

Infant formula with galacto-oligosaccharides (OM55N) stimulates the growth of indigenous bifidobacteria in healthy term infants

T. Matsuki, S. Tajima, T. Hara, K. Yahagi, E. Ogawa and H. Kodama

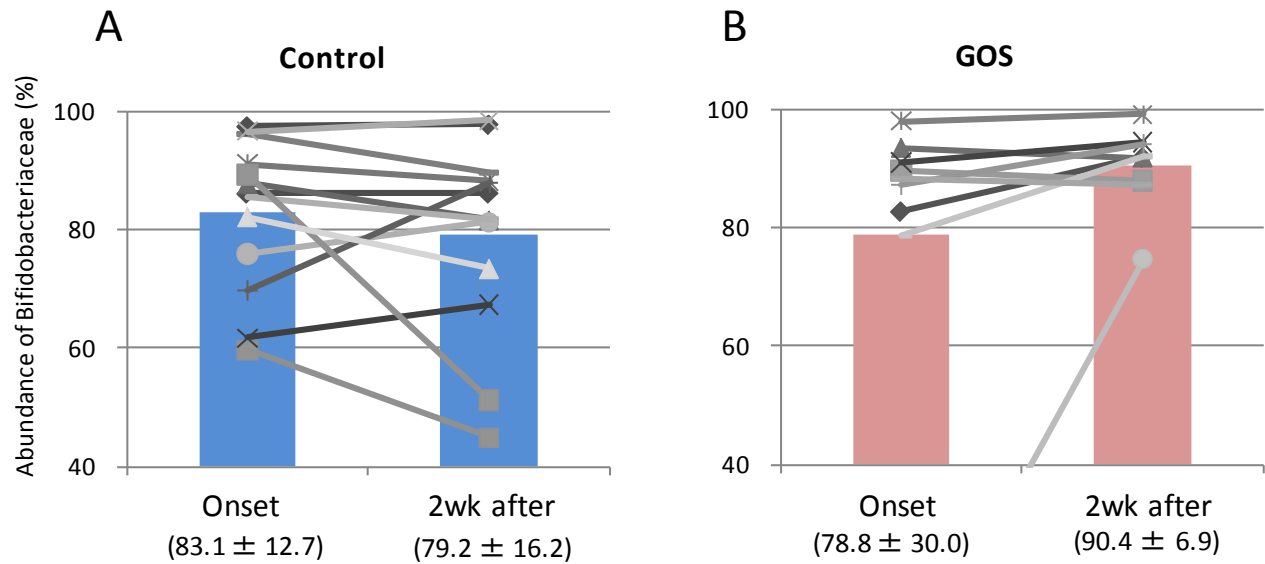


Figure S1. Change in *Bifidobacteriaceae* abundance at onset and at 2 weeks after the intervention with the test formula.

The line graph represents the change of each volunteer, and the bar graph represents the average. (A) Control formula group; (B) Galacto-oligosaccharide (GOS) formula group.

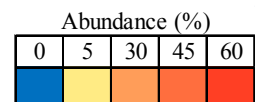
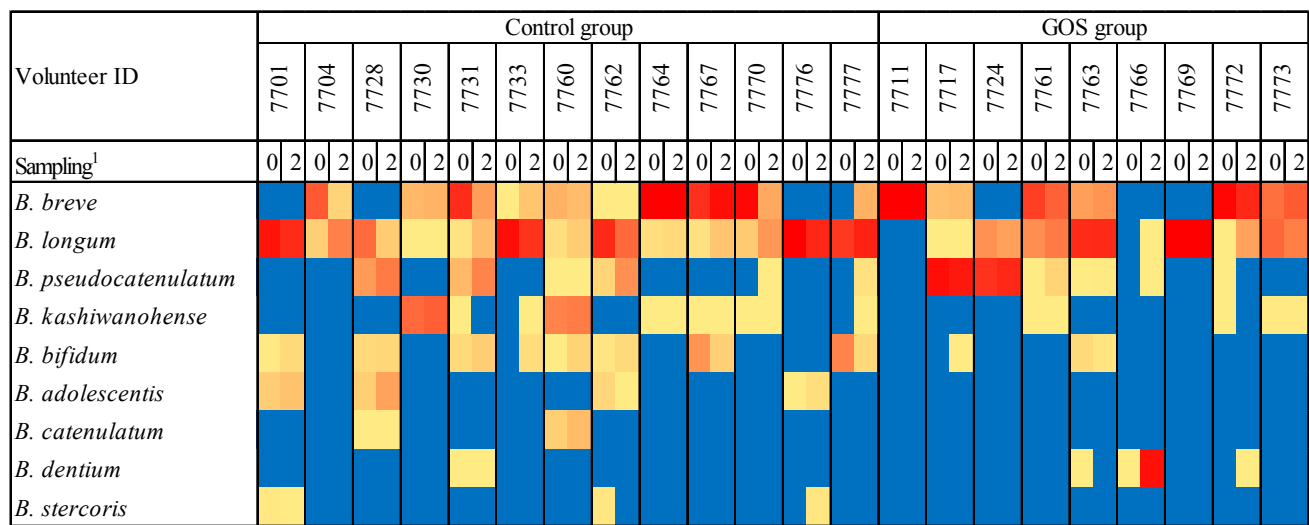
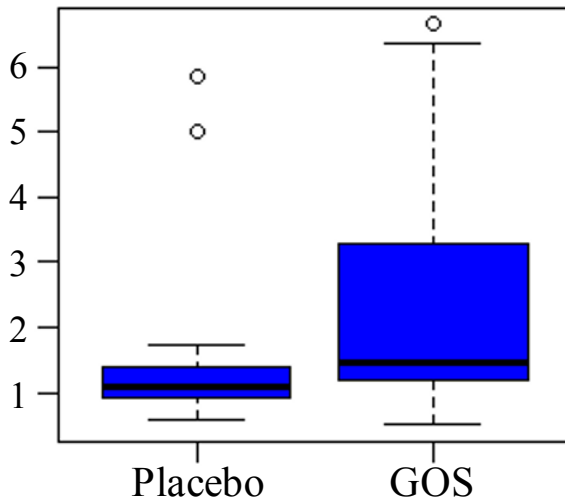


Figure S2. Heat map analysis of the *Bifidobacterium* species.

¹ 0 = trial onset; 2 = 2 weeks after.

GOS = galacto-oligosaccharide supplemented formula.

A



B

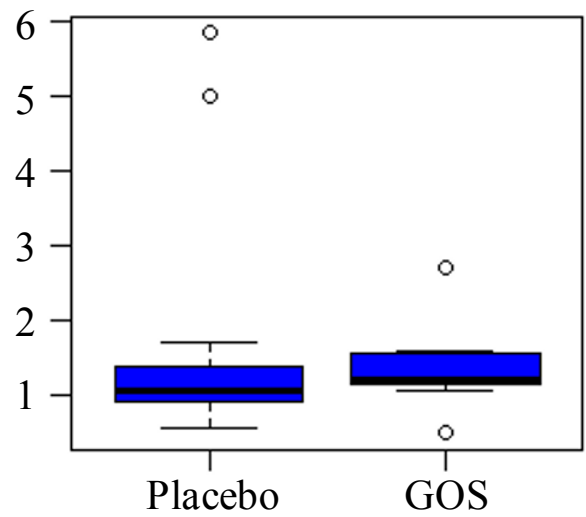


Figure S3. Faecal frequency. The number of defecations per day was recorded during the test period, averaged, and completed between the control and galacto-oligosaccharide (GOS) group. (A) Comparison of all the infants (infants without bifidobacteria were included (n=14 in the control vs n=16 in the OM55N group; $P=0.062$). (B) Comparison of infants (infants without bifidobacteria were excluded (n=13 in the control vs n=9 in the OM55N group; $P=0.347$).

Table S1. Summary of the baseline characteristics of the infants who completed the study.

	Control group (n = 14)	OM55N group (n = 16)	<i>P</i> -value ¹
Sex (male/female)	9/5	12/4	0.729
Delivery (vaginal/Caesarean)	11/3	5/11	0.014
Sibling (+/-)	11/3	8/8	0.215
Birth weight (g)	3105 ± 365	2859 ± 483	0.087
Gestation (weeks)	39 ± 1	38 ± 2	0.066
Feeding started (day)	41 ± 7	42 ± 6	0.991

¹ Differences between the control and galacto-oligosaccharide (GOS) (OM55N) groups were determined using the Wilcoxon rank sum or Pearson chi-square tests.

Table S2. Baseline characteristics of all the infants enrolled in this study.

Subject ID	Sex	Delivery	Sibling	Birth weight (g)	Gestation (weeks)	Feeding started (day)	Remarks
Control group (enrolled, 18; completed, 14)							
7701	Female	Vaginal	+	3070	40	44	
7704	Male	Caesarean	-	3528	40	47	
7728	Female	Vaginal	+	2546	39	45	
7730	Male	Vaginal	+	3010	39	42	
7731	Male	Vaginal	+	3634	37	43	
7733	Female	Vaginal	+	3282	39	43	
7760	Male	Vaginal	+	3330	38	54	
7762	Female	Vaginal	-	3228	40	35	
7764	Female	Caesarean	+	2350	37	46	
7767	Male	Vaginal	+	2656	39	37	
7770	Male	Vaginal	+	3190	39	45	
7776	Male	Vaginal	+	3332	39	31	
7777	Male	Vaginal	+	3245	40	37	
7778 ^a	Male	Caesarean	-	3070	38	29	
7713	Female	Vaginal	-	3100	40	41	Excluded: Introduction of breast milk
7718	Female	Vaginal	-	3106	39	45	Excluded: Introduction of breast milk
7768	Male	Vaginal	-	3404	39	43	Excluded: Parental decision
7771	Female	Vaginal	-	2970	38	47	Excluded: Introduction of breast milk
GOS group (enrolled, 17; completed, 16)							
7702 ^a	Male	Caesarean	+	2874	37	42	
7711	Female	Vaginal	-	2950	39	46	
7717	Male	Caesarean	-	2768	38	49	
7722 ^a	Male	Caesarean	-	2534	36	37	Twin pair A
7723 ^a	Male	Caesarean	-	2368	36	37	Twin pair A
7724	Female	Caesarean	+	2414	37	46	
7734 ^a	Female	Caesarean	-	2256	35	41	
7761	Male	Caesarean	+	3316	41	33	
7763	Male	Vaginal	-	3140	39	54	
7765 ^a	Male	Vaginal	+	3014	40	38	
7766	Male	Vaginal	-	3156	41	36	
7769	Male	Vaginal	+	3946	36	42	
7772	Male	Caesarean	+	2494	38	37	
7773	Female	Caesarean	+	3144	38	47	
7774 ^a	Male	Caesarean	-	2460	37	38	Twin pair B
7775 ^a	Male	Caesarean	-	2604	37	38	Twin pair B
7732	Male	Vaginal	+	3178	39	53	Excluded: Introduction of probiotics

^a Infant volunteers who had no bifidobacteria at the trial onset were excluded.

GOS = galacto-oligosaccharide supplemented formula.

Table S3. The sugar composition of OM55N.¹

		Total sugar (%) ²	Galacto-oligo saccharides (%) ²
Monosaccharides	Glc	22.4	-
	Gal	8.6	-
Disaccharides	Gal β 1-4Glc (lactose)	12.7	-
	Gal β 1-3Glc	3.8	3.8
	Gal β 1-2Glc	2.4	2.4
	Gal β 1-6Glc	5.0	5.0
	Others	3.6	3.6
	Gal β 1-6Gal β 1-4Glc	5.0	5.0
Trisaccharides	Gal β 1-4Gal β 1-4Glc	18.5	18.5
	Others	10.9	10.9
	Gal β 1-6Gal β 1-4Gal β 1-4Glc	3.9	3.9
Tetrasaccharides	Gal β 1-4Gal β 1-4Gal β 1-4Glc	0.8	0.8
	Others including trace amounts of higher oligosaccharides	2.5	2.5
Total		100	56.4

¹ Original data from the Generally Regarded as Safe Notice Inventory of the United States Food and Drug Administration (GRAS Notice No. GRN 334).

² Calculated based on the dry mass.

Table S4. The genus- and group-specific primers used in this study.

Target organism	Primer	Sequence (5' to 3')	Product size (bp)	References
<i>Clostridium coccooides</i> group	g-Ccoc477-F	AAATGACGGTACCTGACTAA	440	Matsuki <i>et al.</i> , 2004
	g-Ccoc895-R	CTTTGAGTTTCAATTCTTGCAGAA		
<i>Clostridium leptum</i> subgroup	sg-Clept933-F	GCACAAGCAGTGGAGT	239	Matsuki <i>et al.</i> , 2004
	sg-Clept1164-R	CTTCCTCCGTTTTGTCAA		
<i>Bacteroides fragilis</i> group	g-Bfra148-F2	A YAGCCTTTCGAAA GRAAGAT	495	Matsuki <i>et al.</i> , 2004
	g-Bfra626-R	CCAGTATCAACTGCAATTTTA		
<i>Bifidobacterium</i>	g-Bifid153-F	CTCCTGGAAACGGGTGG	550	Matsuki <i>et al.</i> , 2004
	g-Bifid699-R	GGTGTCTTCCCATA TCTACA		
<i>Atopobium</i> cluster	g-Atopo292-F	GGGTGAGAGACCGACC	190	Matsuki <i>et al.</i> , 2004
	g-Atopo488-R	CGGRGCTTCTTCTGCAGG		
<i>Prevotella</i>	g-Prevo-F	CACRGTAACGATGGATGCC	513	Matsuki <i>et al.</i> , 2004
<i>Eubacterium cylindroides</i> group	g-Ecylin-1	GTGAYGGTAKCTTACCAGA	416	Matsuki, 2007
	g-Ecylin-2	CTTGCGTGCATACTCCC		
<i>Veillonella</i>	g-Veillo-1	GRAGAGCGATGGAA GCTT	459	Matsuki, 2007
	g-Veillo-2	CCGTGGCTTTCTATTCC		
<i>Fusobacterium</i>	g-Fuso595-F	AAGTCTGATGTGAAAATGC	536	Matsuki, 2007 Rinttila <i>et al.</i> , 2004
	g-Fuso1113-R	TGGTAACATACGA WAGGG		
<i>Lactobacillus</i> group	g-Lact(Rin)-1	AGCAGTAGGGAATCTTCCA	341	Rinttila <i>et al.</i> , 2004
	g-Lact(Rin)-2	CACCGCTACACATGGAG		
<i>Enterobacteriaceae</i>	En-Isu3F	TGCCGTA ACTTCGGGAGAAGGCA	428	Matsuda <i>et al.</i> , 2007
	En-Isu3R	TCAAGGACCA GTGTTCA GTGTC		
<i>Enterococcus</i>	Ec-ssu1 F	CCCATCAGAAGGGGATAACACTT	115	Matsuda <i>et al.</i> , 2007
	Ec-ssu1R	ACCGCGGTCCATCCATC		
<i>Staphylococcus</i>	g-Staph-F	TTTGGGCTACACACGTGCTACAATGGACAA	79	Matsuda <i>et al.</i> , 2009
	g-Staph-R	AACA ACTTTATGGGATTTGCW TGA		
<i>Streptococcus</i>	g-Str-F	AGCTTAGAAGCAGCTATTCA TTC	309	Sakaguchi <i>et al.</i> , 2010
	g-Str-R	GGATACACCTTTCGGTCTCTC		

References

- Matsuda, K., Tsuji, H., Asahara, T., Kado, Y. and Nomoto, K., 2007. Sensitive quantitative detection of commensal bacteria by rRNA-targeted reverse transcription-PCR. *Applied and Environmental Microbiology* 73: 32-39.
- Matsuda, K., Tsuji, H., Asahara, T., Matsumoto, K., Takada, T. and Nomoto, K., 2009. Establishment of an analytical system for the human fecal microbiota, based on reverse transcription-quantitative PCR targeting of multicopy rRNA molecules. *Applied and Environmental Microbiology* 75: 1961-1969.
- Matsuki, T., 2007. Development of quantitative PCR detection method with 16S rRNA gene-targeted genus- and species-specific primers for the analysis of human intestinal microflora and its application. *Japanese Journal of Bacteriology* 62: 255-261.
- Matsuki, T., Watanabe, K., Fujimoto, J., Takada, T. and Tanaka, R., 2004. Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. *Applied and Environmental Microbiology* 70: 7220-7228.
- Rinttila, T., Kassinen, A., Malinen, E., Krogus, L. and Palva, A., 2004. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *Journal of Applied Microbiology* 97: 1166-1177.
- Sakaguchi, S., Saito, M., Tsuji, H., Asahara, T., Takata, O., Fujimura, J., Nagata, S., Nomoto, K. and Shimizu, T., 2010. Bacterial rRNA-targeted reverse transcription-PCR used to identify pathogens responsible for fever with neutropenia. *Journal of Clinical Microbiology* 48: 1624-1628.

Table S5. The 16S rRNA gene amplicon analysis of the gut microbiota in all infants at the trial onset.

	Control group (n = 14)		OM55N group (n = 16)		<i>P</i> -value ¹	
	Abundance (%)	No detected (%)	Abundance (%)	No detected (%)	Abundance	% detected
Bacterial taxon (family)						
<i>Bifidobacteriaceae</i>	77.1 ± 25.3	13 (93%)	44.3 ± 45.9	9 (56%)	0.153	0.039
<i>Enterobacteriaceae</i>	4.4 ± 5	13 (93%)	25.3 ± 31.2	14 (88%)	0.355	1.000
<i>Enterococcaceae</i>	5.7 ± 12.9	12 (86%)	5.2 ± 6.4	14 (88%)	0.371	1.000
<i>Streptococcaceae</i>	2.1 ± 3	13 (93%)	3.9 ± 4.8	16 (100%)	0.179	0.467
<i>Veillonellaceae</i>	2.4 ± 4.9	11 (79%)	3.1 ± 4.3	14 (88%)	0.376	0.642
<i>Bacteroidaceae</i>	1.5 ± 2.7	8 (57%)	2.5 ± 6.5	4 (25%)	0.164	0.156
<i>Staphylococcaceae</i>	0.4 ± 0.8	10 (71%)	4.5 ± 8.7	13 (81%)	0.151	0.675
Alpha-diversity						
Chao1 index	30.8 ± 8		28.2 ± 9.2		0.423	
PD	5.2 ± 1.3		5.2 ± 1.5		0.552	
Shannon index	2.2 ± 0.8		2.1 ± 0.7		0.790	

¹ Differences between the control and galacto-oligosaccharide (GOS) (OM55N) group at the trial onset were determined using the Wilcoxon rank sum (abundance) or Pearson chi-square tests (% detected).

PD = phylogenetic diversity.

Table S6. The 16S rRNA gene amplicon analysis data of all samples in this study (family level analysis).

ID	Abundance(%)								Alpha-diversity			Reads
	<i>Bifidobacteriaceae</i>	<i>Enterobacteriaceae</i>	<i>Enterococcaceae</i>	<i>Streptococcaceae</i>	<i>Veillonellaceae</i>	<i>Bacteroidaceae</i>	<i>Staphylococcaceae</i>	Other bacteria	Chao1 index	PD	Shannon index	
Control group onset												
7701	86.1	5.7	2.3	1.9	0.5	0.0	0.1	3.4	36.6	5.6	2.4	1,472
7704	59.7	18.3	17.8	0.7	0.4	0.0	3.1	0.1	21.0	3.7	2.3	1,066
7728	87.8	4.8	0.7	0.5	0.1	3.6	0.3	2.3	36.7	6.6	2.7	3,596
7730	61.8	10.8	1.9	11.6	3.7	10.0	0.0	0.2	35.9	6.2	3.1	2,921
7731	91.2	0.1	0.8	4.2	3.5	0.1	0.0	0.1	33.9	5.1	2.5	3,443
7733	76.0	2.1	1.3	0.6	0.0	0.0	0.1	19.8 ^c	28.4	6.2	2.1	3,480
7760	69.8	4.6	5.7	0.0	18.4	1.6	0.0	0.0	38.3	4.4	3.7	2,385
7762	85.7	0.0	0.0	2.6	0.3	1.4	0.3	9.8	38.9	6.9	2.2	1,567
7764	96.2	2.5	1.1	0.2	0.0	0.0	0.0	0.0	16.1	2.7	0.8	2,527
7767	97.5	0.5	0.1	1.0	0.0	0.7	0.1	0.3	26.7	4.9	1.6	1,962
7770	89.3	5.9	0.4	0.8	0.6	0.3	0.7	2.0	32.6	6.5	1.5	2,236
7776	82.2	2.9	0.0	1.1	0.3	2.9	0.1	10.5	38.6	5.9	1.3	1,824
7777	96.6	0.0	0.0	2.3	0.1	0.0	0.2	0.6	16.7	3.1	1.3	3,543
7778 ^b	0.0	3.2	47.4	1.6	5.4	0.0	0.7	41.6 ^d	30.3	5.4	3.2	1,389
ALL infant	77.1 ± 25.3	4.4 ± 5	5.7 ± 12.9	2.1 ± 3	2.4 ± 4.9	1.5 ± 2.7	0.4 ± 0.8	6.5 ± 11.6	30.8 ± 8	5.2 ± 1.3	2.2 ± 0.8	2,387 ± 885
Final population	83.1 ± 12.7	4.5 ± 5.2	2.5 ± 4.9	2.1 ± 3.1	2.1 ± 5	1.6 ± 2.8	0.4 ± 0.8	3.8 ± 6	30.8 ± 8.3	5.2 ± 1.4	2.1 ± 0.8	2,463 ± 871
OM55N group onset												
7702 ^b	0.0	59.3	10.3	7.2	3.1	0.0	0.2	19.8 ^e	36.6	5.1	3.1	3,180
7711	82.8	3.6	7.8	0.9	2.1	0.9	1.6	0.2	20.0	5.1	1.2	1,819
7717	89.7	1.3	0.3	4.7	0.1	1.1	0.0	2.8	48.3	8.8	1.7	2,719
7722 ^b	0.0	71.6	0.0	4.8	11.2	0.0	7.5	4.9	20.3	3.4	2.2	1,889
7723 ^b	0.0	81.2	1.9	6.9	8.6	0.0	0.4	0.9	31.3	5.0	1.5	2,260
7724	93.5	0.0	0.5	2.0	1.7	0.0	0.5	1.8	23.1	4.1	1.8	2,375
7734 ^b	0.0	0.0	18.0	19.1	0.2	0.0	0.3	62.3 ^f	39.2	4.8	3.4	2,438
7761	91.0	1.2	4.1	1.1	1.2	0.0	0.0	1.4	26.7	4.6	1.8	3,270
7763	98.0	1.1	0.0	0.1	0.0	0.0	0.2	0.7	19.7	3.6	1.6	1,764
7765 ^b	0.0	4.8	4.4	1.5	2.7	18.5	10.8	57.3 ^g	42.0	7.7	3.2	2,221
7766	0.2	59.9	0.3	3.1	14.2	19.7	0.2	2.3	30.7	6.9	2.6	2,318
7769	87.2	0.5	0.4	8.0	0.8	0.0	0.1	3.1	22.4	6.2	1.0	2,679
7772	78.8	11.4	1.3	0.2	2.4	0.0	0.0	6.0	22.8	5.1	1.5	2,294
7773	88.2	0.7	5.8	0.6	0.6	0.0	0.1	4.1	31.0	5.0	2.2	3,060
7774 ^b	0.0	51.3	20.8	2.0	0.0	0.0	22.0	3.9	17.2	3.6	2.5	1,464
7775 ^b	0.0	57.2	7.4	0.9	0.1	0.0	28.1	6.3	20.4	4.2	2.4	1,802
ALL infant	83.1 ± 12.7	4.5 ± 5.2	2.5 ± 4.9	2.1 ± 3.1	2.1 ± 5	1.6 ± 2.8	0.4 ± 0.8	3.8 ± 6	30.8 ± 8.3	5.2 ± 1.4	2.1 ± 0.8	2,463 ± 871
Final population	78.8 ± 30	8.9 ± 19.5	2.3 ± 2.9	2.3 ± 2.6	2.6 ± 4.4	2.4 ± 6.5	0.3 ± 0.5	2.5 ± 1.8	27.2 ± 8.9	5.5 ± 1.6	1.7 ± 0.5	2,478 ± 510
Control group 2 weeks after												
7701	86.1	6.4	0.3	1.4	0.0	0.0	0.2	5.7	32.7	4.3	2.8	1,184
7704	44.9	25.6	23.1	1.6	0.0	0.0	4.7	0.1	23.0	3.5	2.6	1,353
7728	81.6	1.6	8.6	0.4	0.4	0.3	0.0	7.1	36.0	6.1	3.0	3,185
7730	67.4	10.6	3.8	12.0	2.1	3.6	0.1	0.3	46.0	6.7	2.8	3,339
7731	88.2	1.5	0.4	7.3	2.2	0.2	0.0	0.3	32.5	6.0	2.8	2,527
7733	81.3	1.9	0.5	3.2	0.3	8.7	0.4	3.8	37.1	8.0	2.7	2,869
7760	87.8	2.3	5.6	0.4	3.2	0.5	0.0	0.2	46.3	5.5	3.6	3,050
7762	81.6	2.7	0.0	2.4	0.0	2.1	0.6	10.7	35.7	6.7	2.4	2,242
7764	89.6	3.9	5.9	0.4	0.1	0.0	0.0	0.0	18.7	3.2	1.4	2,003
7767	97.7	0.9	0.3	0.5	0.0	0.1	0.1	0.6	28.7	4.6	1.6	1,762
7770	51.3	15.3	7.1	19.9	0.2	4.7	0.4	1.1	36.1	6.9	3.0	1,884
7776	73.5	6.4	0.4	0.8	0.0	3.6	0.0	15.2 ^h	31.8	6.0	1.9	2,471
7777	98.4	0.6	0.1	0.7	0.0	0.0	0.0	0.2	16.7	3.0	2.0	4,240
7778 ^b	0.0	4.5	53.4	2.0	1.8	0.0	0.8	37.5 ⁱ	34.5	6.7	2.9	1,269
ALL infant	73.5 ± 26.3	6 ± 7	7.8 ± 14.5	3.8 ± 5.7	0.7 ± 1.1	1.7 ± 2.6	0.5 ± 1.2	5.9 ± 10.2	32.6 ± 8.7	5.5 ± 1.6	2.5 ± 0.6	2,384 ± 888
Final population	79.2 ± 16.2	6.1 ± 7.3	4.3 ± 6.4	3.9 ± 5.9	0.7 ± 1.1	1.8 ± 2.7	0.5 ± 1.3	3.5 ± 4.9	32.4 ± 9	5.4 ± 1.6	2.5 ± 0.6	2,470 ± 862
OM55N group 2 weeks after												
7702 ^b	44.7	27.4	21.2	2.2	3.5	0.0	0.6	0.4	28.1	6.2	2.5	1,137
7711	91.9	2.2	1.2	2.8	0.4	1.4	0.0	0.0	12.5	4.5	0.6	2,179
7717	87.9	3.6	0.0	3.7	0.5	0.2	0.0	4.0	50.8	7.4	1.8	2,948
7722 ^b	0.0	30.7	0.7	25.0	40.6	0.0	0.5	2.5	25.0	4.8	2.4	1,653
7723 ^b	0.0	67.3	0.0	4.3	4.8	0.0	0.2	23.5 ^j	27.9	4.6	1.9	1,947
7724	91.8	1.6	0.5	0.2	1.9	0.0	0.6	3.3	24.0	5.5	1.8	2,728
7734 ^b	88.1	0.0	1.7	3.7	0.5	0.0	0.1	5.9	32.1	4.3	2.3	3,589
7761	94.5	1.0	2.8	0.4	0.2	0.0	0.0	1.2	28.7	5.3	2.1	3,918
7763	99.1	0.2	0.0	0.0	0.0	0.0	0.3	0.3	14.1	2.8	1.5	1,631
7765 ^b	0.0	5.6	0.1	0.3	0.2	4.8	3.9	85.1 ^k	25.2	7.3	2.0	1,151
7766	74.6	11.0	6.9	2.7	3.3	0.0	0.1	1.4	22.7	4.8	1.5	1,344
7769	94.1	0.2	0.2	2.7	0.1	0.0	0.1	2.5	22.1	5.8	0.7	1,601
7772	92.1	3.7	1.3	0.1	1.7	0.0	0.0	1.2	24.7	4.9	1.8	1,718
7773	87.4	2.5	8.9	0.5	0.4	0.0	0.0	0.4	26.0	4.0	2.3	2,124
7774 ^b	98.9	0.6	0.4	0.1	0.0	0.0	0.1	0.0	10.3	2.5	1.1	2,443
7775 ^b	99.7	0.0	0.0	0.1	0.0	0.0	0.3	0.0	7.0	1.6	1.1	2,941
ALL infant	71.5 ± 37.8	9.9 ± 18	2.9 ± 5.5	3 ± 6.1	3.6 ± 10	0.4 ± 1.2	0.4 ± 1	8.2 ± 21.3	23.8 ± 10.2	4.8 ± 1.6	1.7 ± 0.6	2,191 ± 840
Final population	90.4 ± 6.9	2.9 ± 3.3	2.4 ± 3.3	1.5 ± 1.5	0.9 ± 1.1	0.2 ± 0.5	0.1 ± 0.2	1.6 ± 1.4	25.1 ± 11	5 ± 1.3	1.6 ± 0.6	2,243 ± 824

^a Abundance of the top seven bacterial families are shown.

^b Infant volunteers who had no bifidobacteria at week 0 were excluded from the galacto-oligosaccharides evaluation.

^c *Porphyromonadaceae* accounted for 18.0%.

^d *Actinomycetaceae*, *Clostridiaceae*, and *Propionibacteriaceae* accounted for 16.3%, 11.7%, and 11.3%, respectively.

^e *Clostridiaceae* accounted for 19.7%.

^f *Lachnospiraceae* accounted for 55.8%.

^g *Lachnospiraceae* and *Erysipelotrichaceae* accounted for 21.9% and 25.4%, respectively.

^h *Coriobacteriaceae* accounted for 14.8%.

ⁱ *Peptostreptococcaceae* accounted for 26.9%.

^j *Clostridiaceae* accounted for 22.8%.

^k *Erysipelotrichaceae* accounted for 63.9%.

Table S7. Summary of the 16S rRNA gene amplicon analysis data for the *Bifidobacterium* species.

	Control group (n = 13)		OM55N group (n = 9)		<i>P</i> -value ¹	
	Onset	2 week after	Onset	2 week after	Control vs GOS at onset	Change from onset to 2 wk after
<i>B. breve</i>	29.7 ± 34.8	21.9 ± 27.2	33.6 ± 33.5	33.4 ± 32.9	0.894	0.561
<i>B. longum</i>	33.7 ± 33.4	33.1 ± 26.0	29.0 ± 32.3	31.8 ± 32.1	0.421	0.948
<i>B. pseudocatenulatum</i>	4.0 ± 8.7	8.3 ± 15.2	15.4 ± 30.6	16.4 ± 30.1	0.302	0.693
<i>B. kashiwanohense</i>	6.2 ± 15.1	6.6 ± 16.2	0.1 ± 0.3	0.1 ± 0.3	0.502	0.262
<i>B. bifidum</i>	6.2 ± 12.0	4.5 ± 3.9	0.7 ± 2.0	0.3 ± 0.8	0.064	0.203
<i>B. adolescentis</i>	2.1 ± 4.0	3.3 ± 7.6	0.0 ± 0.0	0.0 ± 0.0	0.115	0.496
<i>B. catenulatum</i>	0.8 ± 2.7	1.3 ± 4.4	0.0 ± 0.0	0.0 ± 0.0	0.494	0.494
<i>B. dentium</i>	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	8.3 ± 24.8	0.342	0.661
<i>B. stercoris</i>	0.3 ± 0.7	0.2 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.494	0.899

¹ Differences between the control and galacto-oligosaccharide (GOS) (OM55N) group at the trial onset were determined using the Wilcoxon rank sum (abundance) or Pearson chi-square tests (% detected).

Table S8. Real-time PCR data of all samples in the study. ^a

ID	qPCR						
	<i>Bifidobacterium</i>	<i>Enterobacteriaceae</i>	<i>Enterococcus</i>	<i>Streptococcus</i>	<i>Veillonella</i>	<i>B.fragilis</i> group	<i>Staphylococcus</i>
Control group onset							
7701	10.1	9.2	8.6	9.4	8.0	<u>4.9</u>	7.4
7704	9.6	9.2	10.3	9.1	7.3	<u>4.9</u>	8.9
7728	9.9	8.8	9.1	9.1	7.1	8.4	7.2
7730	9.7	9.3	9.6	10.1	8.9	8.9	6.5
7731	9.9	7.2	7.8	9.9	8.7	6.4	6.7
7733	9.8	8.5	10.1	9.4	6.7	<u>4.9</u>	7.7
7760	9.6	8.7	10.0	7.6	8.6	8.1	5.7
7762	9.5	7.3	<u>4.9</u>	9.7	<u>4.9</u>	8.4	8.1
7764	9.5	8.6	9.1	8.2	6.4	<u>4.9</u>	5.8
7767	9.4	7.7	7.1	9.3	<u>4.9</u>	7.7	7.1
7770	9.6	8.9	7.5	9.4	<u>4.9</u>	7.5	8.0
7776	9.5	8.8	<u>4.9</u>	9.4	7.9	8.5	6.4
7777	9.7	5.8	<u>4.9</u>	9.8	8.1	<u>4.9</u>	7.6
7778 ^b	<u>4.9</u>	8.9	12.5	9.0	8.5	<u>4.9</u>	7.3
ALL infant	9.3 ± 1.3	8.3 ± 1	8.3 ± 2.3	9.2 ± 0.7	7.2 ± 1.5	6.7 ± 1.7	7.2 ± 0.9
Final population	9.7 ± 0.2	8.3 ± 1	8 ± 2	9.3 ± 0.7	7.1 ± 1.5	6.8 ± 1.7	7.2 ± 0.9
OM55N group onset							
7702 ^a	<u>4.9</u>	9.7	8.6	10.2	9.1	<u>4.9</u>	7.0
7711	10.3	8.6	10.2	9.1	8.9	7.8	8.4
7717	10.4	8.5	8.8	10.1	7.4	8.4	5.7
7722 ^a	<u>4.9</u>	9.2	<u>4.9</u>	9.0	8.4	<u>4.9</u>	8.0
7723 ^a	<u>4.9</u>	9.6	7.8	9.5	8.9	<u>4.9</u>	7.2
7724	9.5	6.0	9.7	9.3	7.8	<u>4.9</u>	7.8
7734 ^a	<u>4.9</u>	<u>4.9</u>	10.6	10.1	7.2	<u>4.9</u>	7.4
7761	9.8	8.1	9.4	8.9	8.3	<u>4.9</u>	6.6
7763	9.7	8.2	5.5	8.7	6.0	6.6	7.7
7765 ^a	<u>4.9</u>	8.2	9.1	8.8	7.1	8.7	8.3
7766	6.6	10.4	7.6	8.8	9.1	8.7	7.7
7769	9.7	8.0	8.6	10.2	8.1	<u>4.9</u>	7.7
7772	9.5	9.3	9.1	8.7	7.2	<u>4.9</u>	6.0
7773	9.4	8.0	9.8	9.0	8.0	<u>4.9</u>	6.6
7774 ^a	<u>4.9</u>	9.6	9.1	10.7	<u>4.9</u>	<u>4.9</u>	9.1
7775 ^a	<u>4.9</u>	10.0	9.0	9.9	6.9	<u>4.9</u>	9.4
ALL infant	7.4 ± 2.5	8.5 ± 1.4	8.6 ± 1.6	9.4 ± 0.7	7.7 ± 1.2	5.9 ± 1.6	7.5 ± 1
Final population	9.4 ± 1.1	8.4 ± 1.2	8.8 ± 1.5	9.2 ± 0.6	7.9 ± 0.9	6.2 ± 1.7	7.1 ± 0.9
Control group 2 weeks after							
7701	10.0	9.0	9.0	9.7	7.2	<u>4.9</u>	7.2
7704	9.2	9.4	9.4	9.2	6.2	<u>4.9</u>	8.6
7728	9.8	8.2	10.4	8.8	7.8	7.1	6.8
7730	9.7	9.1	9.6	10.0	8.5	8.1	6.5
7731	9.7	8.3	7.6	10.1	8.2	6.5	5.5
7733	9.7	8.1	8.6	10.1	7.8	9.2	7.8
7760	10.0	8.4	10.2	8.8	7.9	7.9	6.5
7762	8.9	8.6	<u>4.9</u>	9.6	<u>4.9</u>	8.0	8.1
7764	9.4	8.6	9.8	8.8	6.7	<u>4.9</u>	<u>4.9</u>
7767	9.9	8.4	8.3	9.6	<u>4.9</u>	6.7	7.4
7770	9.0	9.3	8.8	10.4	<u>4.9</u>	8.5	7.7
7776	9.4	9.1	7.5	9.5	7.3	8.6	6.4
7777	10.0	8.7	7.7	9.2	5.1	<u>4.9</u>	6.9
7778 ^a	<u>4.9</u>	9.0	12.0	9.4	8.1	<u>4.9</u>	7.6
ALL infant	9.2 ± 1.3	8.7 ± 0.4	8.8 ± 1.7	9.5 ± 0.5	6.8 ± 1.4	6.8 ± 1.6	7 ± 1
Final population	9.6 ± 0.4	8.7 ± 0.4	8.6 ± 1.5	9.5 ± 0.5	6.7 ± 1.4	6.9 ± 1.6	6.9 ± 1
OM55N group 2 weeks after							
7702 ^a	9.0	9.5	9.4	9.7	8.0	<u>4.9</u>	6.9
7711	10.2	8.5	9.1	9.3	8.0	7.8	7.3
7717	10.4	9.2	7.5	10.0	7.8	8.3	5.7
7722 ^a	<u>4.9</u>	8.8	7.1	10.1	9.4	<u>4.9</u>	6.8
7723 ^a	<u>4.9</u>	9.6	<u>4.9</u>	9.5	8.5	<u>4.9</u>	6.8
7724	9.7	8.3	10.3	8.5	8.2	<u>4.9</u>	7.7
7734 ^a	9.7	<u>4.9</u>	9.4	9.7	7.0	<u>4.9</u>	7.5
7761	9.8	7.9	9.7	8.3	6.4	<u>4.9</u>	6.1
7763	9.7	7.6	<u>4.9</u>	8.7	6.9	5.5	7.3
7765 ^a	<u>4.9</u>	8.5	7.2	8.4	6.2	7.8	7.9
7766	9.4	9.7	8.7	9.6	8.1	7.3	7.2
7769	9.8	7.8	9.0	10.3	7.1	<u>4.9</u>	7.6
7772	9.5	9.0	9.7	8.9	8.5	<u>4.9</u>	5.8
7773	9.6	8.7	10.3	9.2	8.2	<u>4.9</u>	6.2
7774 ^a	9.5	8.2	7.4	7.8	<u>4.9</u>	<u>4.9</u>	7.5
7775 ^a	9.3	5.7	<u>4.9</u>	7.9	<u>4.9</u>	<u>4.9</u>	8.0
ALL infant	8.8 ± 2	8.2 ± 1.3	8.1 ± 1.9	9.1 ± 0.8	7.4 ± 1.3	5.7 ± 1.3	7 ± 0.7
Final population	9.8 ± 0.3	8.5 ± 0.7	8.8 ± 1.7	9.2 ± 0.7	7.7 ± 0.7	5.9 ± 1.4	6.8 ± 0.8

^a The detection limit of qPCR in log 4.9.

^b Infant volunteers who had no bifidobacteria at trial onset were excluded.

Table S9. The faecal pH and short-chain fatty acid concentrations of all samples in this study.

ID	pH	Short-chain fatty acid							Total
		Acetate	Lactate	Propionate	Succinate	Formate	Butyrate	Valerate	
Control group onset									
7701	5.6	69.2	17.9	6.5	1.5	0.0	0.0	0.0	95.0
7704	7.3	18.7	0.0	2.8	0.0	5.9	0.0	0.0	27.4
7728	6.7	31.5	10.1	3.2	32.5	2.1	0.0	0.0	79.3
7730	6.5	29.2	0.0	26.4	0.0	0.0	0.0	0.0	55.6
7731	6.0	56.7	17.7	8.6	1.1	17.0	0.0	0.0	101.2
7733	7.6	28.0	0.0	16.5	25.4	0.0	0.0	0.0	69.9
7760	6.2	61.8	0.0	15.0	0.0	1.7	0.0	0.0	78.4
7762	6.3	70.3	0.0	9.8	1.1	5.9	17.8	3.7	108.5
7764	5.8	25.7	7.0	1.2	2.2	0.0	0.0	0.0	36.1
7767	5.5	78.6	68.1	0.0	5.4	5.4	0.0	0.0	157.4
7770	5.9	74.3	32.2	18.8	3.6	0.0	0.0	0.0	129.0
7776	6.0	40.3	3.2	15.3	31.7	0.0	3.1	0.0	93.6
7777	5.7	64.7	31.0	0.0	0.0	6.2	0.0	0.0	101.9
7778 ^a	7.6	26.7	4.3	8.7	1.1	20.0	0.0	0.0	60.9
ALL infant	6.3 ± 0.7	48.3 ± 21.5	13.7 ± 19.3	9.5 ± 8	7.5 ± 12.3	4.6 ± 6.4	1.5 ± 4.8	0.3 ± 1	85.3 ± 35
Final population	6.2 ± 0.6	49.9 ± 21.4	14.4 ± 19.9	9.5 ± 8.3	8 ± 12.6	3.4 ± 4.9	1.6 ± 4.9	0.3 ± 1	87.2 ± 35.7
OM55N group onset									
7702 ^a	5.7	34.2	18.2	21.6	2.5	0.0	0.0	0.0	76.5
7711	6.2	54.8	7.5	14.1	5.7	4.4	0.0	0.0	86.5
7717	6.2	123.0	16.7	31.3	0.0	0.0	9.8	0.0	180.9
7722 ^a	7.1	12.0	3.6	4.8	4.3	3.6	0.0	0.0	28.3
7723 ^a	6.6	22.1	0.0	11.7	4.5	0.0	0.0	0.0	38.3
7724	6.1	39.7	33.9	0.0	1.1	6.3	0.0	0.0	80.9
7734 ^a	6.4	71.0	2.8	7.4	0.0	0.0	13.6	0.0	94.8
7761	6.0	81.9	19.9	17.0	1.7	0.0	0.0	0.0	120.5
7763	6.5	47.8	26.9	1.4	19.5	0.0	0.0	0.0	95.6
7765 ^a	6.2	46.4	22.6	31.1	10.5	4.1	1.3	0.0	116.1
7766	6.5	28.8	0.0	17.1	0.0	0.0	1.3	0.0	47.2
7769	6.1	80.6	12.0	22.2	0.0	0.0	0.0	0.0	114.8
7772	6.0	47.8	17.7	3.9	0.0	0.0	6.8	0.0	76.2
7773	6.2	33.1	3.2	8.3	0.0	1.8	3.4	0.0	49.9
7774 ^a	6.4	26.3	5.9	0.0	8.3	0.0	4.5	0.0	44.9
7775 ^a	6.6	17.3	0.0	1.7	8.1	0.0	0.0	0.0	27.2
ALL infant	6.3 ± 0.3	47.9 ± 29	11.9 ± 10.6	12.1 ± 10.5	4.1 ± 5.4	1.3 ± 2.1	2.5 ± 4.2	0 ± 0	79.9 ± 41
Final population	6.2 ± 0.2	59.7 ± 30.2	15.3 ± 11	12.8 ± 10.4	3.1 ± 6.4	1.4 ± 2.4	2.4 ± 3.6	0 ± 0	94.7 ± 40.9
Control group 2 weeks after									
7701	7.6	47.2	0.0	7.7	3.1	0.0	0.0	0.0	58.1
7704	7.4	9.0	0.0	2.5	0.0	1.5	0.0	0.0	13.0
7728	8.1	25.8	0.0	18.4	3.8	7.4	0.0	0.0	55.5
7730	6.6	47.9	4.1	25.2	20.4	4.2	0.0	0.0	101.9
7731	5.7	37.7	65.4	1.2	2.3	4.0	0.0	0.0	110.5
7733	5.9	90.4	32.9	19.9	18.0	8.4	0.0	0.0	169.7
7760	6.2	70.3	2.4	19.0	0.0	4.2	0.0	0.0	95.9
7762	6.1	95.2	22.0	15.0	0.0	7.0	15.5	3.2	157.9
7764	5.6	57.4	24.0	5.4	1.4	0.0	0.0	0.0	88.2
7767	5.5	66.1	47.8	0.0	9.3	6.3	0.0	0.0	129.4
7770	6.3	127.4	14.9	48.5	0.0	0.0	0.0	0.0	190.8
7776	6.0	57.2	3.6	26.2	15.9	0.0	4.1	0.0	107.0
7777	5.8	73.7	45.2	0.0	6.0	9.8	0.0	0.0	134.7
7778 ^a	8.2	14.2	0.0	6.9	0.0	5.8	0.0	0.0	27.0
ALL infant	6.5 ± 0.9	58.5 ± 32.5	18.7 ± 21.6	14 ± 13.6	5.7 ± 7.3	4.2 ± 3.4	1.4 ± 4.2	0.2 ± 0.9	102.8 ± 52.2
Final population	6.4 ± 0.8	62 ± 31.1	20.2 ± 21.8	14.5 ± 14	6.2 ± 7.4	4.1 ± 3.5	1.5 ± 4.4	0.2 ± 0.9	108.7 ± 49.3
OM55N group 2 weeks after									
7702 ^a	6.9	32.9	1.3	7.0	6.5	6.0	0.0	0.0	53.8
7711	5.8	40.4	37.7	1.3	5.5	11.5	0.0	0.0	96.3
7717	5.8	73.6	21.8	19.9	20.5	4.1	0.0	0.0	139.8
7722 ^a	6.0	27.3	24.9	37.7	6.5	0.0	0.0	0.0	96.4
7723 ^a	6.5	29.7	2.3	9.1	2.8	1.2	19.4	0.0	64.5
7724	5.5	68.6	34.1	8.1	0.0	10.3	0.0	0.0	121.2
7734 ^a	6.5	70.1	7.1	5.7	1.2	2.3	5.8	0.0	92.1
7761	6.6	55.2	4.1	10.2	1.2	3.7	0.0	0.0	74.5
7763	7.2	21.2	9.0	1.2	8.6	1.1	0.0	0.0	41.1
7765 ^a	5.8	62.9	0.0	29.4	10.2	2.1	8.3	0.0	113.0
7766	7.2	32.7	0.0	12.1	0.0	6.8	6.4	0.0	57.9
7769	5.9	74.2	30.8	14.2	2.4	0.0	1.1	0.0	122.7
7772	5.8	69.4	27.7	10.2	0.0	1.3	6.2	0.0	114.7
7773	6.3	58.2	5.4	9.3	0.0	6.3	7.4	0.0	86.6
7774 ^a	5.6	53.6	55.1	0.0	6.2	10.2	0.0	0.0	125.0
7775 ^a	5.7	31.2	14.3	0.0	1.2	12.8	0.0	0.0	59.5
ALL infant	6.2 ± 0.6	50.1 ± 18.9	17.2 ± 16.5	11 ± 10.4	4.5 ± 5.4	5 ± 4.3	3.4 ± 5.3	0 ± 0	91.2 ± 30
Final population	6.2 ± 0.6	54.8 ± 19.3	18.9 ± 14.4	9.6 ± 5.9	4.2 ± 6.8	5 ± 4.1	2.3 ± 3.3	0 ± 0	95 ± 32.8

^a Infant volunteers who had no bifidobacteria at onset were excluded.

Table S10. Comparison of gut microbiota between infants delivered vaginally and via Caesarean section.

	Vaginal group (n = 16)		Caesarean (n = 14)		P value ^a	
	Onset Abundance	No detected (%)	Onset Abundance	No detected (%)	Abundance	% detected
Abundance (%)						
Bifidobacteriaceae	74.5 ± 30.6	15 (94%)	42.6 ± 45.1	7 (50%)	0.091	0.012
Enterobacteriaceae	6.7 ± 14.5	15 (94%)	25.7 ± 30.9	12 (86%)	0.257	0.586
Enterococcaceae	1.6 ± 2.3	13 (81%)	9.8 ± 13.0	13 (93%)	0.016	0.602
Streptococcaceae	2.5 ± 3.1	15 (94%)	3.7 ± 5.0	14 (100%)	0.580	1.000
Veillonellaceae	3.0 ± 5.4	13 (81%)	2.5 ± 3.5	12 (86%)	0.886	1.000
Bacteroidaceae	3.7 ± 6.5	11 (69%)	0.1 ± 0.3	1 (7%)	0.001	0.001
Staphylococcaceae	0.9 ± 2.7	13 (81%)	4.5 ± 9.0	10 (71%)	0.330	0.675
Alpha-diversity						
Chao1 index	31.1 ± 7.9		27.5 ± 9.2		0.257	
PD	5.7 ± 1.2		4.7 ± 1.4		0.015	
Shannon index	2.1 ± 0.8		2.2 ± 0.7		0.854	

PD = phylogenetic diversity

^a Differences between the control and galacto-oligosaccharide groups at trial onset were determined using the Wilcoxon rank sum or Pearson chi-square tests.