $R = 0.35$ ,  $P = 0.011$ ), and 3\_1\_2\_1 (Mantel's  $R = 0.37$ ,  $P = 0.005$ ) were significantly associated with specific microbiome compositions.

To examine the interactions of the gut microbiome and the colonic mucin-derived *O*-glycan species, a Pearson correlation was calculated for all taxa and structures in the samples, as well as the total abundance and proportion of colonic mucin-derived *O*-glycan species (Table 1). *Bifidobacteriaceae* abundance



**Fig. 3.** PCoA of colonic mucin-derived *O*-glycan composition among samples using a Bray–Curtis dissimilarity index; control (Con) samples are shown as gray points, and EVC001-fed infant samples are shown as teal points. 63.3% of total variation was explained in the first two principal components (PC1 and PC2). PERMANOVA comparisons identified a significant difference between the two groups, with respect to colonic mucin-derived *O*-glycan composition ( $R = 12.4$ ;  $P = 0.001$ ).

**Table 1.** Colonic mucin-derived *O*-glycan structure, composition, mass, and volume in samples from the two treatment groups.

Glycan code	Composition	Neutral mass	Log <sub>10</sub> volume control [Mean (±SD)]	Log <sub>10</sub> volume EVC001-fed [Mean (±SD)]	Holm–Sidak adjusted <i>P</i> value
1_0_0_1	1HexNAc-1NeuAc	512	7.19 (7.08)	5.44 (5.55)	0.010659
1_1_0_1	1HexNAc-1Hex-1NeuAc	675	5.96 (6.01)	4.06 (4.53)	0.09968
2_0_0_1	2HexNAc-1NeuAc	716	5.93 (6.16)	0 (0)	0.258702
2_1_1_0	2HexNAc-1Hex-1Fuc	735	6.17 (6.23)	0 (0)	0.09968
2_1_0_1	2HexNAc-1Hex-1NeuAc	878	5.9 (6.11)	5.5 (5.56)	0.502866
2_1_2_0	2HexNAc-1Hex-2Fuc	879	6.45 (5.95)	5.46 (5.51)	3.13E-06
3_1_1_0	3HexNAc-1Hex-1Fuc	936	6.43 (6.31)	5.32 (5.43)	0.015609
2_1_1_1	2HexNAc-1Hex-1Fuc-1NeuAc	1024	7.31 (7.01)	5.42 (5.54)	0.000189
2_1_1_2	2HexNAc-1Hex-1Fuc-2NeuAc	1315	7.08 (7.26)	6.71 (7.02)	0.502866
3_1_0_2	3HexNAc-1Hex-2NeuAc	1372	6.43 (6.52)	5.25 (5.4)	0.141386
3_1_2_1	3HexNAc-1Hex-2Fuc-1NeuAc	1373	7.52 (7.28)	5.77 (5.85)	0.000895

**Table 2.** Correlational analysis comparing microbiome composition (Mantel test) and specific bacterial families (Spearman’s  $\rho$ ) with colonic mucin-derived *O*-glycans.

Mucin-glycan Structures	Mantel <i>r</i>	Mantel <i>P</i> -value	Bacterial family	Spearman’s $\rho$	<i>P</i> -value (FDR)
Total	0.39	0.01	<i>Bifidobacteriaceae</i>	−0.66	0.04
			<i>Enterobacteriaceae</i>	0.63	0.04
			<i>Pasteurellaceae</i>	0.61	0.04
1_0_0_1	0.22	0.05	<i>Bifidobacteriaceae</i>	−0.65	0.03
			<i>Enterobacteriaceae</i>	0.63	0.03
			<i>Bacteroidaceae</i>	0.63	0.03
1_1_0_1	0.3	0.027			
2_0_0_1	0.03	0.826			
2_1_0_1	0.07	0.590			
2_1_1_0	0.46	0.003	<i>Clostridiaceae</i>	0.7	0.01
			<i>Planococcaceae</i>	0.63	0.03
			<i>Bifidobacteriaceae</i>	−0.63	0.03
2_1_1_1	0.42	0.003	<i>Bacteroidaceae</i>	0.69	0.01
			<i>Bifidobacteriaceae</i>	−0.67	0.01
2_1_1_2	0.19	0.109			
2_1_2_0	0.69	0.001	<i>Bifidobacteriaceae</i>	−0.82	7.85E-05
			<i>Bacteroidaceae</i>	0.72	0.003
			<i>Enterobacteriaceae</i>	0.71	0.003
3_1_0_2	0.25	0.062			
3_1_1_0	0.35	0.011			
3_1_2_1	0.37	0.005			

was significantly and negatively correlated with the abundance of colonic mucin-derived *O*-glycans, as a whole (Spearman’s  $\rho = -0.66$ ,  $P = 0.04$ ), whereas *Bacteroidaceae* was significantly and positively correlated with the abundance of 1\_0\_0\_1, 2\_1\_1\_1, 3\_1\_2\_1, and 2\_1\_2\_0 (Table 2). Interestingly, *Enterobacteriaceae*, who are unlikely to degrade mucus themselves, were significantly correlated with the overall abundance of colonic mucin-derived *O*-glycans (Spearman’s  $\rho = 0.061$ ,  $P = 0.04$ ) as well as 1\_0\_0\_1 (Spearman’s  $\rho = 0.63$ ,  $P = 0.03$ ), and 2\_1\_2\_0 (Spearman’s  $\rho = 0.71$ ,  $P = 0.003$ ). Similarly, *Clostridiaceae*

(Spearman’s  $\rho = 0.7$ ,  $P = 0.01$ ) and *Planococcaceae* (Spearman’s  $\rho = 0.63$ ,  $P = 0.03$ ) were significantly correlated with 2\_1\_1\_0. While *Pasteurellaceae* were significantly correlated with the overall abundance of colonic mucin-derived *O*-glycans, they were not associated with specific *O*-glycan species. Interestingly, three of these colonic mucin-derived *O*-glycan species (1\_1\_1\_0, 3\_1\_1\_0 and 3\_1\_2\_1) were significantly associated with microbiome compositions, but no individual taxa were responsible for this association.

The broad associations between diminished abundance, number, and proportion of colonic mucin-

derived *O*-glycans and colonization by *B. infantis* EVC001 was reflected in the negative correlations between the abundance of *Bifidobacteriaceae* and both total colonic mucin-derived *O*-glycan abundance and four of the mucin-derived *O*-glycan species monitored here. Similarly, the abundance of a known mucin-degrading family, *Bacteroidaceae*, was positively correlated with the abundance of these same structures. Many *Bacteroides* species allocate a large proportion of their genome to harvesting polysaccharides, including mucin [27], and the significant positive correlation with colonic mucin-derived *O*-glycan concentrations supports these previous findings, even though 16S rRNA gene sequencing here does not confidently distinguish between OTUs at the species level. Many of the genes associated with polysaccharide utilization common to mucin-degrading *Bacteroides* are highly active on mucin glycoproteins, including the *O*-glycan cores found in human colonic mucin, as evidenced here. *Bacteroides* can grow on mucin a sole carbon source and have specific transcriptional responses to incubation with mucin [13]. In particular, *Bacteroides* broadly possess enzymes from glycosyl hydrolase (GH) family GH 84, GH 85, GH 89, GH 101, and GH 129 that are active on mucin glycoproteins and may facilitate the release of these glycans from the mucin protein [13,27,28]. The release of these structures may facilitate the growth of taxa which are potentially pathogenic (e.g., *Clostridiaceae* and *Enterobacteriaceae*), and sialylated and fucosylated glycans derived from host mucin have been shown to play a major role in disease pathogenesis by *Salmonella*, *Clostridium difficile*, and cytotoxic *Escherichia coli* [9] and taxa that act as reservoirs of virulence factors in the infant gut [29].

## Conclusion

The human gastrointestinal epithelium is protected by a layer of mucus. Mucin, as a glycoprotein, is coated with a wide variety of conjugated glycans that can serve as a carbon source for mucolytic bacteria, and their release may facilitate the growth of other taxa such as *Enterobacteriaceae* and *Clostridiaceae*. Specifically, human colon mucins contain unique *O*-glycan structures that can be utilized only by few mucolytic taxa, and in breastfed infants, the predominant mucolytic taxon was *Bacteroidaceae*. These bacteria harbor multiple GH-encoding genes that facilitate the liberation of colonic mucin *O*-glycan from glycoproteins. Our results show that gut microbiomes of infants colonized by *B. infantis* have diminished mucin degradation, as evidenced by reduced abundance and diversity

of freed colonic mucin-derived *O*-glycans, as well as the negative correlations between these and *Bifidobacteriaceae*. *B. infantis* is not able to cleave colonic mucin-derived *O*-glycans, and their colonization is associated with diminished populations of *Bacteroidaceae* [17]. Infants with a gut microbiome with higher relative abundance of mucolytic taxa have enhanced mucin degradation, and this may have long-term health consequences, or increase their susceptibility to infection from taxa harboring virulence factors via cross-feeding [6,9,29]. Further studies are required to determine the specific negative effects of mucin degradation, the role of these taxa in diminishing host gut barrier function, and any functional consequences to the host.

## Acknowledgements

Spectra were collected in the laboratory of Carlito Lebrilla, to whom the authors are extremely grateful. The authors also thank Cora Morgan for critical editing of the manuscript.

## Author contributions

SK performed the analysis of the mass spectra. SF and GC performed statistical tests and microbiome analyses. All authors contributed to the study, read, and approved the final manuscript.

## Conflict of interest

SAF and GC are employed by Evolve Biosystems, Inc., which funded the study from which these samples were derived.

## References

- 1 Brockhausen I and Stanley P (2017) O-GalNAc Glycans. In *Essentials of Glycobiology [Internet]* (Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW and Etzler ME, eds), Chapter 10, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- 2 Robbe C, Capon C, Coddeville B and Michalski JC (2004) Structural diversity and specific distribution of *O*-glycans in normal human mucins along the intestinal tract. *Biochem J* **384**, 307–316.
- 3 Holmén Larsson JM, Karlsson H, Sjövall H and Hansson GC (2009) A complex, but uniform O-glycosylation of the human MUC2 mucin from colonic biopsies analyzed by nanoLC/MS n. *Glycobiology* **19**, 756–766.



- 4 Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM and Henriessat B (2013) The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res* **42**, D490–D495.
- 5 Tailford LE, Crost EH, Kavanaugh D and Juge N (2015) Mucin glycan foraging in the human gut microbiome. *Front Genet* **6**, 81.
- 6 Ng KM, Ferreyra JA, Higginbottom SK, Lynch JB, Kashyap PC, Gopinath S, Naidu N, Choudhury B, Weimer BC, Monack DM et al. (2013) Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. *Nature* **502**, 96.
- 7 Kindon H, Pothoulakis C, Thim L, Lynch-Devaney K and Podolsky DK (1995) Trefoil peptide protection of intestinal epithelial barrier function: cooperative interaction with mucin glycoprotein. *Gastroenterology* **109**, 516–523.
- 8 Fu J, Wei B, Wen T, Johansson ME, Liu X, Bradford E, Thomsson KA, McGee S, Mansour L, Tong M et al. (2011) Loss of intestinal core 1–derived O-glycans causes spontaneous colitis in mice. *J Clin Invest* **121**, 1657–1666.
- 9 Dejea CM, Fathi P, Craig JM, Boleij A, Taddese R, Geis AL, Wu X, DeStefano Shields CE, Hechenbleikner EM, Huso DL et al. (2018) Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. *Science* **359**, 592–597.
- 10 Liu B and Newburg DS (2013) Human milk glycoproteins protect infants against human pathogens. *Breastfeed Med* **8**, 354–362.
- 11 German JB, Freeman SL, Lebrilla CB and Mills DA (2008) Human milk oligosaccharides: evolution, structures and bioselectivity as substrates for intestinal bacteria. In *Personalized Nutrition for the Diverse Needs of Infants and Children* (Bier DM, German JB and Lönnerdal B, eds), pp. 205–222. Karger Publishers, Basel, Switzerland.
- 12 Marcobal A and Sonnenburg J (2012) Human milk oligosaccharide consumption by intestinal microbiota. *Clin Microbiol Infect* **18**, 12–15.
- 13 Marcobal A, Barboza M, Sonnenburg ED, Pudlo N, Martens EC, Desai P, Lebrilla CB, Weimer BC, Mills DA, German JB et al. (2011) Bacteroides in the infant gut consume milk oligosaccharides via mucus-utilization pathways. *Cell Host Microbe* **10**, 507–514.
- 14 Turroni F, van Sinderen D and Ventura M (2011) Genomics and ecological overview of the genus *Bifidobacterium*. *Int J Food Microbiol* **149**, 37–44.
- 15 Garrido D, Ruiz-Moyano S, Lemay DG, Sela DA, German JB and Mills DA (2015) Comparative transcriptomics reveals key differences in the response to milk oligosaccharides of infant gut-associated bifidobacteria. *Sci Rep* **5**, 13517.
- 16 Smilowitz JT, Moya J, Breck MA, Cook C, Fineberg A, Angkustsiri K and Underwood MA (2017) Safety and tolerability of *Bifidobacterium longum* subspecies infantis EVC001 supplementation in healthy term breastfed infants: a phase I clinical trial. *BMC Pediatr* **17**, 1–11.
- 17 Frese SA, Hutton AA, Contreras LN, Shaw CA, Palumbo MC, Casaburi G, Xu G, Davis JCC, Lebrilla CB, Henrick BM et al. 2017 Persistence of supplemented bifidobacterium longum subsp infantis EVC001 in Breastfed Infants. *mSphere* **2**, e00501–e00517
- 18 Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**, 335–336.
- 19 Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, Mills DA and Caporaso JG (2013) Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nat Methods* **10**, 57.
- 20 Davis JC, Totten SM, Huang JO, Nagshbandi S, Kirmiz N, Garrido DA, Lewis ZT, Wu LD, Smilowitz JT, German JB et al. (2016) Identification of oligosaccharides in feces of breast-fed infants and their correlation with the gut microbial community. *Mol Cell Proteomics* **15**, 2987–3002.
- 21 Robbe C, Capon C, Coddeville B and Michalski JC (2004) Diagnostic ions for the rapid analysis by nano-electrospray ionization quadrupole time-of-flight mass spectrometry of O-glycans from human mucins. *Rapid Commun Mass Spectrom* **18**, 412–420.
- 22 Lozupone C and Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol* **71**, 8228–8235.
- 23 Shaffer M and Lozupone C (2018) Prevalence and source of fecal and oral bacteria on infant, child, and adult hands. *mSystems* **3**, e00192–17.
- 24 Matamoros S, Gras-Leguen C, Le Vacon F, Potel G and de La Cochetiere MF (2013) Development of intestinal microbiota in infants and its impact on health. *Trends Microbiol* **21**, 167–173.
- 25 Karav S and Le Parc A, Leite Nobrega de Moura Bell JM, Frese SA, Kirmiz N, Block DE, Barile D and Mills DA (2016) Oligosaccharides released from milk glycoproteins are selective growth substrates for infant-associated bifidobacteria. *Appl Environ Microbiol* **82**, 3622–3630.
- 26 Thomsson KA, Prakobphol A, Leffler H, Reddy MS, Levine MJ, Fisher SJ and Hansson GC (2002) The salivary mucin MG1 (MUC5B) carries a repertoire of unique oligosaccharides that is large and diverse. *Glycobiology* **12**, 1–14.
- 27 Xu J, Bjursell MK, Himrod J, Deng S, Carmichael LK, Chiang HC, Hooper LV and Gordon JI (2003) A genomic view of the human-Bacteroides thetaiotaomicron symbiosis. *Science* **299**, 2074–2076.

- 28 Comstock LE (2009) Importance of glycans to the host-bacteroides mutualism in the mammalian intestine. *Cell Host Microbe* **5**, 522–526.
- 29 Casaburi G and Frese SA (2018) Colonization of breastfed infants by *Bifidobacterium longum* subsp. *infantis* EVC001 reduces virulence gene abundance. *Hum Microbiome J* **9**, 7–10.

## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Study participant demographics showing the mean ( $\pm$ standard deviation) or number per group.