Original Article

THAI J GASTROENTEROL 2016
Vol. 17 No. 1
Jan. - Apr. 2016
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Intestinal Microbiota in Stool of Thai Healthy Volunteers and Their Drug Resistant Extended Spectrum β-Lactamase (ESBL) and Ciprofloxacin-Resistant (CIP-R) *E.Coli* Before and After Ingestion of Fermented Milk Containing *Lactobacillus Casei* Strain Shirota (Lcs)

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ABSTRACT

Objective: To identify the spectrum of microbiota from stools of Thai volunteers before, during and after administration of fermented milk containing LcS and to see if there is any change in the spectrum of microbiota and the concentration of their extended spectrum beta-lactamase (ESBL+) *E. coli* and ciprofloxacin-resistant (CIP-R) *E. coli.*

Material and Methods: Stool samples were collected from the 20 healthy adult volunteers before and 7 days after taking LcS containing milk product (follow-up period) for 7 days. Stools were analysed for the spectrum of microbiota using YIF-SCAN® PCR for stool organic acids content using HPLC technique and were cultured for entero-pathogenic bacteria using MacConkey agar, TCBS agar and XLD agar. The cultured *E. coli* were analysed for ESBL-production and CIP-R production by double disc method.

Results: The concentration of *Lactobacillus,* especially the *L. casei* subgroup was increased during the LcS ingestion period and decreased after stopping LcS. The concentration of *Bifidobacterium* and acetic acid were significant higher in the LcS ingestion period than baseline period. There was 45% (9/20) of subjects carried ESBL and/or CIP-R *E.coli* at baseline. It was observed that in some cases, there was absence of ESBL-producing *E. coli* and less CIP-R *E.coli* during the LcS ingestion period. In these cases, the re-appearance of ESBL-producing *E.coli* and CIP-R E.coli was observed in the follow-up period which LcS ingestion was stopped.

Conclusion: There were some changes in the spectrum of microbiota, short chain fatty acids and the patterns of *E. coli* antibiotic resistant before, during and after ingestion of *L. casei,* but the consistency of findings needs to be further studied.

Key words : Intestinal microbiota, Thai, *Lactobacillus casei,* antibiotics resistant

[Thai J Gastroenterol 2016; 17(1):31-38.]

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BACKGROUND

Human microbiota is complex and contains more than 10^{12} species of microbes. Their composition can be influenced by many factors, including genetic and environmental factors such as geography, diet, sanitation and frequency of antibiotics use. Every country has its own food profile. In Thailand, Thai foods have strong aromatic and spicy components with strong flavor. Raw meat is not commonly consumed, but raw fresh water fish is popular in some provincial areas. Fruits and vegetables are abundant and all year round. Sanitation in Thailand varies in different geographic regions, but in general may be better than in neighboring developing countries, although as ideal as in Japan and other Western nations. Street vendors often sell precooked food as well as instant cooked food in rather limited sanitary environment. Antibiotics can be purchased from drug stores without prescriptions, and inappropriate uses can lead to emergence of resistant bacteria. Data on microbiota in Thai persons is scanty. In a preliminary study using a Japanese agar preparation for cultivating *Lactobacillus casei* strain Shirota (LcS), the lactobacillus were not recovered from stools of healthy Thai healthy subjects due to overgrowth of indigenous *Escherichia coli*[1]. Addition of an antibiotic in the agar to inhibit indigenous *E.coli* allowed *Lactobacillus* to grow in the original agar as successfully used in Japanese subjects, but not in Thai subjects. This observation led to the postulation that Thai peoples may have a different spectrum of microbiota from Japanese or other Asian subjects. If the spectrum of microbiota among Thai and in Japanese is not different, it may be that the same spectrum of bacteria carries different genes that are resistant to antibiotics. LcS has been shown to alter the microbiota composition and to improve the intestinal microbial ecosystem in healthy individuals. It is interesting to know whether the composition of microbiota or its antibiotic resistant pattern can be modified by LcS ingestion. It was the purpose of this study to identify the spectrum of microbiota from stools of Thai volunteers before and after administration of fermented milk containing LcS, and to observe any change in the concentration of their extended spectrum beta-lactamase (ESBL+) *E.coli and* ciprofloxacin-resistant (CIP-R) *E. coli*. To our knowledge, this is the first report to analyse fecal microbiota of healthy adult volunteers. We also discussed the properties of microbiota of healthy Thai subjects, comparing our findings with previously published data in other geographic populations.

MATERIAL AND METHODS

Materials:

Stool samples were collected from 20 healthy adult volunteers who had no subjective abdominal symptoms (e.g., constipation, diarrhea or abdominal pain). The volunteers had previously and participated in a published study[1] about the viability of *Lactobacillus casei* strain Shirota (LcS) in fecal specimens from healthy Thai subjects regularly taking fermented milk product containing LcS. They were 11 female and 9 male, ages 25-57 years old. The first fecal samples (baseline) were collected on day-10 after abstaining from all *Lactobacillus* containing fermented milk products. The second specimens were collected after the subjects ingested LcS containing milk product for 7 days (ingestion period), and the third specimens at day-7 after also training from taking LcS containing milk product (follow-up period). All collected fecal specimens were kept at -80˚C before analysis.

Methods:

The intestinal microbiota was analyzed by 4 processes:

1. YIF-SCAN®: The basic principle of YIF-SCAN® is the combination between quantitative RT-PCR[2,3,4] and PCR[5,6]. The target bacterial groups were *Lactobacillus casei* strain Shirota, *Clostridium coccoides* group, *Clostridium leptum* subgroup, *Bacteroides fragilis* group, *Bifidobacterium, Atopobium cluster, Prevotella, Clostridium perfringens, Clostridium difficile, Enterobacteriaceae, Lactobacillus*(6 subgroups and 3 species): *L. casei* subgroup, *L. gasseri subgroup, L. plantarum subgroup, L. reuteri* subgroup, *L. ruminis* subgroup, *L. sakei subgroup, L. brevis, L. fermentum, L. fructivorans, Enterococcus, Staphylococcus, Vibrio cholerae/mimicus, V. parahaemolyticus, Campylobacter jejuni /coli* and *Shigella* spp.

2. Organic acid: The total amount of organic acids, namely acetic acid, propionic acid, butyric acid, iso-butyric acid, succinic acid, lactic acid, formic acid, iso-valeric acid and valeric acid, were assessed by HPLC[7].

3. Culture for entero-pathogenic bacteria[8,9]:

For quantitative culture of *Salmonella, Shigella, Plesiomonas, Vibrio and Aeromonas,* the specimens were first diluted to 1:10 with normal saline. Each 100 µL of the diluent was subsequently inoculated on MacConkey agar, TCBS agar and XLD agar, which were incubated at 35˚C in ambient air for 18-24 hours. The 100 µL diluted specimens were then added to RV broth, after incubation at 42˚C for 6 hours, and subcultured on MSRV medium and incubated at 42˚C overnight. For *Campylobacter,* the 100 µL diluted specimens were dropped on 0.45 µm filter membrane placed on top of blood agar and rested at room temperature for 30 minutes before discarding the filter membrane. The blood agar plate was incubated at 42˚C in microaerobic condition. Suspected colonies were identified by conventional biochemical tests.

4. Culture for extended spectrum β-lactamase (ESBL) producing and ciprofloxacin-resistant (CIP-R) *E. coli*: A 100 µL of each 1:10 suspension specimen was inoculated on MacConkey agar supplemented with ceftriaxone 1 µg/mL and MacConkey agar supplemented with ciprofloxacin 4 µg/mL for detection of ESBL-producing *E. coli* and CIP-R E. coli, respectively. The inoculated plates were incubated at 35˚C in ambient air for 24-48 hours. The colonies growing on the antibiotics selective media were speciated by conventional biochemical tests and confirmed as ESBL producing by double-disc method out of the ceftriaxone disc, ceftazidime disc, cefepime disc and amoxicillin-clavulanic acid disc[10].

Statistical analysis:

SPSS 14.0 software (SPSS Japan Inc., Tokyo, Japan) was used for statistical analysis. Comparative tests on bacterial counts were conducted by using non-parametric Wilcoxon signed-rank test. For statistical testing of the detection rates of bacteria, we used Fisher' s exact probability test. The level of significance was considered to be $p<0.05$.

Results

The intestinal microbiota was tested by YIF-SCAN® in the 20 healthy volunteers over the three periods are shown in Table 1. The amount of total bacteria was slightly increased during the ingestion period than in the baseline period, with significant increase in the amount of *Bifidobacterium* and *Atopobium* cluster and a marginally significant increase in the amount of *Clostridium* coccoides subgroup, C. leptum group. The number of Lactobacillus was also shown to be significantly increased during the ingestion period, especially the *L. casei* subgroup. During the follow-up period, the concentration of *Lactobacillus* including the *L. casei* subgroup was significantly decreased.

Fecal organic acids in the 20 healthy volunteers during the three periods are shown in Table 2. There was no statistically significant change in the amount of organic acids during the three periods. However, the amount of acetic acid in the ingestion period was significantly increased. There was an increase in the amount of lactic acid in the ingestion period in one sample, but statistical comparison was not possible as only one sample could be tested.

Regarding quantitative culture for entero-pathogenic bacteria, *Salmonella, Shigella, Plesiomonas, Aeromonas, Vibrio cholera, V. parahaemolyticus, and Campylobacter* spp. were not detected (less than 10^2 CFU/g feces) in all stool specimens and in all three periods.

The concentration of ESBL-producing *E. coli* and CIP-R *E. coli* are shown in Table 3. At baseline, six cases (No. 1, 3, 4, 7, 14, 18) were shown to carry ESBL-producing *E. coli.* The presence of ESBL-producing *E. coli* was suppressed (probably by LcS) and turned negative in five cases (No. 1, 3, 4, 7, 18), while ESBL-producing *E. coli* did not reappear in the follow-up period in all five cases. There were six cases in which ESBL-producing E. coli was not detected in the baseline period, but appeared in the ingestion and/or in the follow-up period (No. 2, 5, 6, 9, 13, 19).

CIP-R *E. coli* in stool samples at baseline were present in seven cases (No.3, 5, 7, 8, 9, 14, 18.). Two cases were suppressed (by LcS) and turned negative, while the other five cases showed bacterial persistence in the ingestion and/or in the follow up period. Six cases without CIP-R *E.coli* at baseline period showed positive CIP-R *E. coli* during the ingestion and/or in the follow-up period (No. 2, 4, 6, 11, 13, 19).

Discussion

In this study, we identified the spectrum of microbiota from stools of Thai volunteers before and after administration of fermented milk containing LcS. We also observed any changes in the concentrations of their extended spectrum beta-lactamase (ESBL+) *E.coli*

Table 1. Intestinal microbiota in healthy subjects regularly taking LcS-fermented milk ($10^{Mean\pm SD}$ CFU/gram of feces).

	Baseline period		Ingestion period		Follow-up period	
	Mean±SD	N (detected/ total)	Mean±SD	N (detected/ total)	Mean±SD	N (detected/ total)
Total bacteria	10.0±0.6	(20/20)	10.4±0.58***	(20/20)	10.3±0.3	(19/19)
Anaerobes						
Clostridium coccoides sub gr.	9.2±0.7	(20/20)	9.5±0.5*	(20/20)	9.4±0.3	(19/19)
C. leptum group	9.3±0.7	(20/20)	9.7±0.6*	(20/20)	9.7±0.4	(19/19)
Bacteroides fragilis group	8.3±0.8	(20/20)	8.3±0.8	(20/20)	8.6±0.5	(19/19)
Bifidobacterium	9.0±0.7	(19/20)	9.4±1.2***	(20/20)	8.8±1.4	(19/19)
Atopobium cluster	9.2±0.6	(20/20)	9.6±0.6***	(20/20)	9.5±0.5	(19/19)
Prevotella	8.3±1.4	(18/20)	8.2±1.3	(20/20)	8.8±1.1	(17/19)
C. difficile	<2.3	(0/20)	<2.3	(0/20)	<2.3	(0/19)
C. perfringens	6.3±1.2	(20/20)	6.4±1.1	(20/20)	6.5±1.5	(19/19)
Facultative anaerobes						
Total *Lactobacillus*	6.6±1.4	(20/20)	7.9±0.9**	(20/20)	7.0±1.4#	(19/19)
L. gasseri subgroup	5.3±1.5	(18/20)	5.6±1.5	(20/20)	5.7±1.3	(19/19)
L. brevis	3.8±0.8	(11/20)	4.2±0.7	(20/20)	4.1±0.9	(11/19)
L. casei subgroup	3.8±0.9	(5/20)	7.4±0.9*	(20/20)	4.5±0.8#	(12/19)
L. fermentum	5.1±1.0	(12/20)	5.4±1.0	(20/20)	5.7±1.1	(14/19)
L. fructivorans	<2.3	(0/20)	<2.3	(0/20)	<2.3	(0/19)
L. plantarum subgroup	4.5±1.2	(18/20)	4.7±1.2	(20/20)	4.7±1.3	(18/19)
L. reuteri subgroup	5.7±1.3	(20/20)	6.5±1.2	(19/20)	6.3±1.2	(18/19)
L. ruminis subgroup	5.8±2.3	(15/20)	6.3±1.9	(17/20)	5.9±2.1	(18/19)
L. sakei subgroup	3.2	(2/20)	4.9±1.8	(6/20)	4.4	(1/19)
Enteerobacteriaceae	7.5±1.0	(20/20)	7.7±0.9	(20/20)	8.1±0.5#	(19/19)
Shigella spp./*E. coli*	7.4±1.0	(20/20)	7.7±0.9	(20/20)	8.0±0.6	(19/19)
Enterococcus	6.9±1.1	(18/20)	7.1±1.0	(20/20)	7.1±0.9	(19/19)
Staphyllococcus	4.9±0.7	(17/20)	4.9±0.9	(20/20)	5.2±0.7	(18/19)
Ingested bacteria						
Lactobacillus casei strain Shirota	<5.1	(0/20)	7.0±1.0	(20/20)	<5.1	(0/19)**

* $p<0.05$, ** $p<0.01$, *** $p<0.001$, Baseline period vs. Ingestion period
# $p<0.05$, ## $p<0.01$, Ingestion period vs. Follow-up period
No. of bacterial cells: non-parametric Wilcoxon signed-rank test
Detection rate: Fisher's exact probability test

and ciprofloxacin-resistant (CIP-R) *E. coli.*

The concentrations of *Bifidobacterium* and acetic acid were significantly higher in the ingestion period than in the baseline period ($p < 0.001$ and $p< 0.01$, respectively). The results were similar to those reported by Spanhaak S *et al*[11] who conducted a study the studies in long-term nursing home patients[12], university students[13] and volunteers[14] with suboptimal healthy. Although the reason for such changes is not clear, it is interesting that they were detected in healthy Thai volunteers whose food habits differed from other countries.

Acetic acid is the most common metabolic product of *Bifidobacterium*. This acid has a strong bactericidal activity against Enterobacteriaceae[15]. In our study, the numbers of Enterobacteriaceae were not altered after ingestion of LcS. Further study is needed to clarify the biological impact of increased acetic acid in healthy Thai subjects.

The concentration of *L. casei* strain Shirota was

Table 2. Fecal organic acids in healthy subjects regularly taking LcS-fermented milk.

	Baseline period		Ingestion period		Follow-up period	
	Mean±SD	N (detected /total)	Mean±SD	N (detected/ /total)	Mean±SD	N (detected/ /total)
Total organic acids	113.3±27.7	(20/20)	125.9±24.3	(20/20)	115.8±33.7	(19/19)
Succinic acid	3.3±5.9	(11/20)	0.5±0.3	(10/20)	2.1±3.9	(12/19)
Lactic acid	3.2+3.1	(8/20)	21.6	(1/20)	2.2	(2/19)
Formic acid	0.6±0.2	(8/20)	1.8±3.0	(10/20)	1.2±0.9	(8/19)
Acetic acid	67.0±14.6	(20/20)	80.3±14.1**	(20/20)	72.0±20.7	(19/19)
Propionic acid	26.4±9.8	(20/20)	24.9±8.6	(20/20)	28.0±8.8	(19/19)
Butyric acid	2.1±1.6	(10/20)	2.7±1.5	(14/20)	3.4±1.5	(14/19)
Isovaleric acid	14.6±5.5	(20/20)	14.5±7.8	(20/20)	18.4±7.3	(19/19)
Valeric acid	4.4±4.3	(5/20)	4.0±2.1	(10/20)	4.9±2.9	(11/19)

* $p<0.01$, *** $p<0.001$, Baseline period vs. Ingestion period
Concentration of organic acids: non-parametric Wilcoxon signed-rank test
Detection rate: Fisher's exact probability test

Table 3. Concentrations of extended spectrum β-lactamase-producing (ESBL+) *E. coli* and ciprofloxacin-resistant (CIP-R) *E. coli* in stool samples during the baseline, the ingestion and the follow up periods.

	Baseline period		Ingestion period		Follow-up period	
Subject No.	ESBL + *E.coli* (CFU/g)	CIP-R *E.coli* (CFU/g)	ESBL + *E.coli* (CFU/g)	CIP-R *E.coli* (CFU/g)	ESBL + *E.coli* (CFU/g)	CIP-R *E.coli* (CFU/g)
1	$1x10^3$	Negative	Negative	Negative	Negative	Negative
2	Negative	Negative	Negative	Negative	$5x10^4$	$4x10^4$
3	$1.5x10^5$	$3x10^4$	Negative	Negative	Negative	$1.5x10^5$
4	$1x10^6$	Negative	Negative	Negative	Negative	$7x10^4$
5	Negative	$4.4x10^5$	$8x10^4$	$1.6x10^5$	$2.4x10^5$	$2x10^6$
6	Negative	Negative	Negative	$2.9x10^5$	$2x10^6$	Negative
7	$1.5x10^5$	$1.9x10^5$	Negative	$1.1x10^5$	Negative	$4x10^6$
8	Negative	$1.4x10^5$	Negative	$2x10^4$	Negative	$2x10^5$
9	Negative	$4x10^5$	$2x10^6$	$1.2x10^6$	Negative	Negative
10	Negative	Negative	Negative	Negative	Negative	Negative
11	Negative	Negative	Negative	$4x10^6$	Negative	$2x10^4$
12	Negative	Negative	Negative	Negative	Negative	Negative
13	Negative	Negative	$1x10^4$	Negative	$4x10^4$	$1.2x10^5$
14	$>10^7$	$9x10^4$	$>10^7$	$5x10^5$	>107	$9x10^4$
15	Negative	Negative	Negative	Negative	Negative	Negative
16	Negative	Negative	Negative	Negative	Negative	Negative
17	Negative	Negative	Negative	Negative	Not done	Not done
18	$1x10^4$	$1x10^4$	Negative	Negative	Negative	Negative
19	Negative	Negative	$2x10^6$	$2x10^6$	Negative	Negative
20	Negative	Negative	Negative	Negative	Negative	Negative

significantly lower in the follow-up period than in the ingestion period ($p < 0.05$). This suggested that the probiotic can thrive in intestinal environment although it cannot grow or replicate. Prebiotics that promote proliferation or activity should be chosen to prolong the of probiotics[16].

As shown in Table 3, there were 45% (9/20) of subjects who carried ESBL-producing and/or CIP-R *E. coli* at the baseline. The percentage of ESBL-producing, CIP-R and both ESBL-producing and CIP-R *E.coli* were 30 (6/20), 35 (7/20) and 20 (4/20) respectively. Such prevalence were lower than those reported in a recent study of 544 healthy Thai adult workers, with finding of ESBL-producing *E. coli* and ciprofloxacin-resistant *E. coli* of 75.5 and 82.7%, respectively[17]. Such differences related to different timing with regard to specimen collection which was 5 years apart, while the change in antibiotics use may be due to a smaller sample size in our study. The absence of ESBL-producing *E. coli* in the ingestion period could be due to the effect of LcS although this observation was weaker in the case of CIP-R *E. coli*. The presence of ESBL-producing and CIP-R *E. coli* in the follow-up period could be due to re-exposure to drug-resistant E.coli in contaminated foods or drinking water. This postulate was in keeping with the study by Boonyasiri *et al.* who reported that fresh meat, such as chicken and pork, was commonly contaminated with ESBL-producing *E. coli*[17].

The human intestinal microbiota contains more than 1014 bacterial cells, and were 50 bacterial phyla and 5000 bacterial species[18,19]. Most are strict anaerobes, thus less than 20% are cultural under standard laboratory settings. The wide variation of human intestinal microbiota may be related to diet, geographic region, age, host genotype or antibiotic uses. There are many techniques to assess the intestinal microbiota[18], such as microbiologic culture, YIF-SCAN®, 16S rRNA or pyrosequencing. In our study,YIF-SCAN® technique by specific target primers asdescribed by Tsuji H *at al.*[20] was chosen. We are aware that results from different techniques are difficult for comparison. However, to our knowledge, ours is the first report on analysis of the faecal mocrobiota in healthy Thai adult volunteers. Thus, based on previously published data in different geographic populations, we discussed the characters of microbiota in healthy Thai subjects.

For anaerobes, the concentration of *B. fragilis* group in stools of Thai volunteers was lower than in Japanese, $10^{8.3 \pm 0.8}$ and $10^{9.6 \pm 0.5}$ cells/gram of feces respectively, but the concentration of *C. perfringens* in Thais was higher than in Japanese, $10^{6.3 \pm 1.2}$ and $10^{4.7 \pm 1.2}$ cells/gram of feces respectively. Nevertheless, the *C. difficile* stool concentration in Thais was very low and indeed below the lowest limit of detection (less than $10^{2.3}$ cells/gram of feces). It was noted that only one Japanese had *C. difficile* concentration of $10^{7.6}$ cells/gram of feces. For facultative anaerobe, the stool concentration of *Enterobacteriaceae* in Thais was higher than in Japanese, $10^{7.5 \pm 1.0}$ and $10^{6.9 \pm 0.9}$ cells/gram of feces respectively. This was the basis for modifying the medium used in our earlier study on the viability of *Lactobacillus* casei strain Shirota (LcS) from feces of healthy Thai subjects who regularly consumed fermented milk product containing LcS(1). In that study, we used lactitol-LBS supplemented with vancomycin and fosfomycin to inhibit the normal flora, especially Enterobacteriaceae, whereas the medium used in Japan for stool culture for Lactobacillus casei strain Shirota (LcS) was lactitol-LBS supplemented with vancomycin only. Nonetheless, we observed no changes in the concentrations of *C. difficile* during the ingestion of LcS and during the follow-up period, although the concentrations of *C. perfringens* and of *Enterobacteriaceae* were increased during the ingestion of LcS and in the follow-up periods.

Comparison was also made between microbiota of healthy human subjects from different geographic regions[21]. The mean quantities of Bacteroides fragilis group in healthy Thai subjects and in healthy Ugandans were similar ($10^{8.3}$ and $10^{8.2}$ CFU/gram of feces, respectively), but were lower than in reports from England and America ($10^{9.7}$ and $10^{9.8}$ CFU/gram of feces, respectively). The mean concentrations of enterococci in Thai subjects and in Ugandans were very close ($10^{6.9}$ and $10^{7.0}$ CFU/gram of feces, respectively), and more than in English and American subjects ($10^{5.7}$ and $10^{5.5}$ CFU/gram of feces, respectively). Interestingly, the concentration of *C. perfringens* in healthy Thai was higher than in all others ($10^{6.3}$ and 10^{4-5} CFU/gram of feces, respectively).

In a study using 16S rRNA gene sequencing and biochemical analysis, the four most predominant bacterial phyla, namely Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria, were found in more than 94.2% of the sequences in all samples[22]. There were differences in the concentrations of intestinal microbiota in European children and in rural African

village children. The difference in the amount of microbiota may be accounted from the differences in food intake in each community. Modern European diet was rich in animal protein, sugar, starch and fat but poor in fiber, whereas African diet was poor in animal protein and fat but rich in starch, fiber and plant polysaccharides.

Another study using pyrosequencing technique to assess Korean, Japan, China and USA intestinal microbiota, reported the presence of 13 bacterial phyla, of which 5 were commonly found in the human intestine, namely Actinobacteria, Bacteroidetes, Firmicutes Fusobacteria and Proteobacteria, Firmicutes and Bacteroidetes being predominant 94.8%[23]. Populations with the highest proportions of Actinobacteria, Bacteroidetes and Firmicutes, were Japanese, Chinese and American respectively. At the genus level, Japanese had the highest proportions of *Bifidobacterium* and *Clostridium*, Koreans had the highest proportions of *Prevotella* and *Faecalibacterium,* and Chinese had the highest proportion of Bacteroides.

Variations of intestinal microbiota in different geographic populations are related to many factors such as diet, bacterial culture techniques, study populations and antibiotic uses. In a study of fecal flora in urban and rural elderly Japanese, it was found that urban subjects had significantly less *Bifidobacterium* but more total anaerobic bacteria, bacilli and *Clostridium* than rural folks[24]. In another study in one-week old neonates in Estonia and Sweden[25], Estonian neonates were found to have significantly higher *Staphylococcus, Enterococcus* and *Enterobacteriaceae* than Swedish neonates. Diet is certained an important factor. Consumers of diet rich in protein, saturated fat, carbohydrate and simple sugar would be colonized with intestinal microbiota rich in *Bacteroides* or *Prevotella*[26]. English people in London and on mixed Western diet had more *Bifidobacterium* and *Bacteroides* but less *Enterococcus, Lactobacillus* and yeasts than vegetarian Ugandans living in London[27]. Vegetarian diets, moreover, are associated with fewer anaerobes and more aerobes and facultative anaerobes, Human volunteers who consumed foods rich in fructo-oligo saccharides, such as bananas, onions and asparagus, were found to have more *Bifidobacterium* in their intestinal microbiota([28] Sucrose was found to increase *Bacteroides* and inulin was found to promote most *Bifidobacterium*[29].

Conclusion

In our study, the concentration of *Lactobacillus,* especially the *L. casei* subgroup, was increased during the LcS ingestion period, and was decreased after stopping LcS. The concentrations of *Bifidobacterium* and of acetic acid were significant higher during the LcS ingestion period than at baseline period. There was 45% (9/20) of subjects who carried ESBL and/or CIP-R E. coli at baseline. It was observed that in some cases, there was an absence of ESBL-producing *E. coli* and less CIP-R *E. coli* during the LcS ingestion period. Reappearance of ESBL-producing *E. coli* and CIP-R E. coli was noted in the follow-up period when LcS ingestion was discontinued. Comparing with published data in other countries, the mean concentration of B. fragilis in Thai stool samples was lower than in Japanese, but the concentration of *C. perfringens* in Thais was higher than in Japanese. *C. difficile* concentration in Thai stool samples was also low. The concentration of Enterobacteriaceae in Thai stool samples was higher than in Japanese. The concentrations of *B. fragilis* and of *Enterococci* were similar to in Ugandans and were different from that of Japanese, English and American.

Potential conflict of interest

None

Acknowledgements

The authors thank Yakult (Thailand) and Yakult (Japan) for providing technical support as well as YIF-SCAN® facilities.

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