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Senior Thai Fecal Microbiota Comparison Between Vegetarians and Non-Vegetarians Using PCR-DGGE and Real-Time PCR

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The fecal microbiotas were investigated in 13 healthy Thai subjects using polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE). Among the 186 DNA bands detected on the polyacrylamide gel, 37 bands were identified as representing 11 species: *Bacteroides thetaiotaomicron*, *Bacteroides ovatus*, *Bacteroides uniformis*, *Bacteroides vulgatus*, *Clostridium colicanis*, *Eubacterium eligenes*, *E. rectale*, *Faecalibacterium prausnitzii*, *Megamonas funiformis*, *Prevotella copri*, and *Roseburia intestinalis*, belonging mainly to the groups of *Bacteroides*, *Prevotella*, *Clostridium*, and *F. prausnitzii*. A dendrogram of the PCR-DGGE divided the subjects; vegetarians and non-vegetarians. The fecal microbiotas were also analyzed using a quantitative real-time PCR focused on *Bacteroides*, *Bifidobacterium*, Enterobacteriaceae, *Clostrium coccoides-Eubacterium rectale*, *C. leptum*, *Lactobacillus*, and *Prevotella*. The non-vegetarian and vegetarian subjects were found to have significant differences in the high abundance of the *Bacteroides* and *Prevotella* genera, respectively. No significant differences were found in the counts of *Bifidabacterium*, Enterobacteriaceae, *C. coccoides-E. rectale* group, *C. leptum* group, and *Lactobacillus*. Therefore, these findings on the microbiota of healthy Thais consuming different diets could provide helpful data for predicting the health of South East Asians with similar diets.

Keywords: Fecal microbiota, PCR-DGGE, quantitative real-time PCR, *Bacteroides*, *Prevotella*

Introduction

The gastrointestinal microbiota of humans represent an enormous number of 10^{11}–10^{14} bacterial cells with approximately 500–1,000 different species [9, 31]. The techniques generally used for studying human microbiotas can be broadly divided into cultivation and molecular methods. However, culture-dependent methods are gradually disappearing, as they are labor- and time-consuming, painstaking as regards detail, incomplete in terms of data, and have a community bias, whereas molecular techniques are becoming more popular owing to their broad coverage, rapidity, and accuracy [6, 9, 27, 28, 31]. The most widely used molecular methods are based on the 16S rRNA sequence, such as fluorescent *in situ* hybridization (FISH), terminal restriction fragment length polymorphism (T-RFLP), denaturing/temperature gradient gel electrophoresis (DGGE/TGGE), and pyrosequencing. Although DGGE is an effective and popular fingerprinting technique for separating bacterial communities in environmental systems, a quantitative real-time PCR is used to quantify interesting gut microbiota [18, 19]. The variation of the microbiota development in the human gastrointestinal tract (GI tract) is influenced by many factors, including the composition of

the gut, the person's age, and the consumption style or diet [4, 10, 13, 14, 23, 33, 34]. Owing to growing health concerns, Thai people are now tending to eat more healthy food, like fruit and vegetables. Vegetables are known as a low fat source containing good amounts of vitamins and minerals. Various publications have also showed that vegetarians or vegans have a lower risk of cancer than meat eaters [7, 24]. Thus, different consumption behaviors may result in a different microbial community and provide key bacterial species that maintain good health. To date, most research has focused on the gut microbiota of Asian adults and elders [13, 14, 25], Asian vegetarians [12, 16], vegetarians in Europe [17, 22], predominantly vegetarian children in Africa [8], and high-carbohydrate-consuming Americans [33]. However, there has been no report on the gut microbiota of Thai vegetarians and non-vegetarians. In a report from the National Cancer Institute (NCI, Thailand), between 2007 and 2011, colorectal cancer was among the top three cancers found in males and females [2]. Consequently, this study was interested in the microbiota of Thais who are vegetarians and Thais who are meat eaters. Two methods, PCR-DGGE and real-time PCR, were used for a comparative analysis of the gut microbiota from each group.

Materials and Methods

Fecal Samples

Six and seven fecal samples were obtained from healthy non-vegetarians and healthy vegetarians, respectively. All the subjects had regular bowel habits, including no change of defecation frequency, no history of gastrointestinal disease, such as gastritis, peptic ulcers, gastric cancer, colorectal cancer, or inflammatory bowel disease (IBD), no diarrhea in the month preceding the sampling, and no family history of colorectal cancer. None of the subjects had received any antibiotic treatment within at least one month prior to this study. The vegetarian volunteers were ovo-lacto vegetarians or lacto-vegetarians and had been vegetarians for at least 3 years before participating in this study. A stool sampling kit consisting of a sample collection tube, cotton swabs, and sterile tissue paper together with a questionnaire about each individual's consumption behavior and consent form were given to each subject. The study protocol and consent documents were approved by the Institute for the Development of Human Research Protections (IHRP) under ethic approval No. IHRP 311.

DNA Extraction

The total bacterial genomic DNA from each sample was extracted using zirconium beads and a QIAamp Stool Mini Kit from Qiagen (Hilden, Germany). In brief, each fecal sample (50 mg) was homogenized in 200 µl of an ASL buffer and 0.3 g of 0.1 mm zirconium beads from Bio Spec Products (OK, USA) by vortexing at 2,000 rpm for 1 min, followed by keeping on ice for 1 min. This step was repeated three times. Thereafter, the total bacterial genomic DNA was extracted according to the Qiagen kit instructions. The DNA was eluted with sterilized pure water and kept at −20°C until use.

PCR-DGGE of 16S rRNA

To access the PCR-DGGE, two primers, namely HDA1-GC and HDA2 [30], were used to amplify the 16S rRNA gene of each sample. The sequences for the primers were as follows: HDA1-GC, 5′-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG T-3′, and HDA2, 5′-GTA TTA CCG CGG CTG CTG GCA C-3′. The PCR-DGGE conditions were as follows: 94°C for 5 min, 30 cycles at 94°C for 40 sec, 58°C for 20 sec, and 72°C for 1 min, and a final elongation at 72°C for 5 min [30]. The expected PCR product size was 250 bp. The PCR-DGGE profile was performed using a Dcode System apparatus from BioRad (CA, USA). The electrophoresis was run using a 25−65% denaturing gradient with 100% corresponding to 7 M urea and 40% formamide in an 8% polyacrylamide gel, at 80 volts for 1 h and subsequently at 100 volts for 6 h at 60°C.

After the PCR-DGGE running step, a dendrogram was constructed to cluster the PCR-DGGE profile. The similarities between the PCR-DGGE profiles were analyzed based on the locations of the DNA bands on the PCR-DGGE gel using SYNGENE Gene Tools ver. 4.03(b) and SYNGENE Gene Directory Application ver. 2.01 (c) from SYNGENE, a division of Synoptic Ltd., Dice's similarity coefficient, and the unweighted pair group method with arithmetic mean (UPGMA).

To determine the bacterial species, the bands of interest were cut and eluted in sterile pure water. Each eluted band was then re-amplified with the HDA1-GC and HDA2 primers and run on the DGGE system at a suitable gradient concentration to check the purity of the cut band. Following the purity check, each band was re-amplified without a GC clamp and then purified using a QIAquick PCR Purification kit (Qiagen). The purified PCR products were analyzed using a direct sequencing analysis performed by 1st BASE, Malaysia. The fragment of interest was identified using BLAST in the NCBI and Eztaxon databases.

Real-time PCR Analysis of Gut Microbiota

Seven bacterial groups were quantified in each subject using a quantitative real-time PCR (LightCycler 480; Roche, The Netherlands) based on previously published specific primers for *Prevotella*, *Bacteroides* (based on the *Bacteroides fragilis* group), *Clostridium leptum* group [20], *Bifidobacterium*, *Clostridium coccoides-Eubacterium rectale* group [29], Enterobacteriaceae [3], and *Lactobacillus* [11]. Genomic DNA (50−100 ng) from each subject was used as the template in a reaction volume of 20 µl. Genomic DNA extracted from *Prevotella nigrescenes* JCM 12250T, *Bacteroides fragilis* ATCC 25285, *Bifidobacterium bifidum* JCM 1255, *Salmonella* Typhimurium TISTR 292, *Blautia producta* JGD 07421, *Clostridium leptum* DSM 753,

and *Lactobacillus casei* subsp. *rhamnosus* ATCC 7469 was used to perform the standard curve for each specific group of *Prevotella*, *Bacteroides*, *Bifidabacterium*, Enterobacteriaceae, *C. coccoides-E. rectale* group, *C. leptum* group, and *Lactobacillus*, respectively. The amplification program consisted of one cycle at 95°C for 5 min and 45 cycles at 95°C for 10 sec; an annealing temperature for 10 sec at 62°C for *Prevotella*, 53°C for the *Bacteroides fragilis* group, 55°C for the *C. leptum* group, 62°C for *Bifidabacterium*, 51°C for the *C. coccoides-E. rectale* group, 57°C for Enterobacteriaceae, and 55°C for *Lactobacillus*; plus an extension step at 72°C for 4–21 sec (the extension time was calculated by dividing the target size by 25 according to the Roche recommendation). SYBGreen was used as the PCR reagent. The size and purity of the targeted PCR products were confirmed by agarose gel electrophoresis. For the real-time PCR data analysis, the copy number of the DNA fragment was calculated to determine the number of interesting specific species found in each sample.

Statistical Analysis

The real-time PCR quantification between groups was analyzed by an Independent-Sample *t*-test using SPSS program ver. 18, based on the different subject numbers of vegetarians and non-vegetarians. The *p* values less than 0.01 were considered statistically significant.

Results

Characterization of Thai Subjects

The fecal samples were randomly collected from six non-vegetarians aged between 53 and 78 years and from seven vegetarians aged between 42 and 61 years. All the non-vegetarians consumed red meat (only pork), white meat such as fish and chicken, and eggs (3.8 eggs/month on average), plus only 50% of this group consumed yoghurt and milk (2 cups/month of yoghurt and 6 glasses of milk/month on average). Meanwhile for the vegetarians, all the subjects drank milk (8.5 glasses of milk/month on average) and consumed yoghurt (12 cups/month on average), except for subjects V5 and V6 who did not consume yoghurt and milk, respectively, as shown in Table 1. In addition, since subjects V1 and V2 consumed both eggs and milk, they were grouped as ovo-lacto vegetarians, whereas the others were grouped as lacto-vegetarians. The vegetarians and non-vegetarians all consumed Thai fruit on a daily basis. The body mass index (BMI) for the vegetarians was 22.75 ± 1.17, and that for the non-vegetarians was 24.92 ± 2.11. The mean age of the vegetarians was 52.14 ± 2.71 years, and that for the non-vegetarians was 62.17 ± 3.85 years.

PCR-DGGE Analysis of Gut Microbiota

The microflora fingerprints for the fecal samples from the 25–65% DGGE are shown in Fig. 1. Among the 186 DNA bands separated on the polyacrylamide gel, most were within 35–55% of the denaturing gradient. DNA fragments of 10–16 and 13–19 bands were found in each sample from the vegetarian group and non-vegetarian group, respectively. All the bands were cut and identified. Based on a ≥97% identity, only 37 bands were successfully identified. The dendrogram of the PCR-DGGE profile shown in Fig. 1 was built according to the location of both the unidentified and identified bands and divided into two clusters; A and B. Cluster A consisted of all the non-vegetarians (subjects N1

Table 1. Personal information for 13 subjects.

Sample	Age (years)	BMI	Yoghurt consumption (cup/month)	Milk consumption (glass/month)	Egg consumption (eggs/month)
N1	53	19.98	2	28	5–6
N2	56	22.96	6	6	3–4
N3	60	20.83	0	0	3–4
N4	56	31.62	4	4	1–2
N5	78	31.25	0	0	5–6
N6	68	22.86	0	0	3–4
V1	45	25.91	12	10	0.5
V2	42	22.58	16	30	8–12
V3	51	19.81	20	2	0
V4	61	17.31	2	2	0
V5	61	25.06	0	12	0
V6	53	24.44	6	0	0
V7	57	23.51	30	4	0

Cup of yoghurt = 110–130 g, and glass of milk = 200 ml.

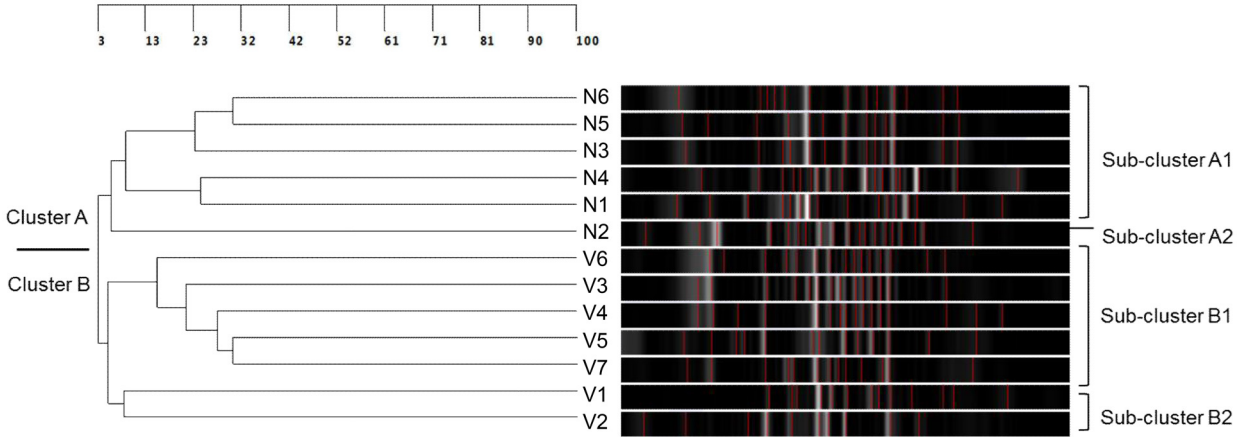

Fig. 1. PCR-DGGE profiles of 13 subjects, shown as a dendrogram of gut microbiota diversity in vegetarians (V) and non-vegetarians (N), indicating similarity among samples calculated using the Dice coefficient and UPGMA algorithm to classify clusters for each community.

The scale shows the percentage of similarity. The red lines indicate the position of the DNA bands detected by the SYNGENE Gene Tools software for clustering the PCR-DGGE profile.

to N6), and cluster B consisted of all the vegetarians (subjects V1 to V7).

Cluster A was divided into two sub-clusters; A1 and A2. *Bacteroides* and *Faecalibacterium prausnitzii* seemed to be the major bacteria detected in sub-cluster A 1, which contained subjects N1, N3, N4, N5, and N6, whereas *Prevotella copri*, *Roseburia intestinalis*, and *F. prausnitzii* were detected in sub-cluster A2, which only contained subject N2 (Table 2).

The *Bacteroides* in sub-cluster A1 were subsequently identified as four different species; *Bacteroides uniformis*, *Bacteroides vulgatus*, *Bacteroides ovatus*, and *Bacteroides thetaiotaomicron*. It should be noted that different combinations of *Bacteroides* species were detected in different subjects. Whereas *Bacteroides vulgatus* was detected in subjects N1, N3, N5, and N6, *Bacteroides uniformis* was found in subjects N3, N5, and N6, but not in subjects N1 and N4, plus *Bacteroides ovatus* and *Bacteroides thetaiotaomicron* were only detected in subject N1 (Table 2). With a 97% identity cut-off, no *Bacteroides* species were detected in subject N4. However, *Bacteroides massiliensis*, with a low identity of 94%, was detected in subject N4. This was why subject N4 was still grouped in sub-cluster A1. In addition to species of *Bacteriodes*, *F. prausnitzii* was also found in subjects N2, N3, N4, N5, and N6, but not in subject N1. *Eubacterium eligenes* and *Megamonas funiformis* with a 97.6% and 100% identity, respectively, were only detected in subject N4.

Table 2. Bacterial composition found in non-vegetarians and vegetarians, determined using PCR-DGGE.

Bacterial species	N1	N2	N3	N4	N5	N6	V1	V2	V3	V4	V5	V6	V7
Bacteroides ovatus	●												
Bacteroides thethaiotaomiocron	●												
Bacteroides uniformis			●		●	●							
Bacteroides vulgatus	●		●		●	●							
Clostridium colicanis											●		
Faecalibacterium prausnitzii		●	●	●	●	●	●	●	●	●	●	●	●
Eubacterium eligens				●									
Eubacterium rectale										●	●		
Roseburia intestinalis		●					●						
Megamonas funiformis				●				●					
Prevotella copri		●					●	●	●	●	●	●	●

N, non-vegetarian subject; V, vegetarian subject; ●, bacteria present in each subject.

It should be noted that the largest microbial portion included unidentified DNA bands with a low identity in the range of 92–94% that belonged to genera *Coprococcus*, *Clostridium*, *Escherichia*, and *Prevotella*, as well as unsuccessfully sequenced DNA bands.

Cluster B included all the vegetarian subjects, V1 to V7. Here, six species were detected and classified as *Clostridium colicanis*, *Eubacterium rectale*, *F. prausnitzii*, *M. funiformis*, *P. copri*, and *R. intestinalis*. The species *P. copri* and *F. prausnitzii* were found in all the subjects (Table 2). This cluster was also divided into two sub-clusters; B1 (subjects V3 to V7) and B2 (subjects V1 and V2), representing the lacto-vegetarians and ovo-lacto-vegetarians, respectively. The subjects in sub-cluster B1 were from the famous Thai vegetarian society in Bangkok called Santi Asoke. Apart from *P. copri* and *F. prausnitzii*, different combinations of bacteria were also detected in each subject. *C. colicanis* was only found in subject V5, whereas *E. rectale* was found in subjects V4 and V5. For sub-cluster B2, the other species detected were *R. intestinalis* and *M. funiformis*, where *R. intestinalis* was found in subject V1, and *M. funiformis* was observed in subject V2.

It should be noted that the largest microbial portion also included unidentified DNA bands that belonged to the genera *Bacteroides*, *Clostridium*, *Escherichia*, and *Prevotella*, with low percentage identity values in the range of 90–96%, as well as unsuccessfully sequenced DNA bands.

Analysis of Gut Microbiota from Thai Subjects Using Quantitative Real-Time PCR

Using a qPCR, standard curves were created using genomic DNA extracted from the following bacterial cultures: *P. nigrescenes* JCM 12250T, *Bacteroides fragilis* ATCC 25285, *Bifidobacterium bifidum* JCM 1255, *S.* Typhimurium TISTR 292, *Blautia producta* JGD 07421, *C. leptum* DSM 753, and *L. casei* subsp. *rhamnosus* ATCC 7469. The seven bacterial groups found in the subjects were quantified using the log copy number/g of feces, as shown in Fig. 2. The results showed that *Prevotella*, the *C. coccoides-E. rectale* group, and Enterobacteriaceae were found mostly in the vegetarian subjects. Moreover, compared with the non-vegetarians, the numbers of *Prevotella* in the vegetarians (10.4–12.8 log copy number/g) were significantly higher (p = 0.005) than those in the non-vegetarians (8.4–9.6 log copy number/g). Among the six non-vegetarian subjects, subject N2 showed an exceptionally high number of *Prevotella* with a 12.2 log copy number/g.

In contrast, the non-vegetarian subjects showed higher numbers of *Bacteroides*, the *C. coccoides-E. rectale* group, and

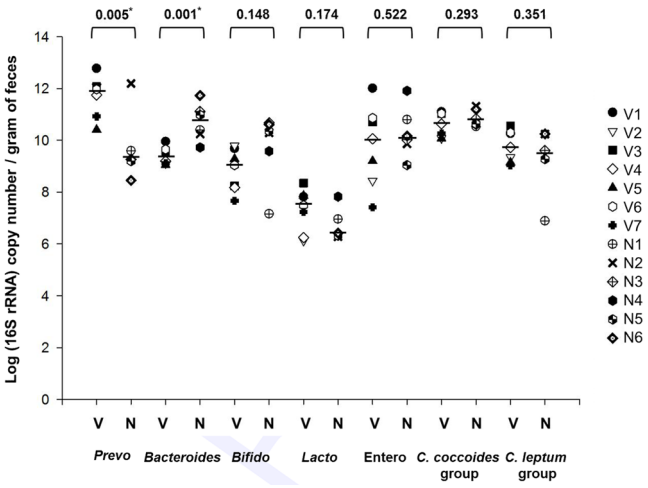

Fig. 2. Populations of bacterial groups in all subjects.
The copy number for each group was determined using a real-time PCR. The horizontal bars represent the median for the vegetarian (V) and non-vegetarian (N) groups. *Prevo*, *Prevotella* sp.; *Bacteroides*, *B. fragilis* group; *Bifido*, *Bifidobacterium* sp.; *Lacto*, *Lactobacillus* sp.; Entero, Enterobacteraceae, and *C. coccoides* group, *C. coccoides-E. rectale* group. Significant differences between the two subject groups were determined by a *t*-test, and the *p*-values are shown at the top of the graph.

Bifidobacterium. The numbers of *Bacteroides* in the non-vegetarians (9.7–11.7 log copy number/g) were significantly higher (p = 0.001) than those in the vegetarians (9.1–9.9 log copy number/g). As previously mentioned, subject N2 showed higher numbers of *Prevotella* and a high level of *Bacteroides* with a 10.2 log copy number/g. Meanwhile, subject N4 showed the highest abundance of Enterobacteriaceae with a 11.9 log copy number/g, but the lowest level of *Bacteroides* with a 9.7 log copy number/g.

No significant differences (p > 0.01) were found between the other bacterial groups studied. The amounts of Enterobacteraceae, *Lactobacillus*, *Bifidobacterium*, the *C. coccoides-E. rectale* group, and the *C. leptum* group did not differ significantly between the vegetarians and non-vegetarians. The copy numbers of *Lactobacillus* detected in the vegetarian subjects (6.1–8.4 log copy number/g) and non-vegetarian subjects (6.3–7.8 log copy number/g) were the lowest among all the specific bacterial groups determined.

Discussion

The microbiotas of two groups of Thai subjects, including seven vegetarians and six non-vegetarians, were first analyzed by PCR-DGGE, which resulted in the classification of clusters A and B that contained the non-vegetarians and

vegetarians, respectively. Subsequently, a qPCR was used for specific bacterial group quantification. From the results, cluster A contained *Bacteroides* as the major bacteria whereas cluster B contained *Prevotella* as the main bacteria. Matijašić *et al.*, [22] also used PCR-DGGE and a qPCR to study microbiotas in vegetarians and omnivores in Slovenia, which revealed a higher ratio of the *C. coccoides* group and *Bacteroides-Prevotella* group in the non-vegetarians. This study also found the *C. coccoides* group in the non-vegetarian subjects, but it was less abundant than the *Bacteroides* group. The different types of diet and lifestyle may have been the cause of these differences in the microbiota.

Cluster A consisted of two sub-clusters; sub-cluster A1 and A2. Subjects N1, N3, N4, N5, and N6 belonged to sub-cluster A1, and subject N2 belonged to sub-cluster A2. This may have been due to the singular presence of *Prevotella* in subject N2. *Prevotella* was also a typical bacteria for cluster B; the vegetarian group. The presence of *Prevotella* in subject N2 may have been due to the type of diet. From a personal interview, it was found that subject N2 consumed high amounts of fruit and vegetables (approx. 200 g/meal). Furthermore, although no *Bacteroides* was detected in subject N2 based on the PCR-DGGE results, the real-time PCR analysis revealed a 10.2 log copy number/g of feces. The inconsistency of these two techniques may have been due to the low identity of *Bacteroides* at 96%, which was not reported in the PCR-DGGE results. Therefore, the results indicated that the non-vegetarians tended to have a higher abundance of *Bacteroides* than the vegetarians. It was also noted that the PCR-DGGE patterns for subjects N3, N5, and N6 were similar. When these three subjects were interviewed, it was found that they always ate their meals together, which may have resulted in the similar detected patterns. Therefore, this observation implies that a similar food intake could be a major factor providing similar microbiota.

Cluster B was divided into two sub-clusters; B1 and B2. The abundant bacterium in this cluster analyzed by PCR-DGGE and qPCR was *P. copri*. *Prevotella* was previously observed in an agrarian society resident in USA [35]. In addition, a genome analysis of *P. copri* DSM 18305 has shown that this species contains cellulase and xylanase genes that may code the enzymes essential for the hydrolysis of cellulose and xylan from plant polysaccharides, respectively [26], which in turn may have been why *P. copri* was found mostly in the subjects who regularly consumed vegetables. Sub-cluster B1 consisted of subjects V3, V4, V5, V6, and V7, all of whom were lacto vegetarians living and working at a Thai vegetarian society, whereas sub-cluster B2 included subjects V1 and V2 who lived in different places. V1 and V2 were both lacto-ovo-vegetarians who consumed different amounts of eggs at 5–6 eggs/year and 2–3 eggs/week, respectively, whereas the other subjects in sub-cluster B1 did not include eggs in their meals. It was also found that *R. intestinalis* and *M. funiformis* were found only in subjects V1 and V2, respectively. However, owing to the low number of subjects analyzed, it was difficult to conclude that egg consumption had any effect on the bacterial types. Thus, a more extensive study is needed to clarify the effect of eggs on the microbiota.

The most prolific genera found in the Thai subjects were *Bacteroides* and *Prevotella*, both of which have already been reported as the genera usually present in the human gut, irrespective of nation or continent [1]. The present results are also consistent with the work of De Filippo *et al*. [8] and Wu *et al*. [34]. De Filippo *et al*. [8] reported that *Prevotella* was the most prolific genus in the gut microbiota of African children from Burkina Faso, where the children were predominantly vegetarians, whereas *Bacteroides* was the most abundant genus detected in the gut microbiota of European non-vegetarian children living in urban Florence, Italy. Wu *et al*. [34] also studied the link between long-term dietary patterns and gut microbiota, and found that the *Bacteroides* enterotype was highly associated with protein and animal fat, whereas the *Prevotella* enterotype was strongly associated with carbohydrate-enriched diets. Moreover, Claesson *et al.* [5] investigated the gut microbiota composition in elderly subjects and reported that healthy community-dwelling subjects consuming low fat/high fiber and moderate fat/high fiber had significantly abundances of *Prevotella* and *Ruminococcus*, whereas most of the long-term resident-care subjects consuming moderate fat/low fiber and high fat/low fiber had a high abundance of *Alistipes*, *Oscillibacter*, and *Bacteroides*. However, the present results are in contrast to the reports of Zimmer *et al*. [36] and Liszt *et al*. [17], which found that *Bacteroides* was the largest genus present in fecal samples of vegetarians. This inconsistency in the results could be attributed to differences in the study methods. Zimmer's study [36] focused on the detection of *Bacteroides* and other bacteria, but not *Prevotella*, and used a culture-based method with different selective media, including U3G agar, Rogosa agar, DIC agar, and SPM agar. As a result, no *Prevotella* was detected in their study. Notwithstanding, their results did show a significantly lower *Bacteroides* count in vegetarians when compared with the control group. In the case of Liszt *et al*. [17], the primers used for the PCR-DGGE and quantitative PCR were specific to *Bacteroides* sp. and *Clostridium* sp.

rather than *Prevotella*, and consequently no *Prevotella* was observed. Furthermore, Kabeerdoss *et al*. [16] quantified the fecal microbiota in vegetarian and omnivorous young women in southern India and reported that the microbial communities, especially the *Bacteroides-Prevotella* group, were similar in both the vegetarians and non-vegetarians. Although these results are different from the current findings, this could be explained by the specificity of the primers used. The primers used by Kabeerdoss were specific to both *Bacteroides* and *Prevotella*, whereas the primers used in the real-time PCR in the present study were only specific to either *Bacteroides* or *Prevotella*.

Since diet, microbiota, and the occurrence of disease are already know to be linked, dietary modulation studies could provide valuble information for understanding diet-microbiota-health [15]. Moreover, the manipulation of microbiotas could be useful for medical applications. One report has suggested that the abundance of *Bacteroides* and *Prevotella* may be useful as a prognostic biomarker of disease [33]. Thus, the *Bacteroides* and *Prevotella* counts related to different diets of the Thai subjects could provide important data for predicting the health condition of South East Asians with similar eating styles. Therefore, more studies are needed on the interaction of diet modulation, the microbial community, and the occurrence of disease.

In conclusion, the present results revealed different tendencies for the microbiota in the non-vegetarians and vegetarians. The non-vegetarians tended to have a higher abundance of *Bacteroides*, whereas the vegetarians had a higher abundance of *Prevotella*. The numbers of *Bifidobacterium* sp., *Lactobacillus* sp., Enterobacteraceae, and the *C. coccoides-E. rectale* group did not differ significantly between the two groups.

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