Gut microbiota of Thai volunteer fecal samples by using quantitative PCR

Vasana Jinatham[1], Niwed Kullawong[2], Ekachai Chukeatirote[1], Kongkiat Kespechara[3], Siam Popluechai[1*]

[1]*School of Science, Mae Fah Luang University, Thasud, Chiang Rai 57100, Thailand*
[2]*School of Health Science, Mae Fah Luang University, Thasud, Chiang Rai 57100, Thailand*
[3]*Sooksatharana (Social Enterprise) Co., Ltd, 88/9, Muang, Phuket 83000, Thailand*
[*]*e-mail: siam@mfu.ac.th*

Abstract

Human gut microbiota are diverse and different among individuals. It has been reported that composition of gut microbiota related to the host physiological conditions and diseases such as obesity, diabetes.Therefore, our study aimed to characterize the composition of gut microbiota including *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria* from fecal samples obtained from 14 Thai volunteers by using qPCR. The volunteers were classified into two groups according to body mass index (BMI),: lean group (BMI≤23) and overweight group (BMI>23). The results showed that relative abundance of *Firmicutes* was dominated in the Lean group whereas those of *Bacteroidetes* and *Proteobacteria* were dominant in the Overweight group. Interestingly, BMI was linearly associated with the presence of the *Proteobacteria*.

Keywords: Gut microbiota, BMI, qPCR, Thai volunteer, fecal sample

Introduction

Human gastrointestinal tract is colonized with various anaerobic bacteria. The bacterial number in colon human is collectively up to 10^{14} cells (Ley et al., 2006). The *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* are dominated in the human gut. Colonic bacteria are important for breaking undigested dietary complex carbohydrates, proteins and fats, fermenting to short chain fatty acids, and synthesis of vitamins. The *Firmicutes* and *Bacteroidetes* are the two most abundant phyla whereas other phyla are *Actinobacteria* and *Proteobacteria* (which includes *Enterobacteriaceae*) (Eckburg et al., 2005). Differences of colonic bacterial compositions can be diverse depend on several factors such as body-mass index, gender, nationality, age and enterotypes. However, it is mostly dependent to dietary intake and the colonic health of the host. In some physiological conditions, the composition of gut bacteria can indicate the colonic health as well as the host health. In Thailand, Ruengsomwong *et al* (2014) found that *Bacteroides* was major abundance in the fecal samples of non-vegetarian whereas *Prevotella* was the major abundance in fecal samples of the vegetarian. Here we aimed to investigate for microbial community inside the gut of Thai healthy volunteers by using qPCR analysis.

Methodology

Volunteers, sample collection and DNA extraction

Four-teen healthy volunteers were recruitedaccording to the MFUhuman ethics license number REH57027. Height, weight, age and genderwere recorded.In addition, all the

volunteers were not restriced for their diet types before sample collection and not administered with antibiotic 2 months before the sample colection. Fecal samples were obtained and total genomic DNAs were extracted from the samples (~0.2 to 0.4 g) by the innuPREP Stool DNA Kit (Analytikjena Biometra). All the extracted DNAwere stored at -20ºCuntil futher use.

Quantitative polymerase chain reaction (qPCR)

Amplification condition of 16S ribosomal RNA genes for each set of primers (Univ-180, Univ-200, Univ-400, *Bacteroidetes, Firmicutes, Actinobacteria*, and *α-* and *γ-Proteobacteria*) was optimized for high specificity and high efficiency using pooled DNA templates (Table 1).Amplification of the 16S rRNA genes was then amplified in a 10 µL reaction containing (100 or 200 µM of each primer, 4 ng of DNA template , SensiFAST^{TM} SYBR No-ROX mix (BIOLINE), and sterile water. Cycling condition used was as follows: a primary denaturation at 95ºC (30 min), 40 cycles of denaturation at 95ºC (5 sec), annealing at each primer Tm (10 sec), extension at 72ºC (20 sec) and melt analysis at 65–95ºC (5 sec/0.5ºC) by using CFX Manager Software in C1000 Touch™ Thermal Cycler machine (Bio-Rad). qPCR efficiency for each primer set was obtained from three points of 10-fold dilutions using pooled samples as a template (40, 4 and 0.4 ng of DNA per reaction were used in duplicate). After each qPCR run, threshold cycle (C_T) was analyzed. The C_T data were ploted as a graph with linear regression fit and the slope and efficiency (E) were calculated. To preciously quantify the total population of all bacteria, geometrical mean of the universal primers was used. Finally, relative quantification analysis was calculated and expresed as proportion (%) (De Gregoris et al., 2011).

Statistical analysis

Statistical analysis was conducted by using SPSS software program (Order: 10-58878). Pearson's correlation was used to measure the linear correlation between parameters at 95% confident interval, significance level $P \leq 0.05$. Independent-samples t-test was performed to determine the difference of mean and Mann-Whitney U-Test was used to determine the difference of the bacterial relative abundance between groups.

Table 1: Primers used in this study

Target phylum/specificity		Primer sequence	Product size (bp)	Tm (ºC)	References
Bacteroidetes		F: CRAACAGGATTAGATACCCT R: GGTAAGGTTCCTCGCGTAT	240	64	(De Gregoris et al., 2011)
Firmicutes		F: TGAAACTYAAAGGAATTGACG R: ACCATGCACCACCTGTC	200	64	
Actinobacteria		F: TACGGCCGCAAGGCTA R: TCRTCCCCACCTTCCTCCG	300	64	
Proteobacteria	*α -proteobacteria*	F: CIAGTGTAGAGGTGAAATTC R: CCCCGTCAATTCCTTTGAGTT	250	60	
	γ- proteobacteria	F: TCGTCAGCTCGTGTYGTGA R: GGTAAGGGCCATGATG	122	60	
All bacteria	Universal 180	F: AAACTCAAAKGAATTGACGG R: CTCACRRCACGAGCTGAC	180	49	
	Universal 200	F: ACTCCTACGGGAGGCAGCAG R: ATTACCGCGGCTGCTGG	200	63	(Fierer et al., 2005)
	Universal 400	F: ACTCCTACGGGAGGCAGCAG R: GGACTACCAGGGTATCTATCCTGTT	449	64	(Nadkarni et al., 2002)

Results

Weight and height of the volunteers were calculated for BMI. The volunteers were classified into two groups according to their BMI, Table 2. The result showed that age and height between the two groups were not significantly different (P>0.05) whereas weight was significantly different (P<0.05). As expectedly, the BMIs between the two group were significantly different (P<0.05) (Figure 2A).

Table 2: Basic information and classification of the volunteers

Group	Age (year)	Weight (kg)	Height (cm)	Male	Female	Total
Lean (BMI≤23)	24.17±4.8	52.5±6.6*	163.7±7.6	2	4	6
Overweight (BMI>23)	26.25±4.8	75.0±17.1*	163.9±7.9	5	3	8
Total	25.36±4.7	65.4±17.6	163.8±7.5	7	7	14

*Mean are different between two groups at significant level (P<0.05) by Independent-samples t-test.

To observe the diversity of gut microbiota composition among the unclassified volunteers, the relative abundance of the detected bacterial phyla was shown as bar graph (Figure 1). The composition of gut microbiota were diverse among the volunteers that presence ofthe Firmicutes was positively correlated with the *Bacteroidetes* at the significant level (r=0.611, P<0.05) (Table 3). Whereas the relative abundance of *Bacteroidetes* was negatively associated with the *Actinobacteria* (r=-0.430, P>0.05). Interestingly, the presence of the *Proteobacteria* was positively correlated with the BMI value (r=0.755, P<0.01) but not with other phyla.

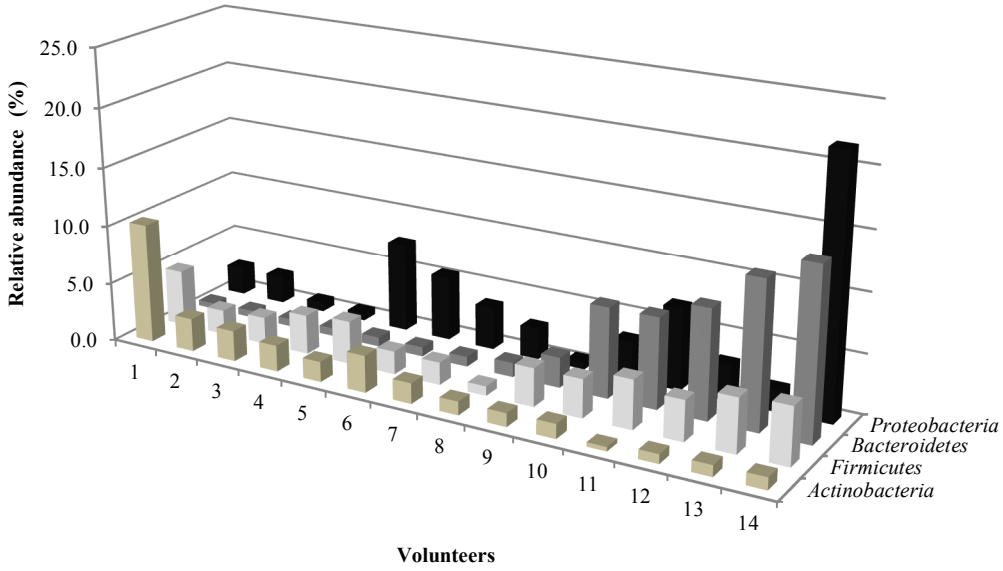

Figure 1: The four phyla of gut microbiota among 14 Thai volunteers

The results were ranged according to the relative abundance (%) of *Bacteroidetes*. *Proteobacteria* data are accumulative value of *α-proteobacteria* and *γ- proteobacteria* primers.

Table 3: Pearson's correlation

	BMI	*Actinobacteria*	*Firmicutes*	*Bacteroidetes*	*Proteobacteria*
BMI	1.000	-0.112	-0.180	0.015	0.755**
Actinobacteria		1.000	0.195	-0.430	0.044
Firmicutes			1.000	0.611*	-0.093
Bacteroidetes				1.000	-0.103
Proteobacteria					1.000

** Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

The distribution of the *Actinobacteria* in the Lean was higher than the Overweight (Figure 2B). However, their relative abundance was not significantly different (P>0.05) in both Lean and Overweight. In addition, distribution and relative abundance of the *Firmicutes* were detected in comparable level between the two groups (Figure 2C). Interestingly, distributions and relative abundances of the *Bacteroidetes* was higher in the Overweight (Figure 2D).

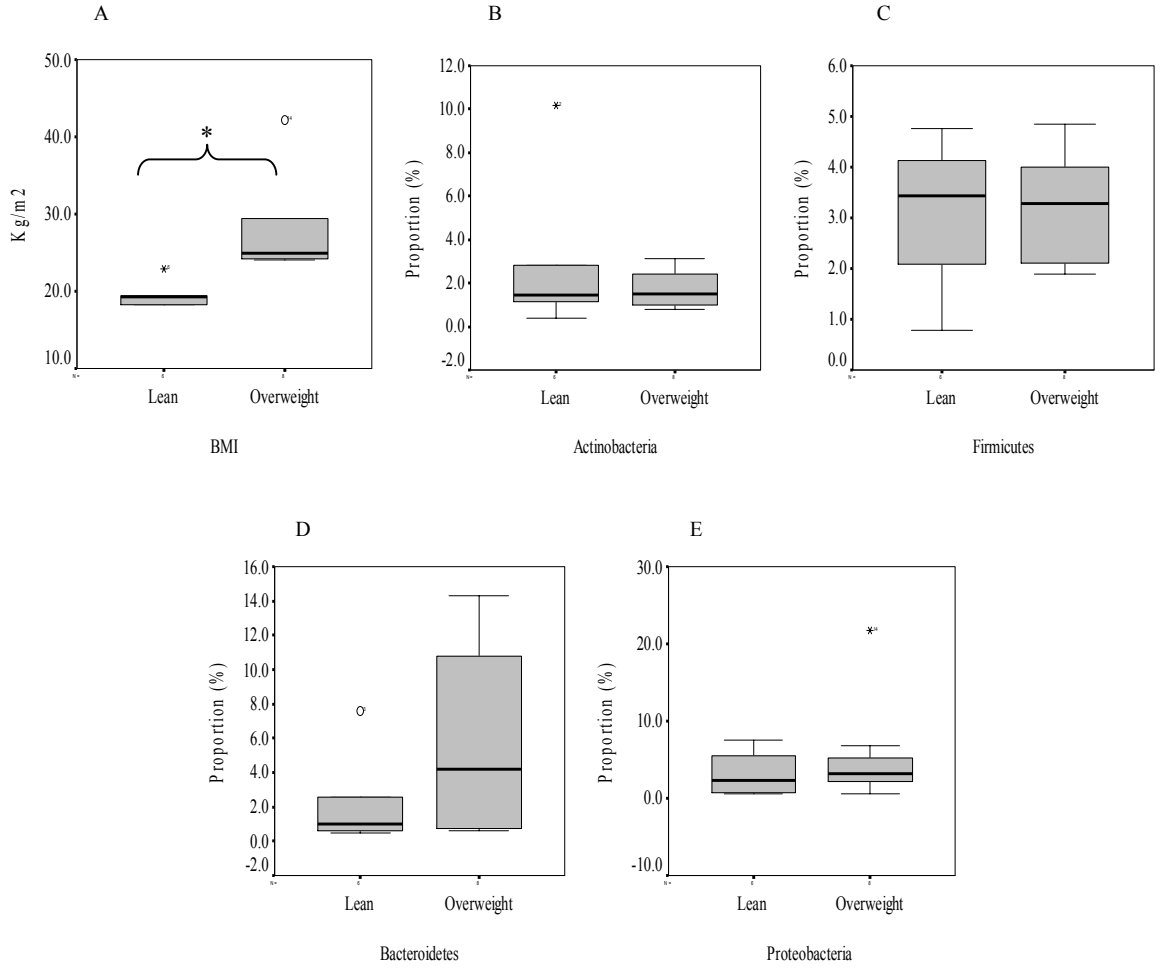

Figure 2: The distribution of phylum relative abundance of 14 Thai volunteers Asterisk (*) indicates the mean of BMI between the two groups are different (P<0.05). For bacterial relative abundance, distributions are not significantly difference (P>0.05) by Mann-Whitney U-Test.

Discussion

In this study, compositions of bacteria colonized in the fecal samples of the healthy volunteers were quantified based on the 16S rRNA analysis. We focused on using a set of primers targeting to the phylum level to observe an overview of the phylum distribution among individuals and between the two different ranges of BMI (Lean and Overweight). Without grouping we found a positive association of the *Firmicutes* and *Bacteroidetes* and they were the two most abundant phyla which were corresponded to previous reported by Ley, et al (2006). This might be the result of co-domination of some groups in the phyla that each other play an important role in exchange of cross-feeding substrates. For example, some *Bacteroides spp*. can degrade amylose and amylopectin to simple sugar (glucose) that provide carbon source for other bacteria. A result was supported by our published data that composition of *Fecalibacterium prausnitzii* (Popluechai et al., 2014), a number of *Firmicutes*, was positively correlated with the *Bacteroidetes* composition in this study. The results also showed that *Actinobacteria* (mostly probiotic *Bifidobacteriam* spp.) dominated in the Lean group than the Overweight which corresponded to the report by Parnell & Reimer (2012). Interestingly, we found that the abundance of potentially pathogenic *Proteobacteria* was positively correlated to the BMI instead of other bacterial phyla. Most of the *Proteobacteria* membersare Gram-negative bacteria whose contain lipopolysaccharide (LPS) as an essential cell wall component. Interestingly, the LPS was recently reported as endotoxin released from the gut bacteria that triggered glycemia, insulinemia and weight gain of whole-body, liver, and adipose tissue in mice (Caniet al., 2007). In addition, the *Bacteroidetes* was also members of the Gram-negative bacteria that dominated in the Overweight group as reported in this study.

Conclusion

We have demonstrated that our quantification system by qPCR had a reliable capacity for analysis of gut microbiota composition in the observed population. This is possible to apply the detection system for study with more specific groups of population such as obese, diabetic and colon cancer volunteers in the near future.

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