

Analysis of the effect of probiotics on shaping human gut microbiota

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Probiotics

According to the currently adopted definition by FAO/WHO, **Probiotics** are “Live microorganisms which confer a health benefit on the host when administered in adequate amounts.”

Lactic acid bacteria (LAB) like *lactobacillus* and *bifidobacteria* are the most common types of microbes used as probiotics.

In 1989 Roy Fuller also suggested that *probiotics beneficially affect the host by improving its intestinal microbial balance*”.

<Cited from Wikipedia>



However, it is largely unknown how probiotics change or improve the microbial composition, the gene content, and the metabolic profile in human gut microbiota.



We have been studying the effect of probiotics on shaping human gut microbiota by using **16S and metagenomic** data, from which variations in the microbial composition and gene content affected by administration of probiotics can be comprehensively evaluated.

Sampling of gut microbiota with and without probiotics

*Healthy volunteers (24 adults) who take commercially available probiotics daily and provide fecal samples according to our experimental plan.

S0: samples without probiotics for 4 weeks before starting administration of probiotics.

S2: samples administrating probiotics twice a day for 4 weeks.

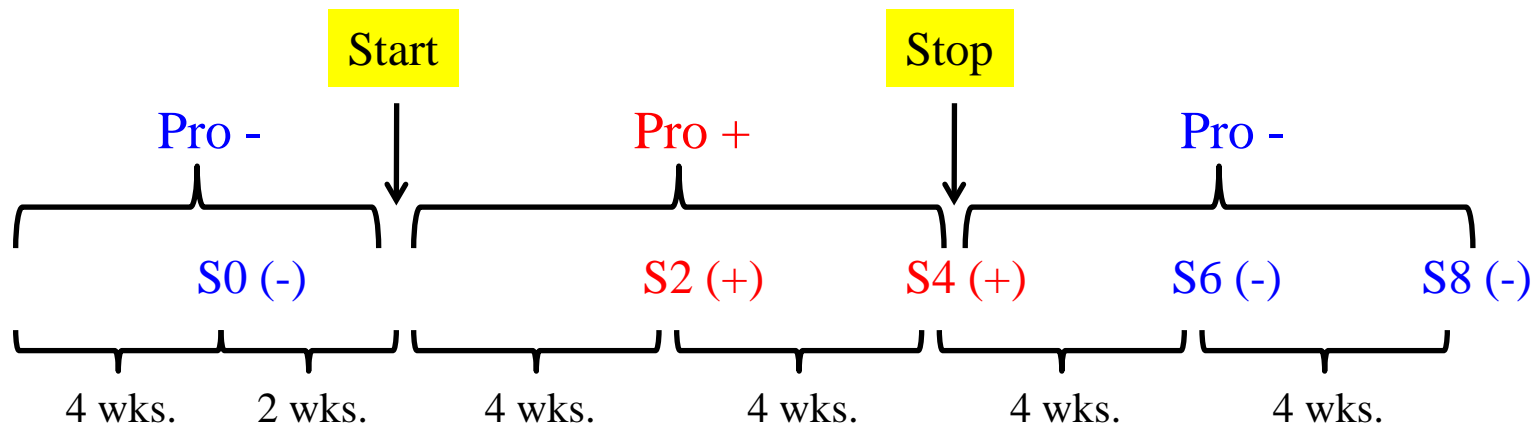
S4: samples administrating probiotics twice a day for 8 weeks.

S6: samples without probiotics for 4 weeks after stopping administration of probiotics.

S8: samples without probiotics for 8 weeks after stopping administration of probiotics.

*Collection of meta data of the individuals

*Sampling of gut microbiota from individuals with and without probiotics



Subjects and samples analyzed

Probiotics	Subject (APr)	Sex	Age	Blood type	BMI	S0	S2	S4	S6	S8
I	1	F	21	B	18.8					
	2	F	23	O	18.6					
	3	F	21	B	19.9					
	5	F	20	A	23.2					
II	9	F	20	B	18.5					
	10	F	21	A	18.0					
	11	F	23	A	19.1					
	12	M	25	O	19.5					
III	16	F	20	B	18					
	17	F	21	O	20.1					
	18	M	21	-	21.5					
	19	M	20	A	19.4					
IV	20	F	21	B	19.8					
	22	F	22	A	19.5					
	23	F	21	B	20.5					
	26	M	21	A	22.2					
V	29	F	23	O	20.9					
	30	F	19	A	17.3					
	31	M	33	AB	28					
	32	M	19	AB	21.8					
VI	35	F	-	-	-					
	37	F	21	A	21.2					
	39	M	23	O	20					
	40	M	19	B	20.1					
Total	9					24	9	9	9	9

Analysis of S0 samples of these 15 subjects was completed.

Analysis of these samples is in progress.

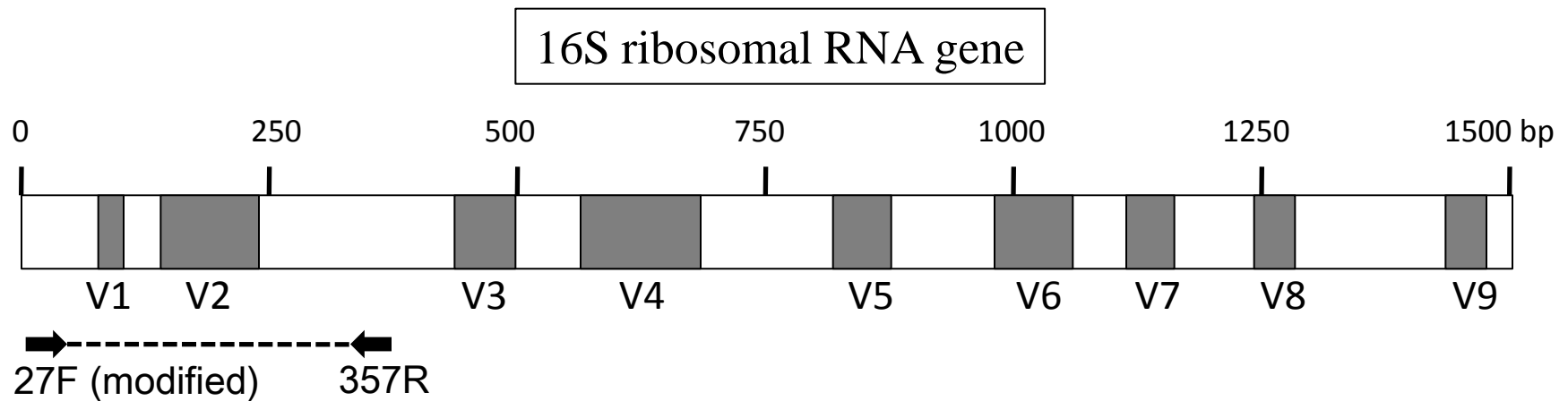
Analysis of all samples (S0 to S8) of these 9 subjects was completed.

Probiotics used in this study

Subject (Apr)	Group	Probiotic strains
1	I	<i>Lactobacillus casei</i>
2		
3		
9	II	<i>Lactobacillus brevis</i>
12		
16	III	<i>Bifidobacterium longum</i> <i>Lactococcus lactis</i> <i>Streptococcus thermophilus</i>
17		
39		
40		

Sequencing of 16S V1-V2 regions by 454 GS FLX and OTU analysis

OTU: Operational Taxonomic Unit



➡ PCR amplified ➡ Sequencing (barcode) by the Roche 454 GS FLX Ti

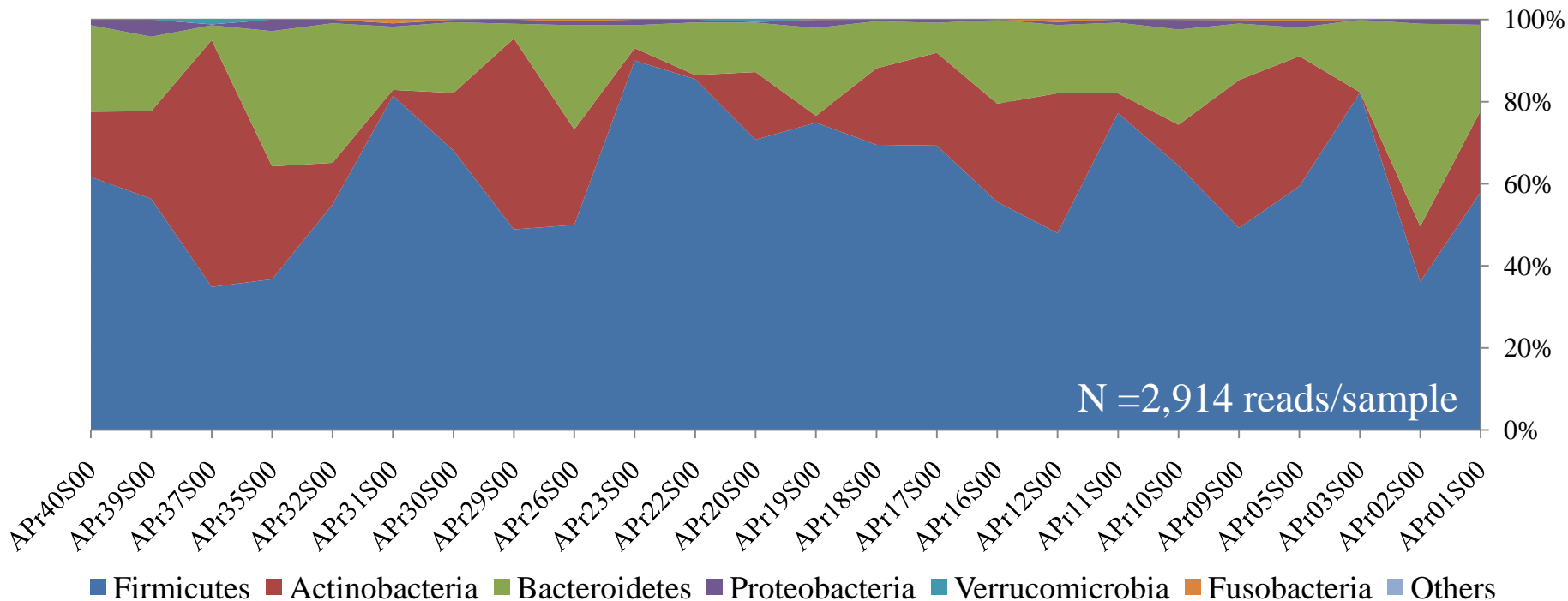
➡ Total 13,1130 reads for 45 sample (S0 to S8 of 9 subjects).
Total 69,936 reads for S0 samples of 24 subjects

2,914 reads/sample

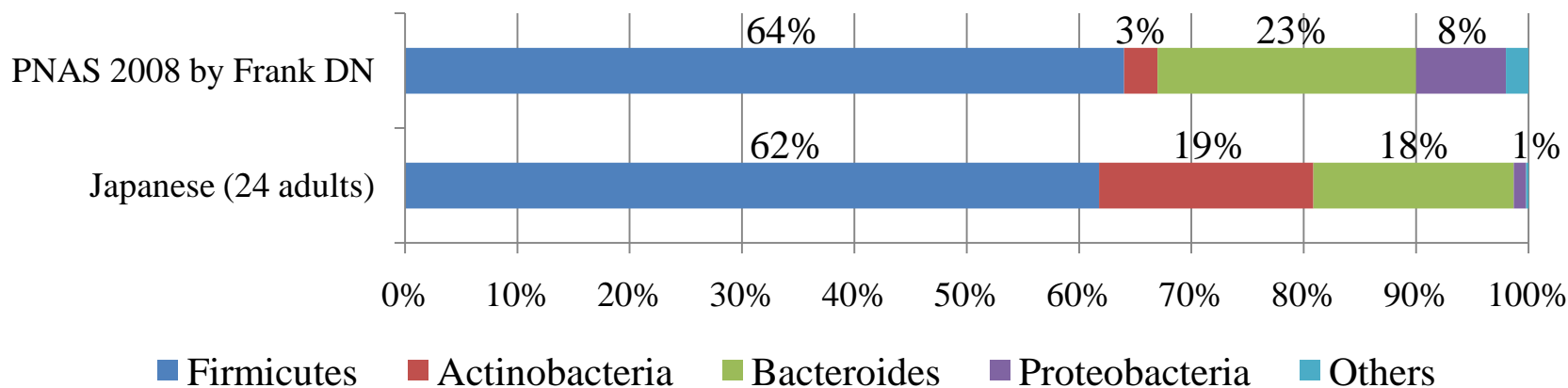
➡ OTU analysis for S0 samples of 24 subjects without probiotics

➡ OTU analysis for 45 samples of 9 individuals with and without probiotics

Microbial composition of 24 Japanese gut microbiota (16S V1-V2 analysis)



Comparison between microbial composition in gut microbiota of Japanese and non-Japanese



OTU analysis of 45 samples of 9 individuals with and without probiotics

Total 13,1130 reads for 45 samples (9 subjects; 2,914 reads/sample).

OTUs obtained by clustering with 97% id.

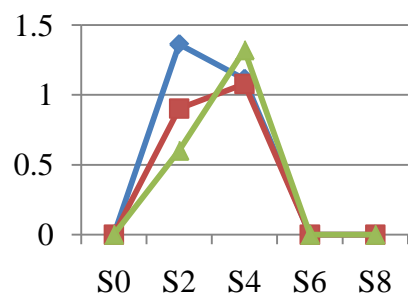
Probiotics	Individual	S0	S2	S4	S6	S8	Total OTUs	OTUs(≥ 10 reads) in either of SNs
<i>L. casei</i>	APr01	125	164	144	137	124	412	75
<i>L. casei</i>	APr02	137	148	236	122	146	439	95
<i>L. casei</i>	APr03	147	169	273	146	297	653	88
<i>L. brevis</i>	APr09	242	159	211	131	117	541	89
<i>L. brevis</i>	APr12	146	171	151	126	136	385	65
<i>B. longum</i>	APr16	137	154	108	230	149	508	100
<i>B. longum</i>	APr17	173	193	206	233	197	544	95
<i>B. animals</i>	APr39	101	192	155	184	148	533	115
<i>B. animals</i>	APr40	329	211	407	198	161	816	99

16S V1-V2 region, N = 2,914 reads/sample, Clustering with 97% id.

Detection of probiotic strains in gut microbiota

OTU id	S0	S2	S4	S6	S8	Probiotic Strains
APr01_OTU00181	0	23	13	0	0	<i>L. casei</i>
APr02_OTU00172	0	8	12	0	0	<i>L. casei</i>
APr03_OTU00230	0	4	21	0	0	<i>L. casei</i>
APr09_OTU00311	0	1	1	0	0	<i>L. brevis</i>
APr12_OTU00254	0	1	1	0	0	<i>L. brevis</i>
APr16_OTU00006	633	135	153	61	202	<i>B. longum</i>
APr16_OTU00300	0	0	10	0	0	<i>Lactococcus lastis</i>
APr17_OTU00014	166	174	126	69	133	<i>B. longum</i>
APr17_OTU00214	0	15	7	0	0	<i>Lactococcus lastis</i>
APr39_OTU00128	0	90	60	0	0	<i>B. animalis</i>
APr39_OTU00156	0	3	16	0	0	<i>Lactobacillus delbrueckii</i>
APr39_OTU00098	0	10	5	0	0	<i>Lactococcus lastis</i>
APr40_OTU00454	0	3	0	0	0	<i>B. animalis</i>

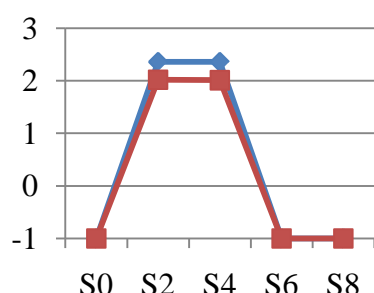
log *Lactobacillus casei*



APr01 (blue diamond)
APr02 (red square)
APr03 (green triangle)

By 16S

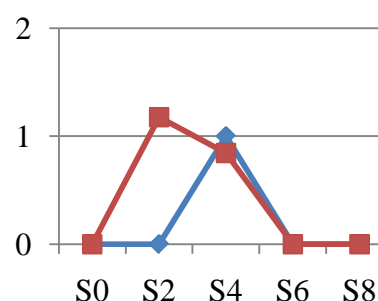
Lactobacillus brevis



APr09 (blue diamond)
APr12 (red square)

By mapping

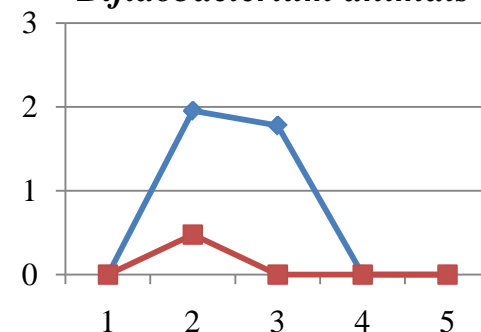
Lactococcus lactis



APr16 (blue diamond)
APr17 (red square)

By 16S

Bifidobacterium animalis

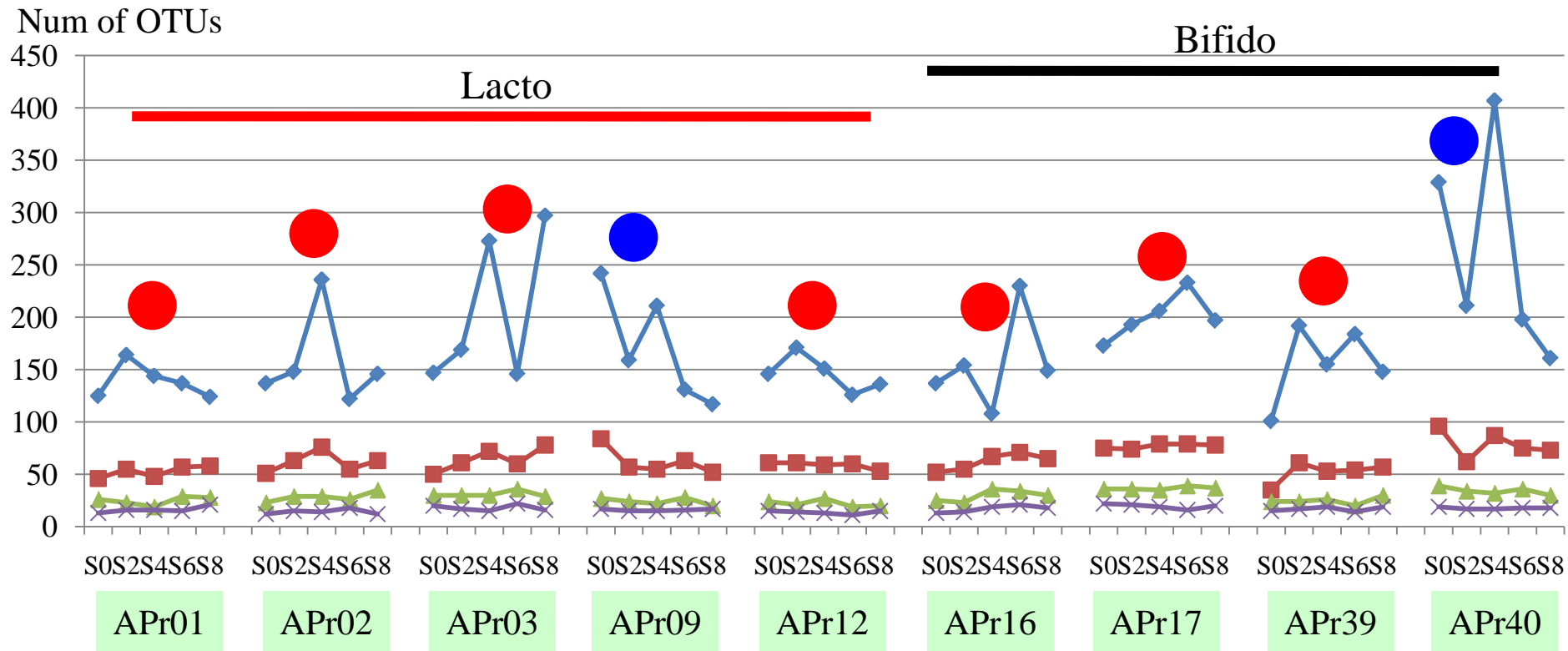


APr39 (blue diamond)
APr40 (red square)

By 16S

Change of OTUs in samples with and without probiotics

◆ Total OTU ■ OTU(≥ 5) ▲ OTU(≥ 20) ✕ OTU(≥ 40)



● Subjects in which OTUs were increased by administrating probiotics (S0→S2)

● Subjects in which OTUs were decreased by administrating probiotics (S0→S2)

The number of OTUs in S0 of ● is more than that in S0 of ●.

Minor species more largely contribute to change of OTUs than major species.

Shared OTUs between samples in an individual

Shared OTUs (%) between SNs

≥97% id

Probiotics	Individual	S0_S2	S2_S4	S4_S6	S6_S8	Ave.
<i>L. casei</i>	APr01	28.0	35.0	39.3	49.1	37.9
<i>L. casei</i>	APr02	28.2	37.1	37.2	45.2	36.9
<i>L. casei</i>	APr03	29.1	31.0	27.8	29.1	29.3
<i>L. brevis</i>	APr09	25.1	33.4	29.3	46.7	33.6
<i>L. brevis</i>	APr12	40.3	42.4	47.0	45.0	43.7
<i>B. longum</i>	APr16	14.0	18.8	33.2	33.8	25.0
<i>B. longum</i>	APr17	43.3	44.0	39.4	41.7	42.1
<i>B. animals</i>	APr39	25.0	31.5	13.8	16.8	21.8
<i>B. animals</i>	APr40	28.4	32.3	32.3	48.7	35.4
	Ave.	29.0	33.9	33.3	39.6	34.0

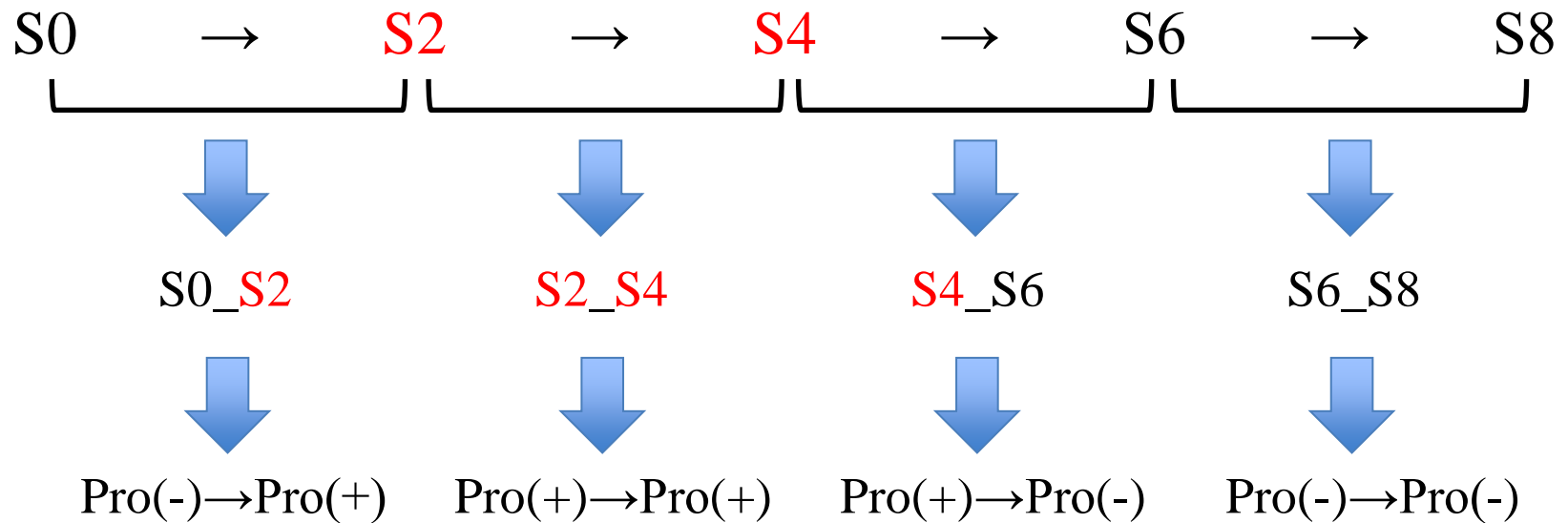
Shared OTUs between S0 and S0 samples in 9 individuals : 15.8% on the average.

*Shared OTUs between samples in the same individual are more than those between unrelated samples (34% vs. 15.8%).

*Shared OTUs between Pro(-) and Pro(+) is smallest (highest variation). ■

*Shared OTUs between Pro(-) and Pro(-), or Pro(+) and Pro(+) is largest (lowest variation). ■

Unifrac distance between samples in an individual

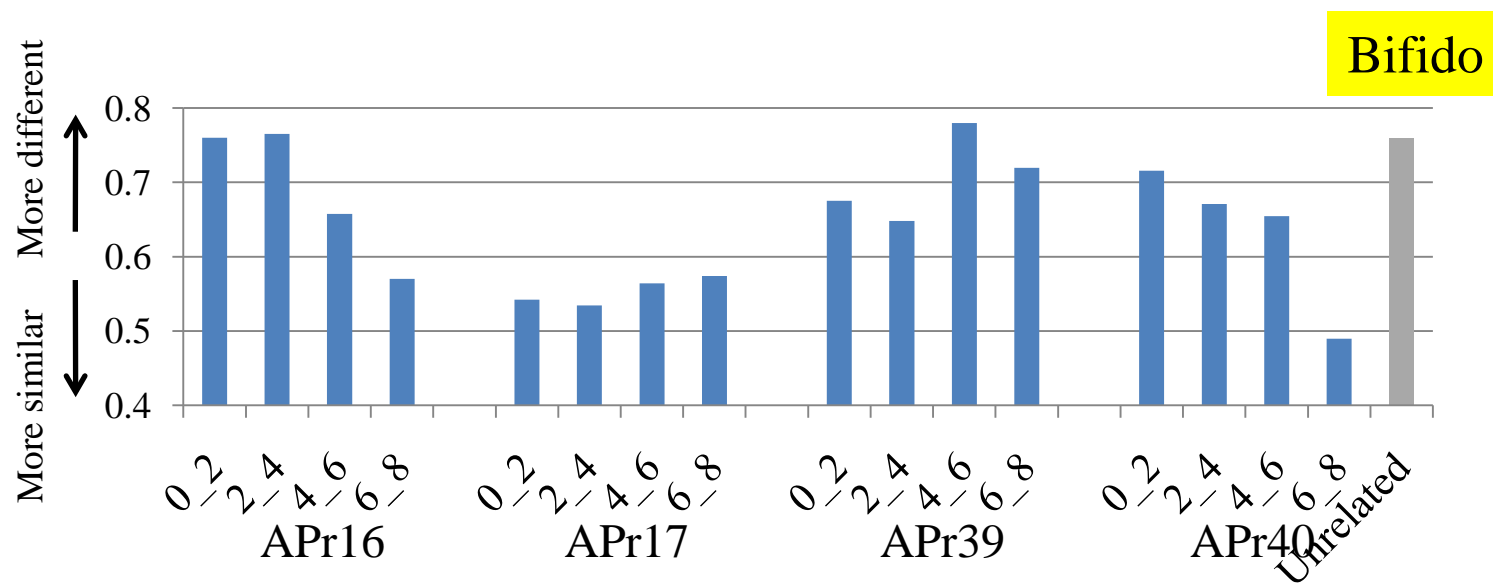
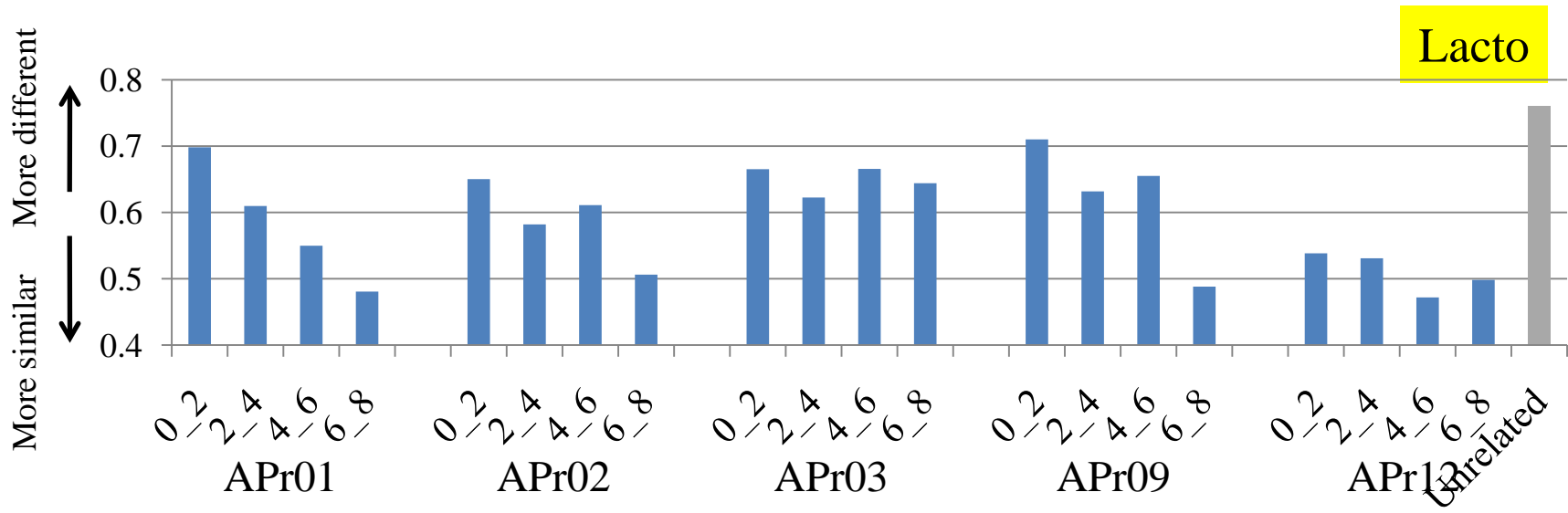


Unifrac distance of

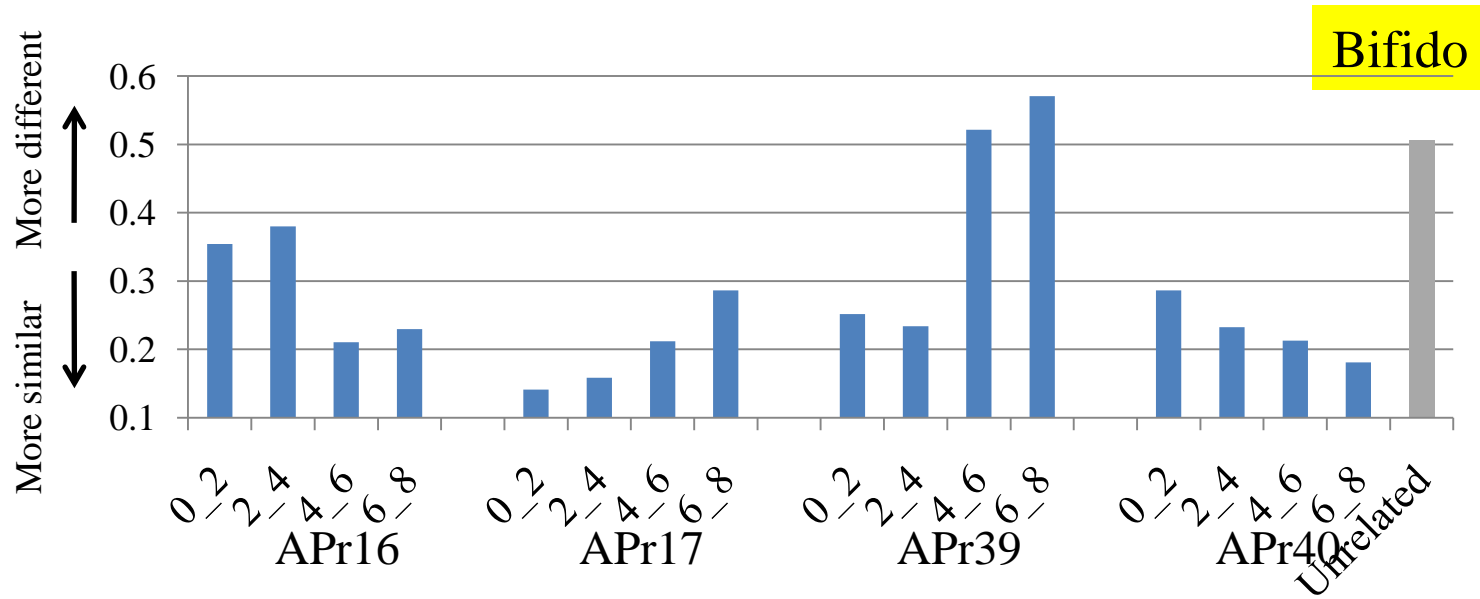
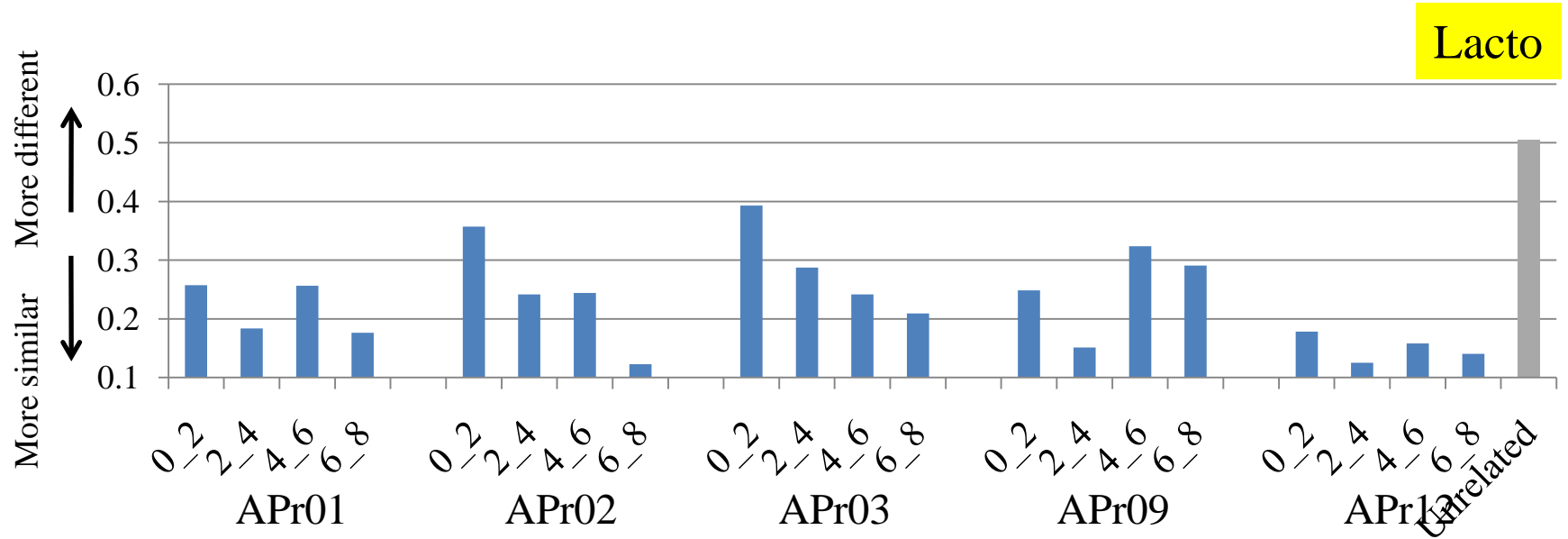
Unweighted: Relative difference in **community membership** between samples

Weighted: Relative difference in **community structure** between samples

Unifrac distance between samples (Unweighted)



Unifrac distance between samples (Weighted)



Relative unifracs distance between samples in an individual

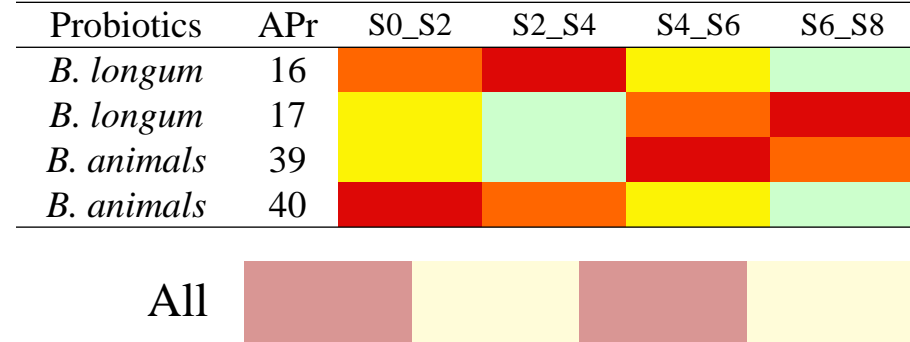
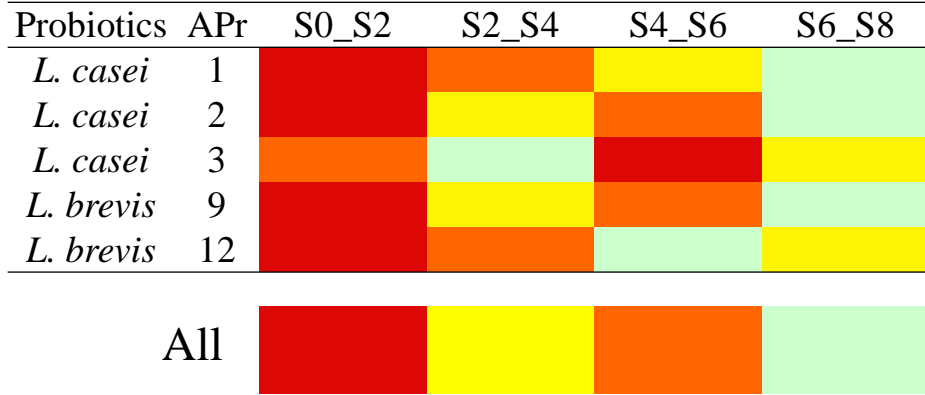
More different ← → More similar



Unweighted

Lactobacillus

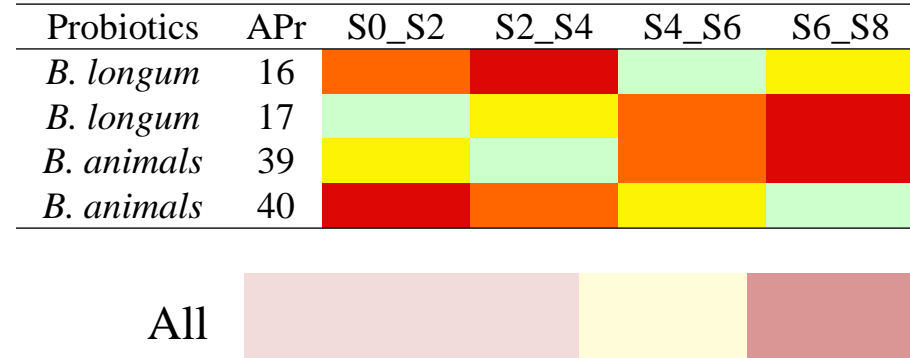
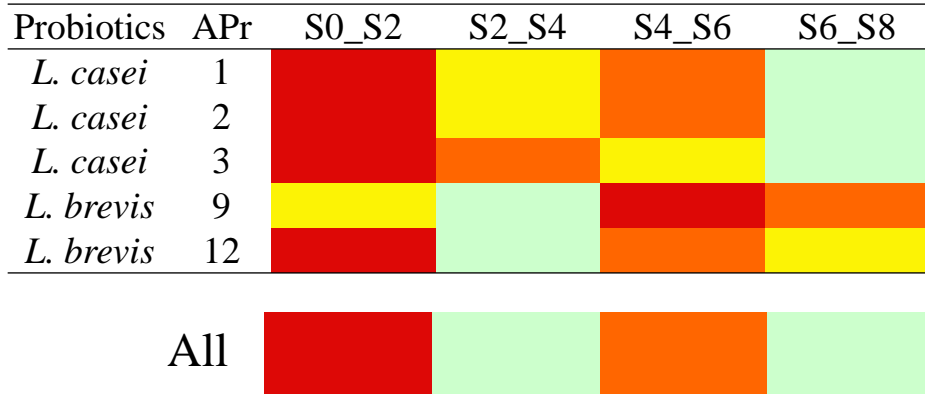
Bifidobacterium



Weighted

Lactobacillus

Bifidobacterium



Summary of analysis by unifrac distance and shared OTUs

Lactobacillus-probiotics

Shared OTUs : S0-S2 < S4-S6 < S2-S4 < S6-S8

Unifrac distance (Unweighted) : S0-S2 > S4-S6 > S2-S4 > S6-S8

Unifrac distance (Weighted) : S0-S2 > S4-S6 > S2-S4 \cong S6-S8

Unifrac distance (Unweighted) > Unifrac distance (Weighted)



- * Difference in gut microbiota between Pro(+) and Pro(-) is larger than that between Pro(-) and Pro(-), and Pro(+) and Pro(+).
- * The degree of variations in gut microbiota: member > structure



Lactobacillus-probiotics have some effect on shaping gut microbiota.

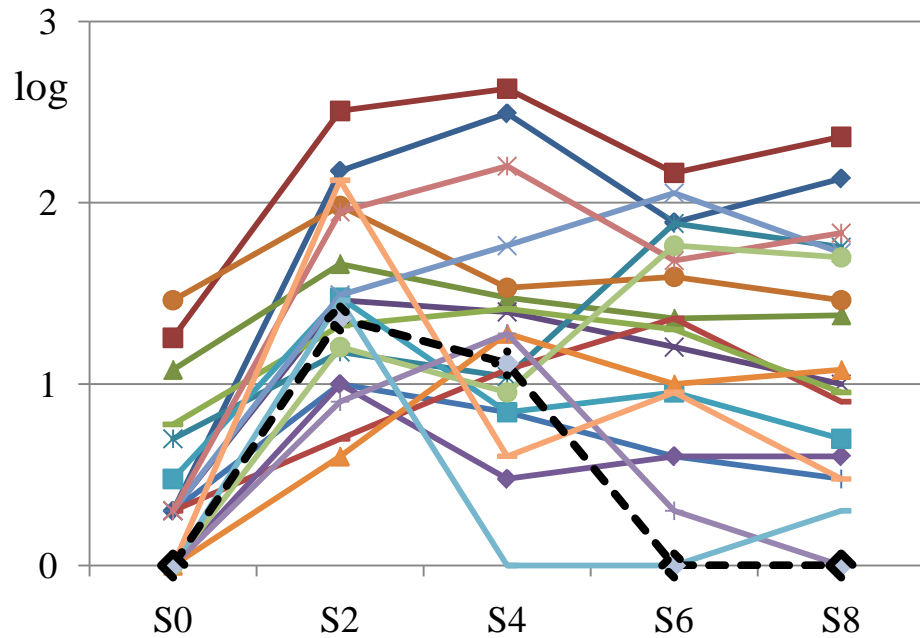
Bifidobacterium-probiotics

- * No particular difference has been found between samples with and without probiotics.



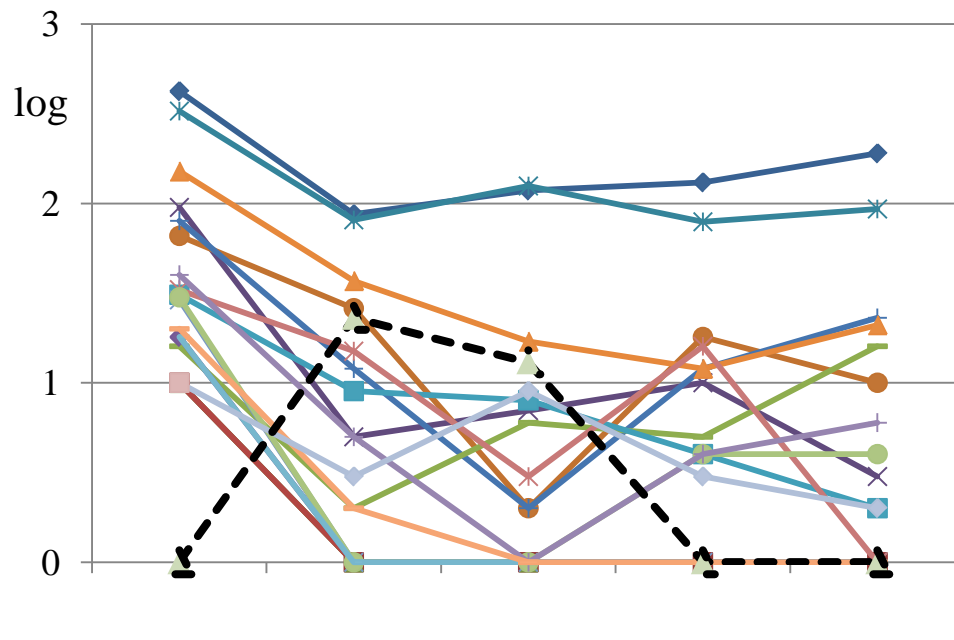
Bifidobacterium-probiotics have less effect on shaping gut microbiota than *Lactobacillus*-probiotics .

Detection of OTUs that increase and decrease by administering probiotics



- APr01_OTU00010
- APr01_OTU00026
- APr01_OTU00038
- APr01_OTU00048
- APr01_OTU00051
- APr01_OTU00053
- APr01_OTU00065
- APr01_OTU00068
- APr01_OTU00082
- APr01_OTU00083
- APr01_OTU00114
- APr01_OTU00120
- APr01_OTU00125
- APr01_OTU00132
- APr01_OTU00165
- APr01_OTU00168
- APr01_OTU00172
- APr01_OTU00177
- APr01_OTU00181

OTUs that increased ≥ 3 -fold in **S2** or **S4** than in S0 in APr01



- xAPr01_OTU00001
- xAPr01_OTU00005
- xAPr01_OTU00007
- xAPr01_OTU00008
- xAPr01_OTU00009
- xAPr01_OTU00012
- xAPr01_OTU00014
- xAPr01_OTU00015
- xAPr01_OTU00016
- xAPr01_OTU00017
- xAPr01_OTU00021
- xAPr01_OTU00022
- xAPr01_OTU00023
- xAPr01_OTU00024
- xAPr01_OTU00034
- xAPr01_OTU00045
- xAPr01_OTU00050
- xAPr01_OTU00070
- xAPr01_OTU00096
- APr01_OTU00181

OTUs that decreased ≥ 3 -fold in **S2** or **S4** than in S0 in APr01

Dashed line is an OTU assigned to *L. case*

OTUs having an increase of ≥ 3 - and ≥ 10 -fold by administrating probiotics
 OTUs having a decrease of ≥ 3 - and ≥ 10 -fold by administrating probiotics

Probiotics	Individual	All OTUs	OUTs having ≥ 10 reads in either of SNs	S2/S0 or S4/S0			S2/S0 or S4/S0		
				Total	Up (≥ 3)	Down (≤ 0.3)	Total	Up (≥ 10)	Down (≤ 0.1)
<i>L. casei</i>	APr01	412	75	39	20	19	25	14	11
<i>L. casei</i>	APr02	439	95	76	47	29	60	41	19
<i>L. casei</i>	APr03	653	88	57	32	25	37	25	12
<i>L. brevis</i>	APr09	541	89	48	16	32	32	6	26
<i>L. brevis</i>	APr12	385	65	27	14	13	10	6	4
<i>B. longum</i>	APr16	508	100	53	37	16	43	32	11
<i>B. longum</i>	APr17	544	95	29	19	10	9	7	2
<i>B. animals</i>	APr39	533	115	45	30	15	31	20	11
<i>B. animals</i>	APr40	816	99	42	17	25	26	3	23
	Total	4831	821	416	232	184	273	154	119
		100%					5.7%	3.2%	2.5%

*About 5.7% of all OTUs increased or decreased by administrating probiotics by ≥ 10 -fold .

Species assignment of OTUs having an increase and a decrease of ≥ 10 -fold by administration of probiotics

Probiotics	Increase & Decrease	Varied OTUs	<i>Firmicutes</i>	<i>Actinobacteria</i>	<i>Bacteroidetes</i>	<i>Proteobacteria</i>
<i>Lactobacillus</i>	Increase	92	79 (85.9)	10 (10.9)	2 (2.2)	1 (1.1)
	Decrease	72	46 (63.9)	6 (8.3)	19 (26.4)	1 (1.4)
			Up ↑	Up ↑	Down ↓	→
<i>Bifidobacterium</i>	Increase	62	46 (74.2)	8 (12.9)	7 (11.3)	1 (1.6)
	Decrease	47	34 (72.3)	3 (6.4)	9 (19.1)	1 (2.1)
			→	Up ↑	→	→
All	Increase	154	125 (81.2)	18 (11.7)	9 (5.8)	2 (1.3)
	Decrease	119	80 (67.2)	9 (7.6)	28 (23.5)	2 (1.7)
			Up ↑	Up ↑	Down ↓	→

Values in parentheses: %

**Lactobacillus*-probiotics increase *Firmicutes* and *Actinobacteria* moderately but decrease *Bacteroidetes* efficiently.

Summary and Collaborators

1. Gut microbiota of Japanese may differ in the microbial composition from that of non-Japanese. The *Actinobacteria* (Most are *Bifidobacterium*) is abundant in gut microbiota of Japanese.
2. Lactobacillus-probiotics had some effect on shaping of gut microbiota.
3. About 6% of all OTUs varied by administering probiotics by ≥ 10 -fold.
4. The *Bacteroidetes* was decreased efficiently by *Lactobacillus*-probiotics.



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