

## **Supplementary information**

### **Title**

Neonatal oral fluid as a transmission route for bifidobacteria to the infant gut immediately after birth

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**Supplemental Table 1. Cell numbers of total bacteria and bifidobacteria in OF samples.**

ID	OF									IF	
	Precultivation				Postcultivation						
	Inoculum		Inoculation ratio	Calculated value		BM		YCFA			
Target	T	B		T	B	T	B	T	B	T	B
1	6	4.8	0.20%	3.3	2.1	7.5		7.2		8.5	10.1
2	4.8		0.20%	2.1		7.3		7.1	6.8	9	10.3
3	5.9	6.4	0.10%	2.9	3.4	8.5	9	8.6	7.8	8	9
4	5.4		0.20%	2.7		8		9		7.6	
5	5.4		0.20%	2.7		7.4	6.1	8.9		8	8.9
6	5.6	4.5	0.10%	2.6	1.5	8.5		8.6		7.7	
7	6		0.10%	3		9.1	9.1	8.9	5.9	7.8	8.9
8	6.9	6.7	0.20%	4.2	4	8.6	6.8	8.1	5	8.8	10.1
9	6.7	4.2	0.20%	4	1.5	8.6		8.8	6.7	8.8	10
10	6.2	4.2	0.20%	3.5	1.5	8.3		8.5	5.4	9.1	10.5
11	6.4	4.3	0.10%	3.4	1.3	5.8		8.7		8.3	9.2
12	6.3	4.8	0.20%	3.6	2.1	9.2	9.1	9.2	8.8	8.8	9.8
13	4.9		0.20%	2.2		8.4	8.1	9.2	7.6	7.6	8.2
14	4.8		0.20%	2.1		8.2	4.2	9	5.4	8.1	
15	4.5		0.10%	1.5		8.2	5.5	8.2		8.5	9.6

The values indicate the cell numbers (Log/mL or g).

Target shown as: T, total cell numbers; or B, *Bifidobacterium*.



**Supplemental Table 3. Detailed information of isolated strains from OF, IF and MF samples.**

Label	Family No.	Isolation Source	Species	Total Sequence Length (Mbp):	Number of Sequences:	Longest Sequences (bp):	N50 (bp):	Gap Ratio (%):	GC content (%):	Number of CDSs:	Average Protein Length:	Coding Ratio (%):	Number of rRNAs:	Number of tRNAs:	Number of CRISPRs:	Cultivation Medium	Carbon source in TOS Agar Plate	DDBJ accession number	Morinaga Culture Collection (MCC) number
ID01IF1	1	Infant feces	<i>B. infantis</i>	2.67	76	164760	94009	0.0075	59.8	2334	323.4	84.7	2	58	0	-	Galactooligosaccharide	BJIA01000001-BJIA01000076	MCC-01971
ID02IF1	2	Infant feces	<i>B. bifidum</i>	2.22	45	242612	118940	0.005	62.8	1868	337.9	85.2	1	55	1	-	Galactooligosaccharide	BJIB01000001-BJIB01000045	MCC-01946
ID02IF2	2	Infant feces	<i>B. longum</i>	2.47	58	233153	124210	0.0042	59.7	2127	333.9	86.2	2	76	2	-	Galactooligosaccharide	BJIC01000001-BJIC01000058	MCC-01972
ID02MF1	2	Maternal feces	<i>B. adolescentis</i>	2.29	23	743896	462236	0.0011	59.3	1943	342.3	87.3	2	59	1	-	Galactooligosaccharide	BJID01000001-BJID01000035	MCC-01943
ID02MF2	2	Maternal feces	<i>B. bifidum</i>	2.22	47	232728	118304	0.002	62.8	1868	336.4	84.8	2	55	1	-	Galactooligosaccharide	BJIE01000001-BJIE01000023	MCC-01947
ID02MF3	2	Maternal feces	<i>B. longum</i>	2.45	66	263956	115024	0.0027	59.9	2080	336.8	86	2	77	2	YCFA	Galactooligosaccharide	BJIF01000001-BJIF01000066	MCC-01975
ID02OF1	2	Neonatal oral fluid	<i>B. adolescentis</i>	2.28	20	1430044	1430044	0.0028	59.3	1952	340.6	87.5	2	59	1	YCFA	Galactooligosaccharide	BJIG01000001-BJIG01000020	MCC-01941
ID02OF2	2	Neonatal oral fluid	<i>B. pseudocatenulatum</i>	2.38	20	757568	505692	0.0011	56.5	1986	344.4	86.3	3	57	1	YCFA	Galactooligosaccharide	BJIH01000001-BJIH01000020	MCC-01992
ID03IF1	3	Infant feces	<i>B. breve</i>	2.26	101	255869	212069	0.0006	58.7	1923	333.8	85.3	1	55	0	-	Galactooligosaccharide	BJII01000001-BJII01000527	MCC-01950
ID03OF1	3	Neonatal oral fluid	<i>B. breve</i>	2.23	28	335081	219798	0.0014	58.7	1928	333.1	86.3	1	55	0	-	Galactooligosaccharide	BJIJ01000001-BJIJ01000028	MCC-01951
ID05IF1	5	Infant feces	<i>B. pseudocatenulatum</i>	2.25	257	81150	15424	0.0001	56.2	1712	338.2	77.2	1	48	5	-	Galactooligosaccharide	BJIK01000001-BJIK01000257	MCC-01994
ID05OF1	5	Neonatal oral fluid	<i>B. pseudocatenulatum</i>	2.25	96	124875	42605	0.0002	56.2	1800	352.4	84.4	2	51	4	BM	Galactooligosaccharide	BJIL01000001-BJIL01000096	MCC-01993
ID07IF1	7	Infant feces	<i>B. bifidum</i>	2.25	81	304615	178434	0.0015	62.8	1846	344.3	84.9	2	56	0	-	2'-Fucosyllactose	BJIM01000001-BJIM01000081	MCC-02030
ID07IF2	7	Infant feces	<i>B. breve</i>	2.34	43	316196	127027	0.0035	58.8	2021	333.4	86.3	2	55	1	-	Galactooligosaccharide	BJIN01000001-BJIN01000043	MCC-01954
ID07IF3	7	Infant feces	<i>B. longum</i>	2.33	37	410666	146688	0.002	59.9	1922	351.9	87	2	54	1	-	Galactooligosaccharide	BJIO01000001-BJIO01000037	MCC-01976
ID07OF1	7	Neonatal oral fluid	<i>B. breve</i>	2.34	43	316196	127027	0.0035	58.8	2021	333.4	86.3	2	55	1	BM	Galactooligosaccharide	BJIP01000001-BJIP01000028	MCC-01953
ID08IF1	8	Infant feces	<i>B. breve</i>	2.43	24	565698	311771	0.0116	58.5	2143	328.7	86.8	2	55	1	-	Galactooligosaccharide	BJIQ01000001-BJIQ01000024	MCC-01957
ID08IF2	8	Infant feces	<i>B. longum</i>	2.33	39	410576	146434	0.0075	59.9	1918	352.7	86.9	2	53	1	-	Galactooligosaccharide	BJIR01000001-BJIR01000039	MCC-01977
ID08OF1	8	Neonatal oral fluid	<i>B. bifidum</i>	2.21	57	215216	96233	0.0016	62.8	1835	342.2	85.2	2	54	0	-	Galactooligosaccharide	BJIS01000001-BJIS01000057	MCC-01958
ID08OF2	8	Neonatal oral fluid	<i>B. breve</i>	2.45	39	594361	283907	0.004	58.4	2148	328.2	86.3	2	55	1	YCFA	Galactooligosaccharide	BJIT01000001-BJIT01000039	MCC-01959
ID09IF1	9	Infant feces	<i>B. breve</i>	2.33	39	285123	108385	0.0018	58.8	1951	342.5	86.2	2	54	1	-	Galactooligosaccharide	BJIU01000001-BJIU01000039	MCC-01963
ID09IF2	9	Infant feces	<i>B. longum</i>	2.35	170	171627	28382	0.006	60.1	1884	342.4	82.3	2	53	4	-	Galactooligosaccharide	BJIV01000001-BJIV01000170	MCC-01978
ID09OF1	9	Neonatal oral fluid	<i>B. breve</i>	2.26	22	405061	162736	0.0042	58.9	1969	331.9	86.7	1	54	0	BM	Galactooligosaccharide	BJIW01000001-BJIW01000022	MCC-01961
ID09OF2	9	Neonatal oral fluid	<i>B. dentium</i>	2.6	67	444170	176860	0.0085	58.6	2183	347.7	87.5	3	55	1	YCFA	L-(+)-arabinose	BJIX01000001-BJIX01000067	MCC-02031
ID09OF3	9	Neonatal oral fluid	<i>B. pseudocatenulatum</i>	2.32	22	899093	287645	0.0008	56.9	1890	354.4	86.8	3	54	2	YCFA	Galactooligosaccharide	BJIY01000001-BJIY01000022	MCC-01995
ID10IF1	10	Infant feces	<i>B. breve</i>	2.22	33	605039	264331	0.0033	58.7	1903	334.4	85.9	2	54	0	-	Galactooligosaccharide	BJJZ01000001-BJJZ01000033	MCC-01964
ID11IF1	10	Infant feces	<i>B. dentium</i>	2.6	26	382911	305666	0.0068	58.5	2157	352.3	87.6	3	55	4	-	Galactooligosaccharide	BJJA01000001-BJJA01000026	MCC-01970
ID12IF1	12	Infant feces	<i>B. breve</i>	2.44	43	562774	134058	0.0016	59.2	2174	322.6	86.1	2	75	1	-	Galactooligosaccharide	BJJB01000001-BJJB01000043	MCC-01966
ID12IF2	12	Infant feces	<i>B. pseudocatenulatum</i>	2.27	46	424289	353901	0.0008	56.7	1844	355.4	86.6	1	54	0	-	D-(+)-Cellobiose	BJJC01000001-BJJC01000046	MCC-02032
ID12IF3	12	Infant feces	<i>B. pseudocatenulatum</i>	2.33	73	335434	115514	0.0052	56.9	1880	354.4	85.8	3	54	2	-	D-(+)-Cellobiose	BJJD01000001-BJJD01000073	MCC-02033
ID12MF1	12	Maternal feces	<i>B. longum</i>	2.53	76	167558	67927	0.0014	59.9	2134	341.5	86.3	2	79	3	YCFA	Galactooligosaccharide	BJJE01000001-BJJE01000076	MCC-01983
ID12MF2	12	Maternal feces	<i>B. pseudocatenulatum</i>	2.29	83	395652	99812	0.0007	56.7	1844	356	86.1	2	54	0	BM	2'-Fucosyllactose	BJJF01000001-BJJF01000083	MCC-02034
ID12MF3	12	Maternal feces	<i>B. pseudocatenulatum</i>	2.28	77	353658	175534	0.0014	56.7	1844	355.7	86.3	2	54	0	BM	2'-Fucosyllactose	BJJG01000001-BJJG01000077	MCC-02035
ID12OF1	12	Neonatal oral fluid	<i>B. longum</i>	2.54	73	185555	68250	0.0028	59.9	2149	339.1	86.2	2	79	3	-	Galactooligosaccharide	BJJH01000001-BJJH01000073	MCC-01979
ID12OF2	12	Neonatal oral fluid	<i>B. pseudocatenulatum</i>	2.27	36	293948	134272	0.0011	56.7	1844	357.7	87.2	2	54	0	BM	Galactooligosaccharide	BJJI01000001-BJJI01000036	MCC-01996
ID13IF1	13	Infant feces	<i>B. breve</i>	2.47	28	323794	177651	0.0071	58.6	2171	326.2	86.1	2	77	2	-	Galactooligosaccharide	BJJJ01000001-BJJJ01000028	MCC-01967
ID13IF2	13	Infant feces	<i>B. breve</i>	2.44	274	131186	27827	0.0099	58.5	2022	321.4	79.8	2	54	2	-	L-(+)-arabinose	BJJK01000001-BJJK01000274	MCC-02036
ID13IF3	13	Infant feces	<i>B. dentium</i>	2.6	67	322579	165925	0.0081	58.6	2182	348.1	87.6	3	55	1	-	L-(+)-arabinose	BJJL01000001-BJJL01000022	MCC-02037
ID13MF1	13	Maternal feces	<i>B. breve</i>	2.36	219	415273	382788	0.0058	58.7	1979	334.8	84.4	2	54	0	BM	2'-Fucosyllactose	BJJM01000001-BJMM01000219	MCC-02038
ID13MF2	13	Maternal feces	<i>B. longum</i>	2.38	36	266064	124325	0.0031	59.8	1995	346.1	86.9	2	60	0	BM	Galactooligosaccharide	BJJN01000001-BJJN01000036	MCC-01986
ID13MF3	13	Maternal feces	<i>B. pseudocatenulatum</i>	2.23	22	752541	413420	0.0045	56.2	1823	355.7	87.2	2	55	1	YCFA	Galactooligosaccharide	BJJO01000001-BJJO01000022	MCC-01988
ID13OF1	13	Neonatal oral fluid	<i>B. breve</i>	2.28	74	338849	218389	0.0045	58.9	1980	331.3	86.1	1	54	0	BM	2'-Fucosyllactose	BJJP01000001-BJJP01000074	MCC-02039
ID13OF2	13	Neonatal oral fluid	<i>B. longum</i>	2.38	43	248213	109588	0.0029	59.8	1992	345	86.5	2	58	0	BM	Galactooligosaccharide	BJJQ01000001-BJJQ01000043	MCC-01984
ID13OF3	13	Neonatal oral fluid	<i>B. pseudocatenulatum</i>	2.23	29	379214	143234	0.0004	56.2	1812	356.6	86.9	2	55	1	YCFA	Galactooligosaccharide	BJJR01000001-BJJR01000029	MCC-01998
ID14OF1	14	Neonatal oral fluid	<i>B. longum</i>	2.46	46	312578	130152	0.0011	59.8	2153	328.9	86.3	2	56	3	-	Galactooligosaccharide	BJJS01000001-BJJS01000046	MCC-01989
ID14OF2	14	Neonatal oral fluid	<i>B. longum</i>	2.38	31	270543	164562	0.0061	59.8	2003	344.5	86.8	2	58	0	-	Galactooligosaccharide	BJJT01000001-BJJT01000031	MCC-01990
ID15IF1	15	Infant feces	<i>B. breve</i>	2.2	26	465231	134114	0.0019	58.6	1900	333.5	86.2	1	55	0	-	Galactooligosaccharide	BJJU01000001-BJJU01000026	MCC-01969
ID15OF1	15	Neonatal oral fluid	<i>B. breve</i>	2.26	20	525267	389540	0.0016	58.9	1965	332.8	86.8	2	54	0	BM	Galactooligosaccharide	BJJV01000001-BJJV01000020	MCC-01968

**Supplemental Table 4. ANI values for candidate strains.**

Candidate No.	Label	Family No.	Isolation Source	Species	ANI Values
1	ID03IF1	3	Infant feces	<i>B. breve</i>	99.97%
	ID03OF1		Neonatal oral fluid		
2	ID05IF1	5	Infant feces	<i>B. pseudocatenulatum</i>	99.95%
	ID05OF1		Neonatal oral fluid		
3	ID07IF2	7	Infant feces	<i>B. breve</i>	99.96%
	ID07OF1		Neonatal oral fluid		
4	ID08IF1	8	Infant feces	<i>B. breve</i>	99.97%
	ID08OF2		Neonatal oral fluid		
5	ID09IF1	9	Infant feces	<i>B. breve</i>	98.19%
	ID09OF1		Neonatal oral fluid		
6	ID12IF2	12	Infant feces	<i>B. pseudocatenulatum</i>	99.97%
	ID12OF2		Neonatal oral fluid		
7	ID13IF1	13	Infant feces	<i>B. breve</i>	98.39%
	ID13OF1		Neonatal oral fluid		
8	ID15IF1	15	Infant feces	<i>B. breve</i>	98.78%
	ID15OB1		Neonatal oral fluid		
9	ID02MF1	2	Maternal feces	<i>B. adolescentis</i>	98.75%
	ID02OF1		Neonatal oral fluid		
10	ID12MF1	12	Maternal feces	<i>B. longum</i>	99.95%
	ID12OF1		Neonatal oral fluid		
11	ID12MF2	12	Maternal feces	<i>B. pseudocatenulatum</i>	99.96%
	ID12OF2		Neonatal oral fluid		
12	ID13MF3	13	Maternal feces	<i>B. pseudocatenulatum</i>	99.97%
	ID13OF3		Neonatal oral fluid		
13	ID13MF2	13	Maternal feces	<i>B. longum</i>	99.99%
	ID13OF2		Neonatal oral fluid		

Supplemental Table 5. Detailed information on the subjects.

Subject ID	Day		Information about infants						Information about mothers							Notes	
	Birthday	Hospital visiting day for 1 month check up	Infant gender	Body weight (g)		Nutritional status		Medication from birth to first 3-4 weeks of life	Gestational age at birth (weeks)	Blood group	Maternal age at birth (year-old)	Maternal defecation during delivery	Habitually intake of milk products	Habitually intake of probiotics	Use of antimicrobials at birth		Drug use during pregnancy
				At birth	At first 3-4 weeks of life	Breastfeeding (%)	Formula feeding (%)										
1	10-Jun-2017	18-Jul-2017	female	3450	3750	75	25	-	40	O	36	-	+	+	-	Gastrointestinal agents, Laxatives, Antihistamine, Antiallergic agents, Dermatologic preparation	Meconium staining
2	12-Jun-2017	18-Jul-2017	female	2660	3492	50	50	-	38	A	38	+	-	+	-	Tocolytic agent, Thyroid hormone preparation, Laxatives	Female infertility, multifetal pregnancy reduction
3	19-Jun-2017	18-Jul-2017	female	3510	4858	70	30	-	39	unknown	31	-	+	+	-	-	
4	19-Jun-2017	20-Jul-2017	male	3475	4396	100	0	-	38	A	35	-	+	-	+	Antihistamine, Antipyretic-analgesics	BMI16.3, GBS(2+)
5	15-Jul-2017	17-Aug-2017	female	3325	4282	80	20	-	39	A	34	-	+	+	-	Tocolytic agent	Pyelonephritis
6	8-Aug-2017	12-Sep-2017	female	2900	3844	70	30	-	41	B	32	+	+	+	-	Tocolytic agent, Antianemic agent	
7	9-Aug-2017	13-Aug-2017	male	4120	5195	100	0	-	40	O	36	-	-	-	-	-	
8	14-Aug-2017	12-Sep-2017	male	3110	4816	100	0	-	39	A	28	-	+	+	-	Laxatives	
9	15-Aug-2017	22-Sep-2017	female	2760	3484	100	0	-	39	A	28	-	+	+	-	Antianemic agent, Laxatives	
10	22-Aug-2017	25-Aug-2017	female	3280	4142	100	0	-	39	B	38	-	+	+	-	-	
11	24-Aug-2017	23-Sep-2017	female	2780	4052	100	0	-	38	B	31	-	-	-	-	Tocolytic agent, Antianemic agent, Gastric mucosal protection agent	
12	30-Aug-2017	27-Sep-2017	female	3135	4398	100	0	-	39	A	37	+	-	-	+	Laxatives, Antipyretic-analgesics, Prophylactic for oral bacterial infection	
13	5-Sep-2017	8-Sep-2017	male	3595	4254	33	67	-	41	A	28	+	+	+	+	Antianemic agent, Antianemic agent, Gastric mucosal protection agent, Laxatives	
14	6-Sep-2017	3-Oct-2017	female	2925	3970	90	10	-	40	O	32	-	+	-	-	-	
15	11-Sep-2017	10-Oct-2017	male	3480	4664	100	0	-	38	O	31	-	-	+	-	Tocolytic agent, Laxatives, Antianemic agent, Gastric mucosal protection agent, Antitussive drugs	







**Supplemental Table 8. List of primer sets.**

Primer	Target	Sequence (5' to 3')	Reference
Tru357F	V3-V4 region of the 16S rRNA gene	CGCTCTTCCGATCTCTGTACGGRAGGCAGCAG	
Tru806R		CGCTCTTCCGATCTGACGGACTACHVGGGTWTCTA	
g-Bifid-F	<i>Bifidobacterium</i>	CTCCTGGAAACGGGTGG	<i>Matsuki T et al.</i> , Appl Environ Microbiol. 68, 11, 5445-5451 (2002).
g-Bifid-R		GGTGTTCCTCCCGATATCTACA	
BiADOG-1a	<i>Bifidobacterium adolescentis</i> group	CTCCAGTTGGATGCATGTC	<i>Matsuki T et al.</i> , Appl Environ Microbiol. 65, 10, 4506-4512 (1999).
BiADOG-1b		TCCAGTTGACCGCATGGT	
BiADOG-2		CGAAGGCTTGCTCCCAGT	
Bflact2	<i>Bifidobacterium animalis</i> ssp. <i>lactis</i>	GTGGAGACACGGTTTCCC	<i>Ventura M et al.</i> , Appl Environ Microbiol. 67, 6, 2760-2765 (2001).
Bflact5		CACACCACACAATCCAATAC	
BiBIF-1	<i>Bifidobacterium bifidum</i>	CCACATGATCGCATGTGATTG	<i>Matsuki T et al.</i> , FEMS Microbiol Lett. 167, 2, 113-121 (1998).
BiBIF-2		CCGAAGGCTTGCTCCCAA	
BiBRE-1	<i>Bifidobacterium breve</i>	CCGGATGCTCCATCACAC	<i>Matsuki T et al.</i> , FEMS Microbiol Lett. 167, 2, 113-121 (1998).
BiBRE-2		ACAAAGTGCCTTGCTCCCT	
BiCATg-1	<i>Bifidobacterium catenulatum</i> group	CGGATGCTCCGACTCCT	<i>Matsuki T et al.</i> , FEMS Microbiol Lett. 167, 2, 113-121 (1998).
BiCATg-2		CGAAGGCTTGCTCCCGAT	
BiDEN-1	<i>Bifidobacterium dentium</i>	ATCCCGGGGGTTCGCCT	<i>Matsuki T et al.</i> , Appl Environ Microbiol. 65, 10, 4506-4512 (1999).
BiDEN-2		GAAGGGCTTGCTCCCGA	
BiLON-1	<i>Bifidobacterium longum</i> ssp. <i>longum</i>	TTCCAGTTGATCGCATGGTC	<i>Matsuki T et al.</i> , Appl Environ Microbiol. 65, 10, 4506-4512 (1999).
BiLON-2		GGGAAGCCGTATCTCTACGA	
INF_clpC_F	<i>Bifidobacterium longum</i> ssp. <i>infantis</i>	ACATCCAGGACCGTAACCTG	In this study
INF_clpC_R		GCTTGTGCAGCTCCGTCT	
OPF-09	RAPD-PCR (primer type 1)	CCAAGCTTCC	
OPF-11	RAPD-PCR (primer type 2)	TTGGTACCCC	