Supplementary Tables

Supplementary Table 1 | Structures and initial concentrations of sugars (Lac and HMOs purified from pooled breastmilk) used in this study, at 1 % (*w/v*).

Oligosaccharide	Structure	Concentration (mM)
Lactose	Galβ1–4Glc	0.75 ± 0.12
2'-FL	Fucα1–2Galβ1–4Glc	3.58 ± 0.54
3-FL	Galβ1–4(Fucα1–3)Glc	4.06 ± 0.59
LDFT	Fucα1–2Galβ1–4(Fucα1–3)Glc	0.49 ± 0.07
LNT	Galβ1–3GlcNAcβ1–3Galβ1–4Glc	1.21 ± 0.24
LN*n*T	Galβ1–4GlcNAcβ1–3Galβ1–4Glc	0.54 ± 0.08
LNFP I	Fucα1–2Galβ1–3GlcNAcβ1–3Galβ1–4Glc	0.56 ± 0.08
LNFP II + III	Galβ1–3(Fucα1–4)GlcNAcβ1–3Galβ1–4Glc (LNFP II)	0.89 ± 0.12
	Galβ1–4(Fucα1–3)GlcNAcβ1–3Galβ1–4Glc (LNFP III)	
LNDFH I	Fucα1–2Galβ1–3(Fucα1–4)GlcNAcβ1–3Galβ1–4Glc	0.68 ± 0.08
Total		12.76 ± 1.52

* Abbreviations: 2'-FL, 2'-Fucosyllactose; 3-FL, 3-Fucosyllactose; LDFT, Lactodifucotetraose; LNT, Lacto-*N*-tetraose; LN*n*T, Lacto-*N*-*neo*tetraose; LNFP, Lacto-*N*-fucopentaose; LNDFH, Lacto-*N*-difucohexaose; Fuc, Fucose; Glc, Glucose; GlcNAc, *N*-Acetylglucosamine; Gal, Galactose

Supplementary Table 2 | Summary of genes related to HMO assimilation in the strains used in this study.

Name	Pathway	Annotation	Classification	*B. longum* subsp. *longum* MCC10007	*B. longum* subsp. *infantis* ATCC 15697	*B. breve* UCC2003	*B. bifidum* JCM 1254	Reference
GalK	Galactose catabolism	Galactokinase	EC 2.7.1.6	MCC10007_0432	Blon_2062	Bbr_0492	JCM1254_16870	(Inoue et al. 2011, Li et al. 2012)
GalE	Galactose catabolism	UDP-glucose 4-epimerase	EC 5.1.3.2	MCC10007_1574	Blon_0538	Bbr_0040	JCM1254_05330	(De Bruyn et al. 2013)
GalT	Galactose catabolism	UDP-glucose-hexose 1-phosphate uridylyl transferase	EC 2.7.7.12	MCC10007_0431	Blon_2063	Bbr_0491	JCM1254_16880	(Inoue et al. 2011)
Pgm	Galactose catabolism	Phosphoglucomutase	EC 5.4.2.2	MCC10007_1664	Blon_1766; Blon_2184	Bbr_0742;Bbr_1595	JCM1254_12450	NA
NagK	GlcNAc catabolism	Predicted *N* -acetyl-glucosamine kinase, ROK family	EC 2.7.1.59	MCC10007_1232	Blon_0879	Bbr_1250	JCM1254_01520	NA
NagA	GlcNAc catabolism	*N* -acetylglucosamine-6-phosphate deacetylase	EC 3.5.1.25	MCC10007_1229	Blon_0882	Bbr_1247	JCM1254_14260	NA
NagB	GlcNAc catabolism	Glucosamine-6-phosphate deaminase	EC 3.5.99.6	MCC10007_1230	Blon_0881	Bbr_1248	JCM1254_14270	NA
LnpA	LNB catabolism	1,3-β-galactosyl-*N* -acetylhexosamine phosphorylase	EC 2.4.1.211	MCC10007_1654	Blon_2174	Bbr_1587	JCM1254_01530, JCM1254_11570	(Kitaoka et al. 2005)
LnpB	LNB catabolism	*N* -acetylhexosamine 1-kinase	EC 2.7.1.162	MCC10007_1653	Blon_2173	Bbr_1586	JCM1254_01500	(Nishimoto and Kitaoka 2007)
LnpC	LNB catabolism	UDP-glucose-hexose 1-phosphate uridylyl transferase	EC 2.7.7.12	MCC10007_1652	Blon_2172	Bbr_1884	JCM1254_01490	(Nishimoto and Kitaoka 2007)
LnpD	LNB catabolism	UDP-glucose 4-epimerase	EC 5.1.3.2	MCC10007_1651	Blon_2171	Bbr_1585	JCM1254_01480	(Nishimoto and Kitaoka 2007)
FumB	Fucose catabolism	L-fucose mutarotase	EC 5.1.3.29	NA	Blon_2305; Blon_2337	NA	NA	(James et al. 2019)
FumC	Fucose catabolism	L-fuco-β-pyranose dehydrogenase	EC 1.1.1.122	MCC10007_0307	Blon_2308; Blon_2339	Bbr_1291; Bbr_1743	NA	(James et al. 2019)
FumD	Fucose catabolism	L-fuconolactone hydrolase	EC 3.1.1.-	MCC10007_0306	Blon_2306	Bbr_1290; Bbr_1741	NA	(James et al. 2019)
FumE	Fucose catabolism	L-fuconate dehydratase	EC 4.2.1.68	MCC10007_0308	Blon_0344;Blon_2309;Blon_2340	Bbr_1292; Bbr_1744	NA	(James et al. 2019)
FumF	Fucose catabolism	2-keto-3-deoxy-L-fuconate aldolase	EC 4.1.2.18	MCC10007_0305	Blon_2338	Bbr_1289, Bbr_1740	NA	(James et al. 2019)
FumG	Fucose catabolism	Predicted lactaldehyde reductase	EC 1.1.1.77	MCC10007_1571	Blon_0540	Bbr_1505	JCM1254_03860	(James et al. 2019)
Bga42A(BbgII)	GH	β-1,3/4/6-galactosidase	GH42	MCC10007_0485	Blon_2016	Bbr_0529	JCM1254_05840	(Goulas et al. 2009, Viborg et al. 2014, Ambrogi et al. 2019)
Bga2A(BbgIV)	GH	β-1,4-galactosidase	GH2	MCC10007_0744	Blon_2334	Bbr_1552	JCM1254_05290	(Goulas et al. 2009, Yoshida et al. 2012, Ambrogi et al. 2019)
BbgIII	GH	Extracellular β-1,4-galactosidase	GH2	NA	NA	NA	JCM1254_06870	(Miwa et al. 2010)
Hex1	GH	β-1,3/4/6-*N* -acetylglucosaminidase	GH20	NA	Blon_0459	Bbr_1556	JCM1254_03400	(Garrido et al. 2012)
Hex1′	GH	β-1,3/4/6-*N* -acetylglucosaminidase	GH20	MCC10007_1361	Blon_0732	NA	NA	(Garrido et al. 2012)
Hex2	GH	β-1,3/4-*N* -acetylglucosaminidase	GH20	NA	Blon_2355	NA	NA	(Garrido et al. 2012)
BbhI	GH	Extracellular β-1,3-*N* -acetylglucosaminidase	GH20	NA	NA	NA	JCM1254_09260	(Miwa et al. 2010)
BiAfcA	GH	α-1,2-L-fucosidase	GH95	MCC10007_0304	Blon_2335	Bbr_1288	NA	(Sela et al. 2012)
BiAfcB	GH	α-1,3/4-L-fucosidase	GH29	NA	Blon_2336	NA	NA	(Sela et al. 2012)
AfcA	GH	Extracellular α-1,2-L-fucosidase	GH95	NA	NA	NA	JCM1254_17170	(Katayama et al. 2004)
AfcB	GH	Extracellular α-1,3/4-L-fucosidase	GH29	NA	NA	NA	JCM1254_05790	(Ashida et al. 2009)
LnbB	GH	Extracellular lacto-*N* -biosidase	GH20	NA	NA	NA	JCM1254_04020	(Wada et al. 2008)
LnbX	GH	Extracellular lacto-*N* -biosidase	GH136	MCC10007_1471	NA	NA	NA	(Sakurama et al. 2013)
GlcP(GalP)	Transport	Glucose; galactose transporter	MFS; SP Family	MCC10007_1663	NA	NA	NA	(Parche et al. 2006)
GlcU	Transport	Glucose transporter	DMT; GRP Family	NA	NA	NA	JCM1254_04340	(Briczinski et al. 2008; Briczinski et al. 2009)
PtsG	Transport	Glucose transporter	PTS; Glc Family	MCC10007_1662	Blon_2183	Bbr_1594	JCM1254_12500	(Parche et al. 2007)
Blon_1383	Transport	Predicted galactose transporter	MFS; SSS Family	NA	Blon_1383	NA	NA	NA
FucP	Transport	Fucose transporter	MFS; FHS Family	NA	Blon_2307	Bbr_1742	NA	(Egan et al. 2014, Higgins et al. 2021)
LacS	Transport	Lactose transporter	MFS; GPH Family	MCC10007_0745	Blon_2331;Blon_2332	Bbr_1551	JCM1254_05300	(O'Connell Motherway et al. 2013)
GltABC	Transport	LNB transporter; predicted LNT transporter	ABC; CUT1 Family	MCC10007_1655-MCC10007_1657	Blon_2175-2177	Bbr_1588-1590	JCM1254_01540-01560	(Suzuki et al. 2008, Garrido et al. 2011, Katoh et al. 2020)
Blon_0883-0885	Transport	LNB transporter*	ABC; CUT1 Family	NA	Blon_0883-0885	NA	NA	(Garrido et al. 2011)
Blon_2345-2347	Transport	LN*n* T transporter*	ABC; CUT1 Family	NA	Blon_2345-2347	NA	NA	(Garrido et al. 2011)
Blon_2342-2344	Transport	LN*n* T (low affinity) transporter*	ABC; CUT1 Family	NA	Blon_2342-2344	NA	NA	(Garrido et al. 2011)
Bbr_1554	Transport	LN*n* T transporter	ABC; CUT1 Family	NA	Blon_0462^^	Bbr_1554	NA	(James et al. 2016)
FL1	Transport	2′-FL; 3-FL transporter	ABC; CUT1 Family	NA	Blon_0341-0343	NA	NA	(Sakanaka et al. 2019)
FL2	Transport	2′-FL; 3-FL; LDFT; LNFPI transporter	ABC; CUT1 Family	MCC10007_0309-MCC10007_0311	Blon_2202-2204	NA	NA	(Sakanaka et al. 2019)
Blon_2350	Transport	Predicted HMO transporter*	ABC; CUT1 Family	NA	Blon_2350	NA	NA	(Garrido et al. 2011)
Blon_2351	Transport	Predicted HMO transporter*	ABC; CUT1 Family	NA	Blon_2351	NA	NA	(Garrido et al. 2011)
Blon_2352	Transport	Predicted HMO transporter*	ABC; CUT1 Family	NA	Blon_2352	NA	NA	(Garrido et al. 2011)
Blon_2354	Transport	Predicted HMO transporter*	ABC; CUT1 Family	NA	Blon_2354	NA	NA	(Garrido et al. 2011)

^^ ortholog is truncated

* Based on *in vitro* binding assays

Strains that possess the listed gene are highlighted in green

Supplementary Table 3 | Quantification of priority effects in pairwise cultures. Priority effects were quantified based on the equation proposed by Vannette and Fukami[30]. The strength of priority effects for species *A* when cultured with species B (P_{AB}) is calculated by the log of the ratio between the absolute abundance (number of gene copies per ng of DNA + 1) of species *A* when it was introduced after species *B*, $D(A)_{BA}$, and when it was introduced before species *B*, $D(A)_{AB}$. $P_{AB} = \ln[\frac{D(A)_{BA}}{D(A)_{AB}}]$.

		Co-Culture Species			
		B. bifidum	*B. breve*	*B. infantis*	*B. longum*
Focal Species	*B. bifidum*		-2.88	-6.80	-1.88
	B. breve	-4.94		-4.84	2.94
	B. infantis	-0.60	-3.60		-3.81
	B. longum	-5.08	0.32	-11.91	

Supplementary Table 4 | PERMANOVA of the covariant relationship between the concentration of sugars remaining in the culture medium at 24 h, and the final community structure *in vitro* (Significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Variables (Sugar remaining in the medium at 24 h)	NMDS1	NMDS2	R^2	p
Fuc	0.94921	-0.31465	0.4349	4.00E-04***
GlcNAc/Glc	0.97376	-0.22759	0.1672	0.050
Gal	-0.90301	-0.42962	0.124	0.091
LNB	0	0	0	1.000
Lac	0	0	0	1.000
2′-FL	0	0	0	1.000
3-FL	-0.90271	-0.43025	0.3518	0.002**
LDFT	-0.90271	-0.43026	0.0989	0.132
LNT	0	0	0	1.000
LN*n*T	0	0	0	1.000
LNFP I	0	0	0	1.000
LNFP II/III	-0.90269	-0.43028	0.2096	0.021*
LNDFH I	-0.9027	-0.43027	0.4453	2.00E-04***

Supplementary Table 5 | PERMANOVA of the covariant relationship between the bifidobacterial community structure at 4 months of age, and the abundances of each bifidobacterial species at the time of birth, based on *in vivo* data from Bäckhed et al. (2015), with taxonomic classifications performed using Kraken2 and Bracken (Significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Variables (*Bifidobacterium* abundances at birth)	NMDS1	NMDS2	R^2	p	
Bifidobacterium actinocoloniiforme	0.000	0.000	0.000	1	
Bifidobacterium adolescentis	0.787	-0.617	0.016	0.432	
Bifidobacterium angulatum	0.994	-0.106	0.003	0.866	
Bifidobacterium animalis	-0.957	-0.290	0.092	0.052	.
Bifidobacterium asteroides	0.000	0.000	0.000	1.000	
Bifidobacterium bifidum	0.150	0.989	0.007	0.656	
Bifidobacterium breve	-0.984	0.177	0.092	0.0499	*
Bifidobacterium catenulatum	0.662	0.750	0.008	0.564	
Bifidobacterium choerinum	0.000	0.000	0.000	1.000	
Bifidobacterium coryneforme	0.000	0.000	0.000	1.000	
Bifidobacterium dentium	0.998	-0.057	0.005	0.607	
Bifidobacterium eulemuris	-0.990	-0.139	0.001	0.920	
Bifidobacterium imperatoris	0.553	0.833	0.007	0.666	
Bifidobacterium indicum	0.000	0.000	0.000	1.000	
Bifidobacterium lemurum	0.862	-0.508	0.002	0.820	
Bifidobacterium longum / *infantis*	0.706	0.708	0.015	0.496	
Bifidobacterium pseudocatenulatum	-0.064	-0.998	0.022	0.374	
Bifidobacterium pseudolongum	-0.980	0.200	0.011	0.418	
Bifidobacterium pullorum	0.995	-0.100	0.008	0.654	
Bifidobacterium saguini	0.026	1.000	0.001	0.927	
Bifidobacterium scardovii	0.902	-0.431	0.001	0.922	
Bifidobacterium subtile	-0.151	-0.989	0.010	0.553	
Bifidobacterium thermophilum	0.840	-0.543	0.004	0.756	

Supplementary Table 6 | List of primers used in this study

Species		Primer Sequence	Reference
Bifidobacterium bifidum	F	5′-CCACATGATCGCATGTGATTG -3′	(Matsuki et al. 1998)
	R	5′-CCGAAGGCTTGCTCCCAAA -3′	
Bifidobacterium breve	F	5′-CCGGATGCTCCATCACAC -3′	(Matsuki et al. 1998)
	R	5′-ACAAAGTGCCTTGCTCCCT -3′	
Bifidobacterium longum	F	5′- ACATCCAGGACCGTAACCTG -3′	(Toda et al. 2019)
subspecies *infantis*	R	5′- GCTTGTGCAGCTCCGTCT -3′	
Bifidobacterium longum	F	5′- TTCCAGTTGATCGCATGGTC -3′	(Matsuki et al. 1998)
subspecies *longum*	R	5′- GGGAAGCCGTATCTCTACGA -3′	

Supplementary Table 7 | PERMANOVA of the covariant relationship between the bifidobacterial community structure at 4 months of age, and the abundances of each bifidobacterial species at the time of birth, based on *in vivo* data from Bäckhed et al. (2015), with taxonomic classifications performed using METAnnotatorX2 (Significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Variables (*Bifidobacterium* abundances at birth)	NMDS1	NMDS2	R^2	p	
Bifidobacterium adolescentis	-0.35181	0.93607	0.0078	0.7615	
Bifidobacterium angulatum	0	0	0	1	
Bifidobacterium animalis	0.51601	0.85658	0.0019	0.957	
Bifidobacterium bifidum	0.73276	-0.68049	0.0087	0.7404	
Bifidobacterium breve	-0.42509	-0.90515	0.327	0.0001	***
Bifidobacterium catenulatum	-0.96156	-0.27459	0.0057	0.8295	
Bifidobacterium dentium	-0.21366	0.97691	0.0786	0.0593	
Bifidobacterium longum / *infantis*	0.34135	0.93994	0.0605	0.1062	
Bifidobacterium pseudocatenulatum	-0.91103	0.41233	0.0557	0.1165	
Bifidobacterium ruminantium	0	0	0	1	
Bifidobacterium scardovii	0	0	0	1	
Bifidobacterium unclassified species	0.8073	-0.59014	0.0072	0.777	

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