SUPPLEMENTARY MATERIAL

TABLE S1: List of *Bifidobacterium* strains used in this study.

Code	Species	Additional strain information[a]	Source
UCC2003	*B. breve*	(1)	Infant nursing stool
ATCC15700	*B. breve*	JCM1192; DSM20213	Infant feces
ATCC15698	*B. breve*	JCM1273; DSM20091	Infant feces
ATCC15701	*B. breve*	JCM7016	Infant feces
JCM7017	*B. breve*		Human feces
JCM7019	*B. breve*		Infant feces
JCM7020	*B. breve*		Infant feces
S-17c	*B. breve*	(2)	Infant feces
S-46	*B. breve*	(2)	Infant feces
SC81	*B. breve*	This study	Infant feces
SC95	*B. breve*	This study	Infant feces
SC139	*B. breve*	This study	Infant feces
SC154	*B. breve*	This study	Infant feces
SC500	*B. breve*	This study	Infant feces
SC506	*B. breve*	This study	Infant feces
SC508	*B. breve*	This study	Infant feces
SC522	*B. breve*	This study	Infant feces
SC559	*B. breve*	This study	Infant feces
SC567	*B. breve*	This study	Infant feces
SC568	*B. breve*	This study	Infant feces
SC573	*B. breve*	This study	Infant feces
SC580	*B. breve*	This study	Infant feces
SC670	*B. breve*	This study	Infant feces
KA179	*B. breve*	This study	Infant feces
ATCC15697	*B. longum subsp. infantis*	JCM1222; DSM20088	Infant feces
JCM10602	*B. animalis subsp. lactis*	DSMZ 10140	Dairy product

[a]The original strain numbers are also noted, if known. JCM, Japan Collection of Microorganisms, ATCC, American Type Culture Collection; DSMZ, German Collection of Microorganisms and Cell Culture.

TABLE S2: MLST genes and primers.

Gene	PCR primer (5´-3´)*,[a]	Expected Amplicon size (bp)	Anneling Temp. (ºC)
clpC	GAG TAC CGC AAG TAC ATC GAG CAT CCT CAT CGT CGA ACA GGA AC	748	63
purF	CAT TCG AAC TCC GAC ACC GA GTG GGG TAG TCG CCG TTG	977	62
gyrB	AGC TGC ACG CBG GCG GCA AGT TCG GTT GCC GAG CTT GGT CTT GGT CTG	811	66
fusA	ATC GGC ATC ATG GCY CAC ATY GAT CCA GCA TCG GCT GMA CRC CCT T	784	66
Iles	ATC CCG CGY TAC CAG ACS ATG CGG TGT CGA CGT AGT CGG CG	789	66
rplB	GGA CAA GGA CGG CRT SCC SGC CAA ACG ACC RCC GTG CGG GTG RTC GAC	498	67
rpoB	GGC GAG CTG ATC CAG AAC CA GCA TCC TCG TAG TTG TAS CC	1057	62

*= Upper sequence, forward primer; Lower sequence, reverse primer.

[a] In the primer sequence R indicates (A/G), S (C/G), Y (C/T).

TABLE S3: Descriptive evolutionary analysis of MLST data.

Gene	Fragment analyzed(bp)*	G+C (mol%)	polymorphic sites	Alle frequencies	π	k
clpC	678 (0,25)	61,97	14	8	0,00404	2,739
purF	855 (0,56)	62,33	65	11	0,01308	11,18116
gyrB	688 (0,33)	60,17	14	12	0,00335	2,30435
fusA	753(0,35)	60,35	14	8	0,00319	2,4058
Iles	743(0,22)	61,51	41	12	0,0148	10,78986
rplB	428 (0,51)	64,75	8	5	0,00273	1,16667
rpoB	965 (0,27)	62,89	16	11	0,00353	3,4058

*Percentage of the gene is given in parenthesis.

π = mean pairwise nucleotide difference per site.

k = mean pairwise nucleotide difference per sequence.

TABLE S4: Allelic profiles of 24 *B. breve* strains analyzed by MLST.

Strains	ST[a]	Allele						
		clpC	*purF*	*gyrB*	*fusA*	*Iles*	*rplB*	*rpoB*
UCC2003	1	1	1	1	1	1	1	1
ATCC15700	2	1	2	2	1	2	2	2
ATCC15698	3	1	3	3	1	3	3	3
ATCC15701	4	2	4	4	1	4	3	4
JCM7017	4	2	4	4	1	4	3	4
JCM7019	5	3	5	5	7	5	3	5
JCM7020	6	4	6	5	8	6	3	6
S-17c	7	1	7	6	1	4	3	2
S-46	8	2	4	4	1	4	3	7
SC81	9	5	8	7	2	7	3	8
SC95	10	6	9	8	1	3	3	3
SC139	11	7	3	8	1	8	3	4
SC154	12	2	1	8	1	3	3	1
SC500	13	1	10	3	2	3	4	9
SC506	14	6	9	8	3	3	1	3
SC508	15	1	1	9	1	9	5	2
SC522	9	5	8	7	2	7	3	8
SC559	16	1	1	10	1	10	3	10
SC567	17	1	11	8	6	11	3	1
SC568	10	6	9	8	1	3	3	3
SC573	18	2	4	11	1	3	3	3
SC580	9	5	8	7	2	7	3	8
SC670	19	1	1	12	5	11	3	11
KA179	20	8	3	8	4	12	3	9

[a]ST Indicates specific sequence type.

Table S5: Genebank accession numbers for glycosyl hydrolase.

Glycosyl hydrolase name	Protein sequences accession numbers
Blon_2335	YP_002323771.1; ZP_06596922.1; ZP_03742645.1; ZP_03167824.1; NP_241708.1;ZP_03474775.1; YP_003010680.1; ZP_04552485.1; ZP_07812017.1
Blon_2336	YP_002323772.1; WP_003795385.1; WP_007588699.1; YP_001297867.1; WP_006775425.1; YP_003822597.1; WP_008706707.1; WP_009776262.1
Blon_0248/0426	YP_002321754.1; ZP_08285605.1; ZP_08026776.1; ZP_06607921.1; ZP_06184004.1; YP_002533924.1; ZP_05989289.1; ZP_02477566.1; YP_001851141.1; ZP_03212758.1; ZP_05280631.1
Blon_0346	YP_002321848.1; ZP_02040503.1; ZP_02079496.1; ZP_08131039.1; ZP_05718978.1; YP_004456548.1; P_003242853.1;YP_002547035.1
Blon_2348	YP_002323784.1; WP_003818390.1; ACH92844.1; WP_003796112.1; ACH92824.1; BAD66680.2
Blon_0646	YP_002322131.1; YP_007554019.1
Blon_0459	YP_002321953.1; YP_007555353.1

TABLE S6: Glycosyl hydrolase gene and qPCR primers.

Primer name	Primer sequence (5'-3')[a]	Expected amplicon size (bp)	Anneling Temp. (ºC)
Blon_2335F	GARATGAAYTAYTGGATG	960	56 ºC
Blon_2335R	TTNCCRTCDATYTGRAANGGNGG		
Blon_2336F	AARCAYCAYGAYGGNTTYTG	600	55 ºC
Blon_2336R	ACYTCNGCNGGRTACCA		
Blon_0248/0426F	TAYGCNGARTGGTAY	210	45 ºC
Blon_0248/0426R	TCRTGRTGYTTNGTNGT		
Blon_0346F	YTNGAYTTYCAYACNWS	740	48 ºC
Blon_0346R	TCRTGRTGYTTNGTNGT		
Blon_2348F	ATHACNGCNGAYATHAC	250	45 ºC
Blon_2348R	TCNACNACYTTRTTYTCRTC		
Blon_0646F	CCACCAGACATGGAACAGTG	220	60 ºC
Blon_0646R	AAATCGCCGAAGGTGATATG		
Blon_0459F	CCCCACCCTCGACTGGCTCA	510	62 ºC
Blon_459R	CTTCGAGGTGGCACAGG		
0248WF	ACCAACAACCAGCAACCAAT	135	56 ºC
0248WR	ATCGAATACGGCACCTTCAG		
0426WF	ACCAACAACCAGCAACCAAT	135	56ºC
0426WR	GACCGCCTTCATGGATAAGA		
RNP-F	AACCTGATGATCGGACGACG	182	60ºC
RNP-R	GGCAAACTGCTCATCCAACG		60ºC
GH29-F	GGACTGAAGTTCGGCGTGTA	160	60ºC
GH29-R	TCGTTGTCCTCCTCCGAGAT		60ºC
GH95-F	CGCGGACTACCGCAGATATT	163	60ºC
GH95-R	ATCGAACATTGCCTCTGCCA		60ºC

[a] In the primer sequence R indicates (A/G), W (A/T), S (C/G), Y (C/T), H (A/C/T), D (A/G/T), N (A/C/G/T).

TABLE S7: Kinetic analysis of bacterial growth in 2% HMO.

	Kinetic parameters in 2% HMO							
Strain	Growth rate (1/h)	SD	Lag time (h)	SD	Generation time (h)	SD	Max. OD (600nm)	SD
UCC2003	6.70E-02	± 7.55E-03	3.070	± 0.132	4.531	± 0.543	0.524	± 0.055
ATCC15700	6.16E-02	± 1.12E-02	5.420	± 0.042	5.007	± 1.001	0.538	± 0.025
ATCC15698	7.98E-02	± 2.14E-03	4.260	± 0.118	3.772	± 0.102	0.656	± 0.065
ATCC15701	5.77E-02	± 2.22E-03	2.445	± 0.881	5.225	± 0.206	0.779	± 0.040
JCM7017	7.19E-02	± 4.50E-03	5.549	± 0.096	4.199	± 0.257	0.656	± 0.021
JCM7019	9.94E-02	± 4.55E-03	7.653	± 0.310	3.033	± 0.135	0.655	± 0.014
JCM7020	8.51E-02	± 2.08E-03	4.016	± 0.083	3.538	± 0.087	0.661	± 0.015
S-17c	7.26E-02	± 7.21E-03	6.161	± 0.237	4.170	± 0.398	0.540	± 0.013
S-46	8.36E-02	± 9.27E-03	4.413	± 0.073	3.627	± 0.378	0.71	± 0.033
SC81	1.07E-01	± 4.81E-03	6.331	± 0.108	2.825	± 0.129	0.715	± 0.033
SC95	1.20E-01	± 6.43E-03	4.655	± 0.047	2.523	± 0.131	0.859	± 0.029
SC139	9.41E-02	± 9.16E-03	5.390	± 0.204	3.219	± 0.297	0.667	± 0.015
SC154	7.54E-02	± 5.22E-03	6.295	± 0.166	4.007	± 0.281	0.768	± 0.031
SC500	5.24E-02	± 3.65E-03	13.512	± 0.362	5.759	± 0.404	0.558	± 0.026
SC506	5.92E-02	± 2.66E-03	3.806	± 0.050	5.088	± 0.222	0.731	± 0.0007
SC508	4.26E-02	± 2.37E-03	5.157	± 0.070	7.086	± 0.390	0.277	± 0.054
SC522	4.88E-02	± 1.14E-02	1.050	± 0.223	6.439	± 1.715	0.698	± 0.047
SC559	6.26E-02	± 1.27E-03	6.311	± 0.137	4.807	± 0.098	0.612	± 0.0015
SC567	5.76E-02	± 5.49E-03	9.953	± 0.765	5.256	± 0.529	0.567	± 0.042
SC568	6.26E-02	± 2.91E-03	6.216	± 0.524	4.815	± 0.220	0.680	± 0.034
SC573	3.31E-02	± 3.45E-03	3.419	± 0.123	9.168	± 0.933	0.306	± 0.014
SC580	6.34E-02	± 3.82E-03	2.045	± 0.204	4.762	± 0.284	0.727	± 0.028
SC670	3.38E-02	± 5.98E-03	9.886	± 0.234	9.083	± 1.505	0.332	± 0.054
KA179	1.13E-01	± 2.83E-03	6.990	± 1.144	2.673	± 0.066	0.606	± 0.038
ATCC15697	2.07E-01	± 3.29E-03	3.930	± 0.051	1.452	± 0.022	1.295	± 0.015
JCM10602	1.10E-02	± 1.45E-03	14.919	± 2.389	27.578	± 3.368	0.180	± 0.025

Supplemental references

1. O'Connell Motherway, M., A. Zomer, S. C. Leahy, J. Reunanen, F. Bottacini, M. J. Claesson, F. O'Brien, K. Flynn, P. G. Casey, J. A. Munoz, B. Kearney, A. M. Houston, C. O'Mahony, D. G. Higgins, F. Shanahan, A. Palva, W. M. de Vos, G. F. Fitzgerald, M. Ventura, P. W. O'Toole, and D. van Sinderen. 2011. Functional genome analysis of *Bifidobacterium breve* UCC2003 reveals type IVb tight adherence (Tad) pili as an essential and conserved host-colonization factor. Proc Natl Acad Sci U S A 108: 11217-22.
2. Roy, D., Ward, P., and Champagne, G. 1996. Differentiation of bifidobacteria by use of pulsed-field gel electrophoresis and polymerase chain reaction. Int J Food Microbiol 29:11-29.

FIGURE S1

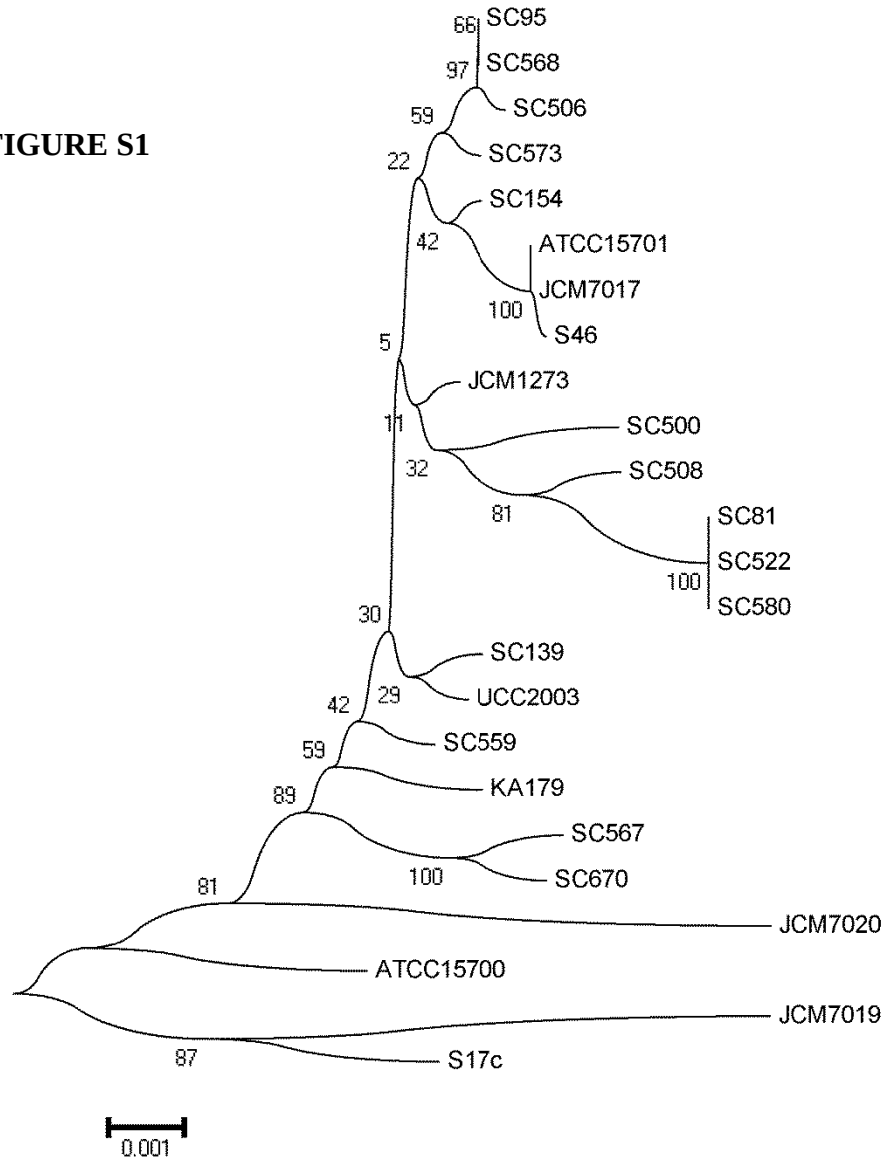

FIGURE S1. Evolutionary relationship of *B. breve* strains used in the study. The tree is drawn to scale, with branch lengths in the same units (number of base substitutions per site) as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary history was inferred using the Minimum Evolution method, followed by 1000 bootstrap replicates.

FIGURE S2

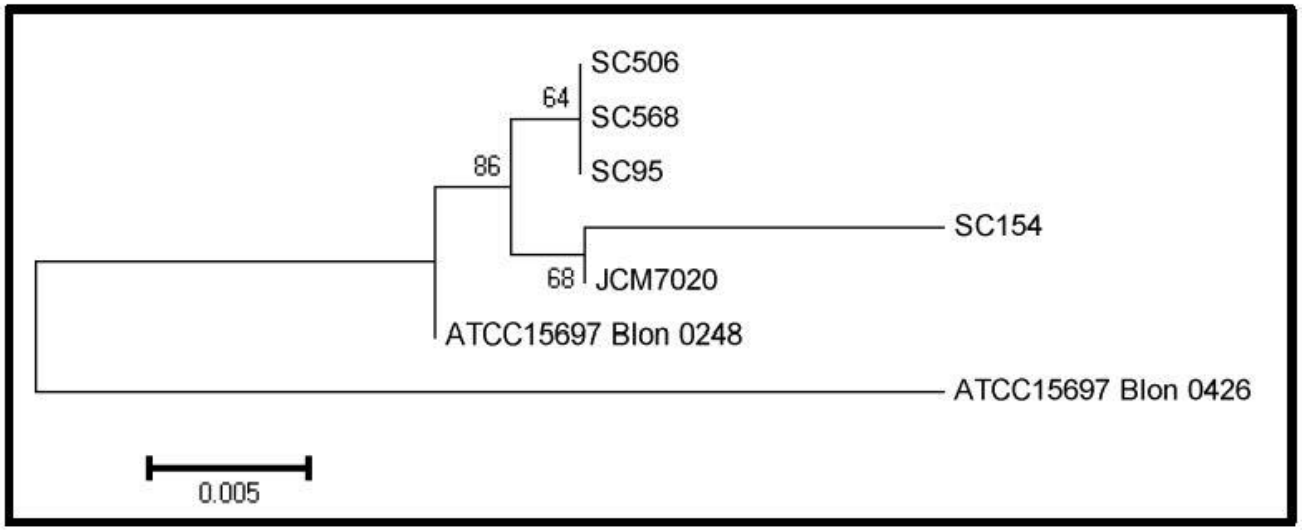

FIGURE S2. Phylogenetic relationship of homologous fucosidase Blon_0248 in *B. breve* strains. The tree is drawn to scale, with branch lengths in the same units (number of amino acid substitutions per site) as those of the evolutionary distances used to infer the phylogenetic tree The evolutionary history was inferred using the Minimum Evolution method, followed by 1000 bootstrap replicates.

FIGURE S3

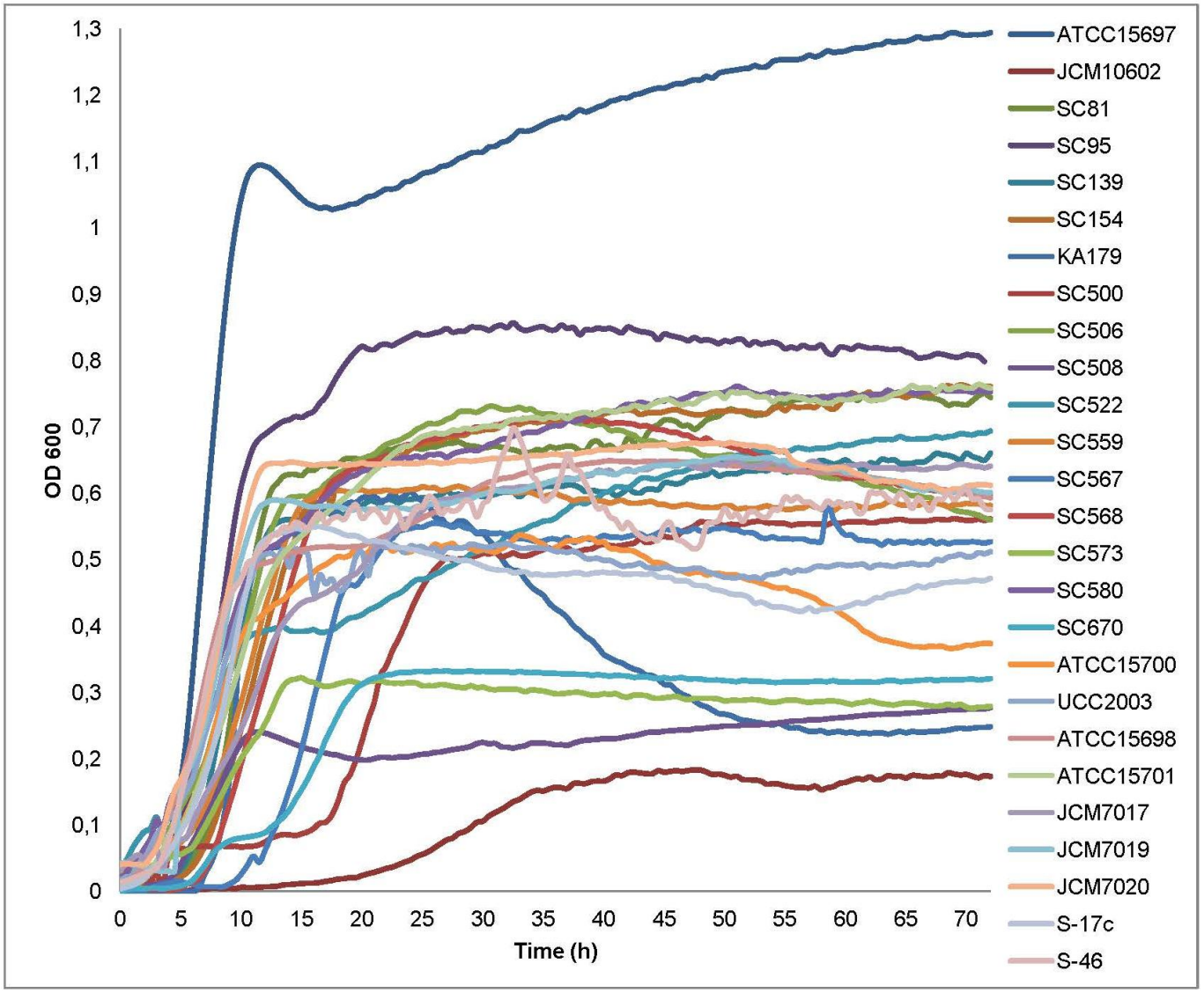

FIGURE S3. Growth of *B. breve* isolates on mMRS medium supplemented with 2% (w/v) HMO. *B. infantis* ATCC 15697 and *B. breve* ATCC 15700 were included as a high and low growth respectively. Growth was measured as OD of the media at 600 nm. Fermentations were carried out in triplicate; controls consisted of inoculated medium lacking of substrates and un-inoculated medium containing substrates which was also used as blank for OD measurements.

FIGURE S4

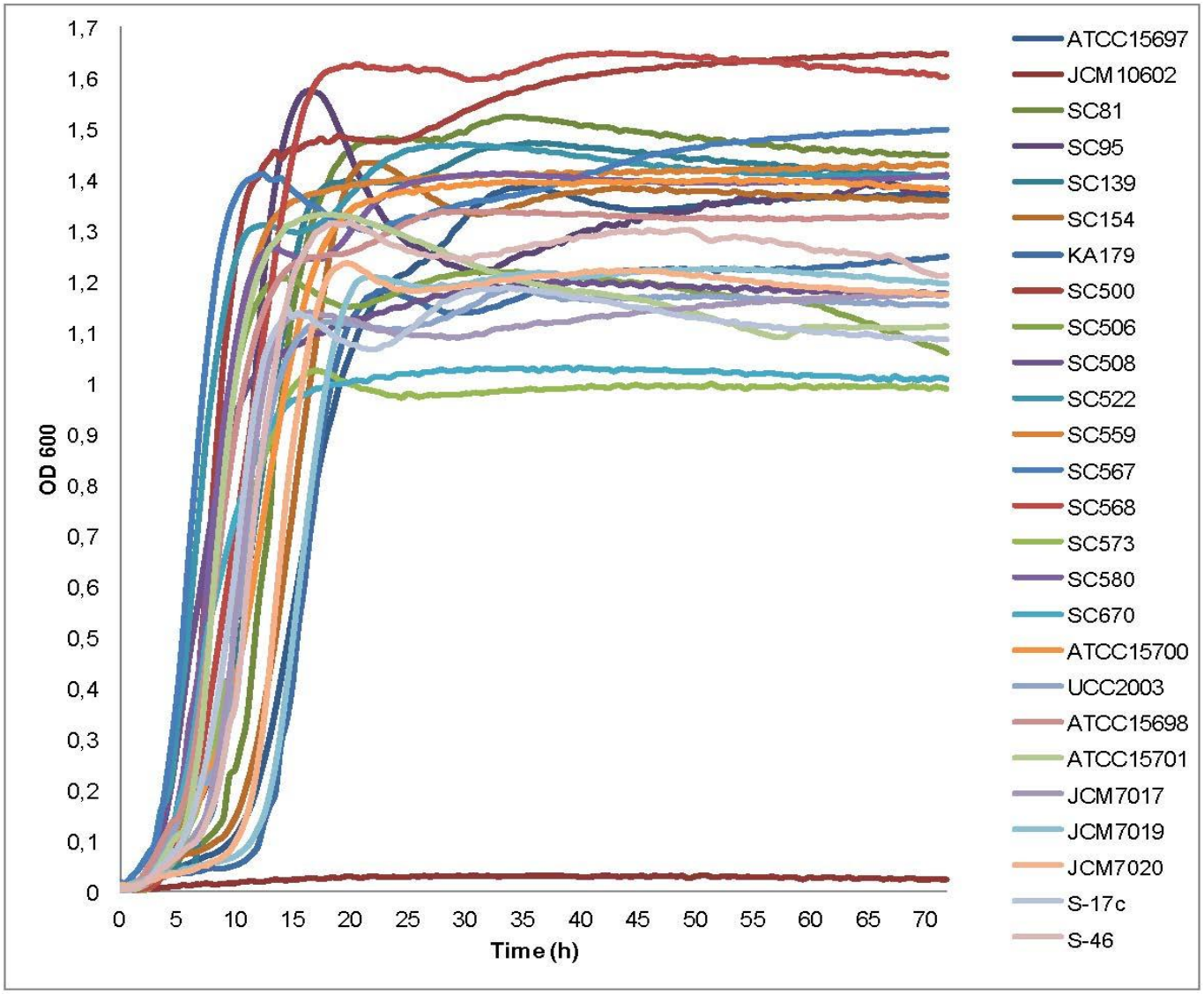

FIGURE S4. Growth of *B. breve* isolates on mMRS medium supplemented with 2% (w/v) LNT. *B. infantis* ATCC 15697 and *B. breve* ATCC 15700 were included as a high and low growth respectively. Growth was measured as OD of the media at 600 nm. Fermentations were carried out in triplicate; controls consisted of inoculated medium lacking of substrates and un-inoculated medium containing substrates which was also used as blank for OD measurements.

.

FIGURE S5

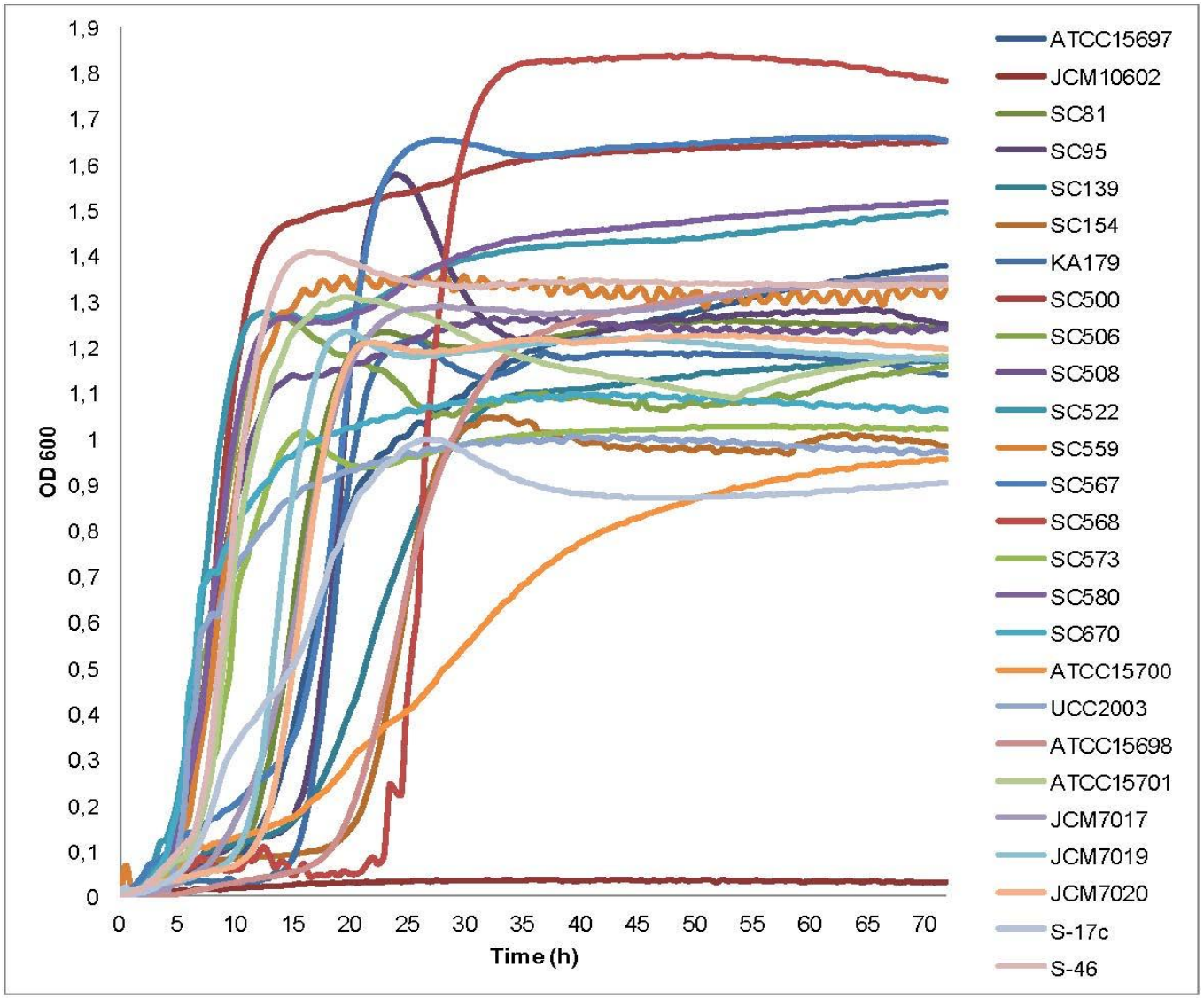

FIGURE S5. Growth of *B. breve* isolates on mMRS medium supplemented with 2% (w/v) LNnT. *B. infantis* ATCC 15697 and *B. breve* ATCC 15700 were included as a high and low growth respectively. Growth was measured as OD of the media at 600 nm. Fermentations were carried out in triplicate; controls consisted of inoculated medium lacking of substrates and un-inoculated medium containing substrates which was also used as blank for OD measurements.

FIGURE S6

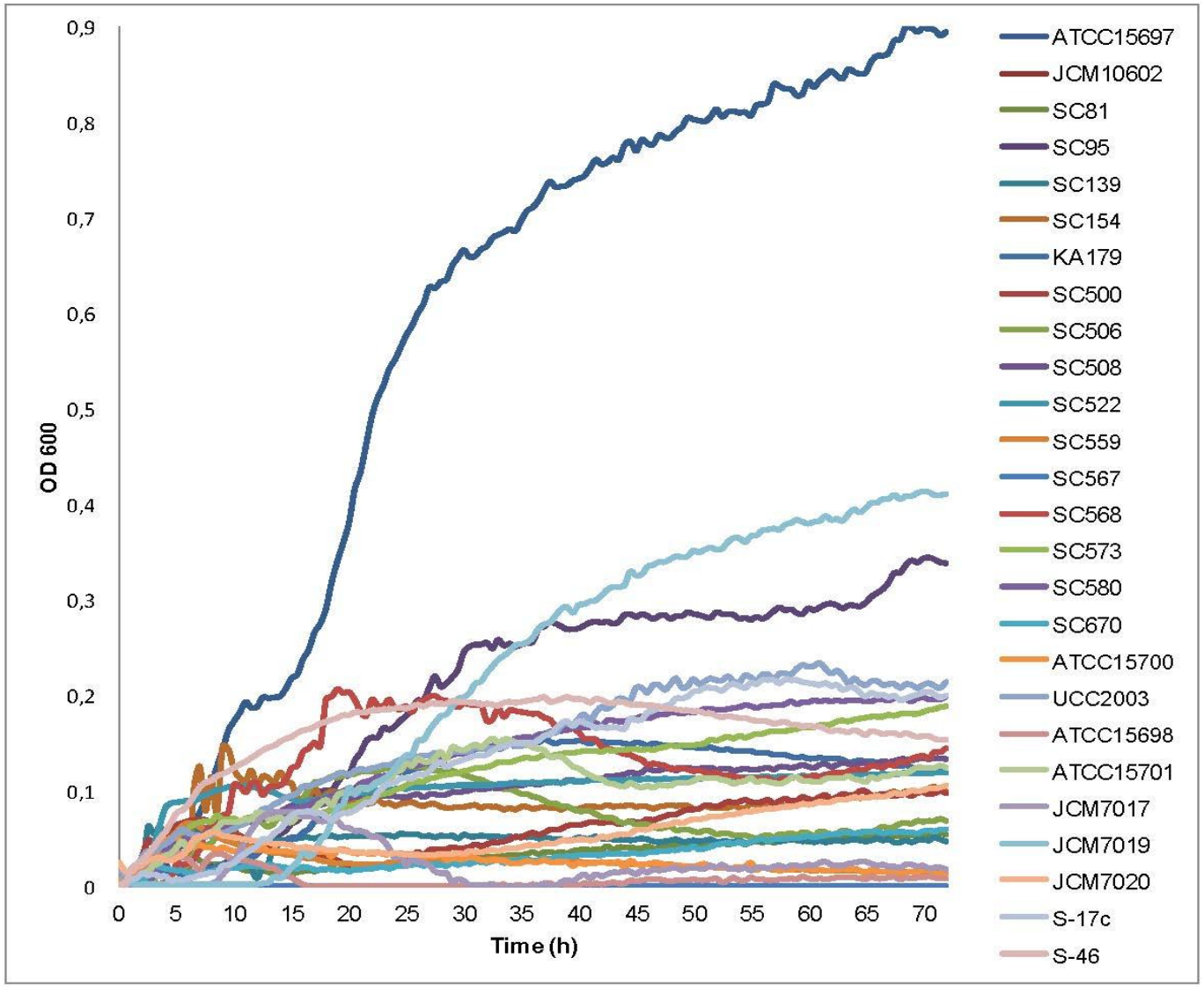

FIGURE S6. Growth of *B. breve* isolates on mMRS medium supplemented with 2% (w/v) 3FL. *B. infantis* ATCC 15697 and *B. breve* ATCC 15700 were included as a high and low growth respectively. Growth was measured as OD of the media at 600 nm. Fermentations were carried out in triplicate; controls consisted of inoculated medium lacking of substrates and un-inoculated medium containing substrates which was also used as blank for OD measurements.

.