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Intra-Specific Composition and Succession of *Bifidobacterium longum* in Human Feces

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Running title: *Intra-specific Composition and Succession of Fecal B. longum*

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Summary

Intra-species analysis of pulsed field gel electrophoresis on human fecal *B. longum* isolates revealed that a majority of 12 Japanese subjects harbored strains of unique PFGE types or subtypes over a 68 week period, suggesting that “indigenous” *Bifidobacterium* strains remain stable for a considerable time in each individual intestinal microbiota.

Key words: Fecal microbiota – *Bifidobacterium longum* – indigenous strain – PFGE

1 Aspects of the species composition of intestinal bifidobacteria have been long
2 studied in terms of its relation to the location, age, gender, and diet of study
3 subjects [2, 3, 8, 9]. However, information about the composition of species or
4 strains per individual over a period of time is limited to the following
5 investigations. Through pulsed field gel electrophoresis (PFGE) analysis,
6 McCartney et al. [7] reported that the bifidobacterial populations of fecal samples
7 collected from two human subjects were stable at strain level throughout a
8 12-month period. Kimura et al. [4] conducted a similar study on 10 human
9 subjects over a 12-month period and also found that each subject harbored a
10 genotypically unique collection of bifidobacterial strains. The evidence suggests
11 that “indigenous” bifidobacterial strains colonize the intestinal microbiota of each
12 human individual. Here we have extended these observations and describe the
13 intra-specific comparison of *Bifidobacterium longum* strains that were isolated
14 from feces of 12 Japanese subjects over a 15 months period by PFGE in order
15 to confirm the succession of “indigenous” strains.

16 Twelve apparently healthy human subjects (subjects 1 to 12) whose age
17 ranged from 15 to 27 years at the commencement of fecal sampling maintained
18 their usual lifestyles and dietary intakes (including yogurt and other probiotic
19 products containing bifidobacterial cultures; collectively termed as “probiotics”
20 hereafter) throughout the study period, which continued for up to 68 weeks (ca.
21 15 months). Nineteen probiotics (7 yogurt and 12 other fermented milk products)
22 produced by 15 different companies and commercially available in the
23 Osaka-Kobe areas in Japan during the study period were also subjected to
24 strain isolation.

1 Fecal samples were collected at the 1st, 23rd, and 68th week from all subjects
2 except subjects 4 and 5 whose fecal samples were collected up to the 23rd week.
3 Briefly, Swab samples (ca. 0.2 to 0.4 g wet weight each) of fresh human feces
4 were each transferred to a tube containing 5 ml of sterile saline as diluent and
5 thoroughly emulsified aseptically with a vortex test-tube mixer. We made a
6 series of 10-fold dilutions (10^{-1} to 10^{-4}) of each emulsified sample with the same
7 diluent and, from each dilution, 0.1 ml was spread onto a *Bifidobacterium*
8 selective plate media, TOS agar (TOS; Yakult Pharmaceutical Inc. Co., Ltd,
9 Tokyo, Japan) with the aid of sterile T-shaped plastic rods (Nissui
10 Pharmaceutical Co., Ltd., Tokyo). Meanwhile, a loopful of probiotic samples
11 (ca. 0.1g wet weight each) was inoculated in 5 ml of TOS without agar as an
12 enrichment broth. The broth was then incubated anaerobically in Anaero-Pack
13 (Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan) at 37° C for 48h. After
14 incubation, 20 µl of each culture was spread onto TOS. The TOS plates thus
15 inoculated were incubated anaerobically in Anaero-Pack (Mitsubishi) at 37° C
16 for 3 days. After incubation, 10 opaque colonies formed on the TOS plates were
17 randomly selected as tentative “*Bifidobacterium*” colonies and subjected to
18 subsequent genetic identification and DNA fingerprinting of *B. longum* strains.

19 Firstly, a PCR assay described by Matsuki et al. [6] that was designed to
20 amplify species-specific sequences in 16S rRNA gene of *B. longum* was
21 performed on genomic DNAs prepared from the isolates. PFGE typing was
22 subsequently performed on 174 fecal and 47 probiotics’ isolates that were
23 identified as *B. longum*, essentially following the methodology described by
24 McCartney et al. [7]. A total of 105 PFGE patterns were observed for the fecal

isolates examined. Meanwhile, 47 probiotics' strains showed 5 distinct PFGE patterns. The software analysis on the PFGE patterns of the isolates revealed the presence of 35 PFGE types (BL 1 to BL 35 at 50 % similarity level, which could be further assigned to "subtypes" at 80% similarity level (i.e. BL23a, BL23b and BL23c) on the dendrogram (Tables 1a and 1b). The fecal isolates were designated to 79 subtypes and the probiotics isolates to 5 subtypes.

The distribution of PFGE types and subtypes of *B. longum* isolated from the 12 subjects' fecal samples at the 1st, the 23rd and 68th week are shown in Tables 1a and 1b.

Each subject harbored subject-specific PFGE types or subtypes although subtypes clonally close to each other at 50% similarity level occurred among the subjects. All of the subject specific clones showed less than 80% similarity with those probiotics subtypes although the subtypes in several subjects were marginally related at 50% similarity level to the probiotic subtypes. There appears to be a subject-specific "congregation" of subtypes in most subjects. Apparent successions of the subject-specific PFGE type or subtypes over the period (ca. 6 months) from the 1st to the 23rd week were observed in 7 subjects, 5 of which harbored them to the 68th week. Apparent successions of the subject-specific PFGE subtype(s) were also observed over the period (ca. 11 months) from the 23rd to 68th week for 4 subjects. The evidence further substantiates the view of Reuter [10] that autochthonous (or indigenous) *Bifidobacterium* microbiota will remain stable in human intestine throughout life.

Interestingly, the fecal isolates seldom shared the same PFGE profile (at 80% similarity level) with any of the probiotic isolates while the subjects had not been

1 advised to refrain from consuming any *B. longum* commercially available in
2 Japan during the study period. A subsequent interview revealed that Subjects 8,
3 9, 10, and 11 had eaten a certain yogurt product containing *B. longum* almost
4 daily. Although those probiotic strains may have existed in these hosts'
5 microbiota but their concentrations were too low to be detected by the isolation
6 technique used in the present study (random picking of colonies grown on TOS
7 plates), the present data may support the observations of Kullen et al. [5] and
8 Amann et al. [1] that fecal counts of total bifidobacteria were not affected by the
9 consumption of exogenous bifidobacteria.

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16 **References**

- 17 **1. Amann, M. M., Kullen, M. J., Martini, M. C., Bustam, F. F., Brady, L. J.:**
18 Consumption of exogenous bifidobacteria does not alter fecal bifidobacteria
19 and breath hydrogen excretion in humans. J. Nutr. 128: 996-1002 (1998).
- 20 **2. Benno, Y., Sawada, K., Mitsuoka, T.:** The intestinal microflora of infants:
21 composition of fecal flora in breast-fed and bottle-fed infants. Microbiol.
22 Immunol. 28: 975-986 (1984).

- 1 **3. Finegold, S. M., Attebery, H. R., Sutter, V. L.:** Effect of diet on human fecal
2 flora: comparison of Japanese and American diets. *Am. J. Clin. Nutr.* 27:
3 1456-1469 (1974).
- 4 **4. Kimura, K., McCartney, A. L., McConnell, M. A., Tannock, G. W.:** Analysis
5 of fecal populations of bifidobacteria and lactobacilli and investigation of the
6 immunological responses of their human hosts to the predominant strains.
7 *Appl. Environ. Microbiol.* 63: 3394-3398 (1997).
- 8 **5. Kullen, M. J., Amann, M. M., O'Shaughnessy, M. J., O'Sullivan, D. J.,**
9 **Busta, F. F., Brady, L. J.:** Differentiation of ingested and endogenous
10 bifidobacteria by DNA fingerprinting demonstrates the survival of an
11 unmodified strain in the gastrointestinal tract of humans. *J. Nutr.* 127: 89-94
12 (1997).
- 13 **6. Matsuki, T., Watanabe, K., Tanaka, R., Fukuda, M., Oyaizu, H.:** Distribution
14 of bifidobacterial species in human intestinal microflora examined with 16S
15 rRNA-gene-targeted species-specific primers. *Appl. Environ. Microbiol.* 65,
16 4506-4512 (1999).
- 17 **7. McCartney, A. L., Wenzhi, W., Tannock, G. W.:** Molecular analysis of the
18 composition of the bifidobacterial and lactobacillus microflora of humans. *Appl.*
19 *Environ. Microbiol.* 62: 4608-4613 (1996).
- 20 **8. Moore, W. E. C., Holdeman, L. V.:** Human fecal flora: the normal flora of 20
21 Japanese-Hawaiians. *Appl. Microbiol.* 27: 961-979 (1974).
- 22 **9. Mueller, S., Saunier, K., Hanisch, C., Norin, E., Alm, L., Midtvedt, T.,**
23 **Cresci, A., Silvi, S., Orpianesi, C., Verdenelli, M. C., Clavel, T., Koebnick,**
24 **C., Zunft, H. J., Dore, J., Blaut, M.:** Differences in fecal microbiota in

different European study populations in relation to age, gender, and country: a cross-sectional study. Appl. Environ. Microbiol. 72:1027-33 (2006).

10. Reuter, G: The *Lactobacillus* and *Bifidobacterium* microflora of the human intestine: composition and succession. Curr. Issues. Intest. Microbiol. 2: 43-53 (2001).

Legends of Figures

Fig. 1. PFGE dendrogram showing clustering (by UPGMA and the Dice coefficient) of *B. longum* strains isolated from fecal samples of 12 human subjects collected at the 1st, 23rd, and 68th week and 19 probiotics commercially available.

★The PFGE types or subtypes of probiotics strains. The bandwidth tolerance was critically set at 1.5%.

Fig. 2. Distribution of the *B. longum* strains of various PFGE types and subtypes detected in fecal samples collected from 12 human subjects at the 1st, 23rd, and 68th week.

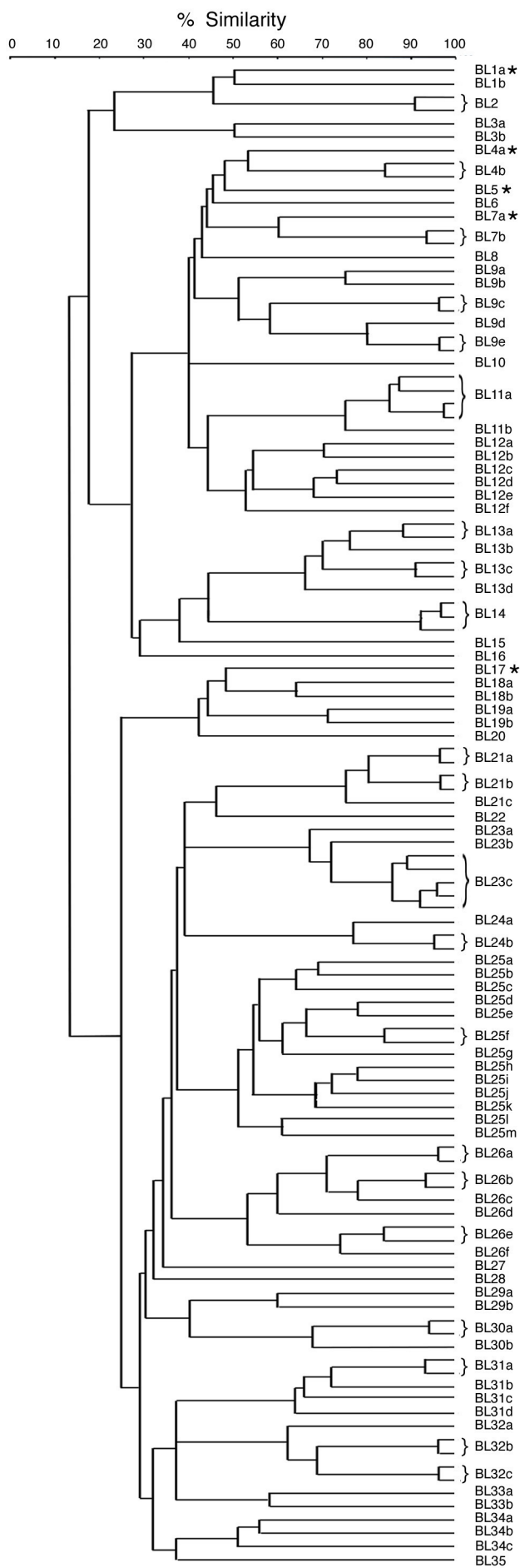


Fig. 1

PFGE types and subtypes

		Subject 1		
		1st	23rd	68th
BL21	b	6		
BL23	a	1		
BL32	a		1	
	b		4	2
	c		1	2

		Subject 2		
		1st	23rd	68th
BL3	a	1		1
BL27				1
BL31	a	1	1	1
	b			1
	c			1
	d		1	

		Subject 3		
		1st	23rd	68th
BL21	a	1	2	
BL23	b		1	
	c	6	1	4

		Subject 4		
		1st	23rd	
BL4	b		4	
BL6		2		
BL14		2	1	
BL29	a	1		
	b	1		
BL30	a		2	
	b		1	
BL33	a		1	
	b		1	
BL34	b	1		
		Subjec5		
		1st	23rd	
BL2		2		
BL10			1	
BL18	a		1	
	b		1	
		Subject 6		
		1st	23rd	68th
BL1	b	2		
BL19	a			1
	b	2	1	
BL22		2		
		Subject 7		
		1st	23rd	68th
BL11	a	1	3	4
	b		1	
BL35			3	2
		Subject 8		
		1st	23rd	68th
BL7	b	2		
BL8			1	
BL12	a	5	1	1
	b		1	
	c	1		
	d		1	
	e		1	
BL21	c			1
BL21	c		1	
	l	1		
		Subject 9		
		1st	23rd	68th
BL17		1		
BL26	a	2	1	
	b		1	2
	c			2
	d	2		
	e	1	2	2
	f		1	
BL28				1
		Subject 10		
		1st	23rd	68th
BL12	f			1
BL26	a	1		
	b	1	1	
	d	1		
	e	1	1	1
	f			1
	g	1		
	h	1		
	l		1	
	j	1		
	k	1		
	m		1	
		Subject 11		
		1st	23rd	68th
BL9	a	2		
	b	1		
	c	4		
	d		2	
	e	2		
BL13	a		1	2
	b		1	
	c		2	
BL24	a	1		
	b			2
		Subject 12		
		1st	23rd	68th
BL3	b	1		
BL13	d			1
BL15	a	1		
BL20			1	
BL34	a	4		
	c			1

Fig. 2