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Comparison of Gut Microbiota between Lean and Obese Adult Thai Individuals

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Current reports suggest that obesity is a serious global health issue. Emerging evidence has predicted strong links between obesity and the human gut microbiota. However, only a few such studies have been conducted in Asia, and the gut microbiota of lean and obese adult Asians remains largely unexplored. Here, we investigated the potential relationship between gut microbiota, body massindex (BMI), and metabolic parameters in adults from Thailand, where obesity is increasing rapidly. Fecal and blood samples were collected from 42 volunteers who were allocated into lean, overweight, and obese groups. The fecal microbiota was examined by quantitative PCR analysis. Bacteroidetes, Firmicutes, and *Staphylococcus* spp. and methanogens were most abundant in lean volunteers. Overweight volunteers majorly harbored *Christensenella minuta* and *Akkermansia muciniphila*, γ-Proteobacteria, and bacteria belonging to the genus *Ruminococcus*. Methanogens and bacteria belonging to the phylum Bacteroidetes were negatively correlated with adiposity markers (BMI and waist circumference), but positive correlated with high-density lipoprotein, suggesting that they can be used as leanness markers. While some of our results agree with those of previous reports, results regarding the contributions of specific taxa to obesity were inconsistent. This is the first study to report the adult gut microbiota in Southeast Asian populations using molecular techniques and biochemical markers and provides a foundation for future studies in this field.

Keywords: **Fecal gut microbiota, Thailand, obesity, quantitative PCR**

Introduction

Obesity is defined as the accumulation of adipose tissue in the body and is often associated with negative effects including increased levels of cholesterol, triglycerides, as well as, hypertension and insulin resistance [1, 2]. Though obesity itself is considered a non-communicable disease (NCD) [3], it is also a major risk factor for other NCDs including cardiovascular diseases, cancer and type 2 diabetes [4]. The global rate of obesity has doubled since 1980, thus obesity is now considered an epidemic, consequently affecting both industrialized and non-industrialized nations [5]. Imbalance in caloric intake is thought to be one of the primer drivers of obesity, but genetic, environmental and socio-economic factors also have a major influence [6]. Recent studies, mainly focused in western nations, have revealed that the human gut microbiota, a previously overlooked factor, is at play.

The gut microbiota is the collection of eukaryotic and prokaryotic microorganisms forming a complex ecosystem in the intestinal tract. The gene complement of these microbes exceeds that of humans by at least 100-fold and contributes to host physiological and biochemical processes, the best known being fermentation of fiber

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and vitamin biosynthesis [7, 8]. Increasingly, studies demonstrate pivotal roles of gut microbiota in health and disease, while roles of microbial communities to energy homeostasis and adipose tissue formation are being uncovered [4, 9–12]. Though the scientific community has reached a consensus concerning the role of gut microbiota in obesity development, the roles of specific taxa remain elusive and sometimes contradictory despite considerable research effort [9, 13]. For instance, the Bacteroidetes/Firmicutes ratio was initially proposed as an indicator of obesity, but this was later disputed [14, 15].

Nonetheless, associations of other taxa with obese or lean phenotypes seem to be somewhat more consistent, albeit with exceptions. Abundance of Bacteroidetes is often associated with microbiota of lean people [16] with one exception [14], whereas increased *Bacteroides*, *Staphylococcus* and *Clostridium* have been linked to overweight subjects [11]. Along the same lines, *Faecalibacterium prausnitzii* has been found in significantly increased amounts in obese children [17]. Butyrate-producing bacteria along with *Bifidobacterium* and *Methanobrevibacter* are often associated with the lean phenotype [14, 15, 18]. Recently, a mucus-residing bacterium, *Akkermansia muciniphila* was linked to low body weight in both rodents and humans and emerging evidence suggests that it can be used as an indicator species of healthy mucus [19]. A recent study examining 977 twin volunteers showed that *Christensenella minuta*is associated lower body mass index [20].

However, a notable oversight of the above-mentioned studies has been that most of them have focused on populations from the Americas and Europe with only comparably few coming from Asia. Thus, it is not yet clear how the microbiota patterns of obese adult Asian populations differ from those of obese Americans and Europeans. Though some data are available from countries such as Russia, China and Japan [21–23], none exists for adult populations of Southeast Asia including Thailand.

Thailand is a gastronomically distinct country that has traditionally been considered a nation of "lean people". Nonetheless, this is rapidly changing: the current prevalence of overweight and obesity in Thai adults is 32.2%, which is the second highest in Southeast Asian nations [24]. This increase is concerning because Southeast Asian populations are particularly susceptible to abdominal obesity, which is related to insulin resistance. In the present pilot study we investigated the prokaryotic gut microbiota of subjects from Chiang Rai, Northern Thailand using quantitative PCR (qPCR). We used seventeen pairs of primers to quantify specific prokaryotic taxa that have been previously linked to obesity. We also examined relationships between these taxa, BMI and blood profiles.

Materials and Methods

Ethics statement

The human trials of the present study were approved by the Human Ethics Committee of Mae Fah Luang University (License approval number REH57027). Each volunteer signed an informed consent before participating in the study.

Human subjects

All volunteers were Asian and lived in Chiang Rai Province, Thailand at the time of sampling. In total, 42 volunteers participated in the study ranging from 20 to 49 years old. The volunteers had no history of acute or chronic inflammatory disease, no diarrhea episodes one month prior to sampling and had received no antibiotic treatment at least two months before sample collection. The volunteers were classified into lean, overweight and obese groups according to BMI based on the criteria of the WHO for adults <25, 25–30 and >30 kg/m^2 respectively (Table 1).

Anthropometric measurements and metabolic variables

Height (m), weight (kg) and waist circumference (inches) were measured by standard instruments. Systolic (SBP) and diastolic blood pressure (DBP) was measured twice and the blood profiles of the volunteers were assessed. Blood samples were collected in the morning, after fasting for at least 8 hours. Levels of fasting blood sugar (FBS) and lipid profiles – total triglycerides (Tg); total cholesterol (Chol); high-density lipoprotein cholesterol (HDL-c); and low-density lipoprotein cholesterol (LDL-c) – were measured at Mae Fah Luang University Hospital medical laboratory on the day of collection.

Table 1. Physical characteristics of the 42 Thai volunteers used in this study. The difference of mean for each characteristic among groups was calculated using ANOVA, with the exception of number of subjects, which was calculated using χ^2 test. Superscript letters denote comparisons between groups, which were calculated by using Independent Sample *t*-test

Characteristic	Total	Lean	Overweight	Obese	*p* value
Number of subject, n (%)	42 (100)	21 (50.0)	10 (23.8)	11 (26.2)	0.071
Female	28 (66.6)	15 (35.7)	6 (14.3)	7 (16.7)	0.795
Male	14 (33.4)	6 (14.3)	4 (9.5)	4 (9.5)	
Age (years, mean ± SD)	27.60 ± 1.31	27.71 ± 1.93	26.40 ± 2.75	28.45 ± 2.46	0.859
BMI (kg/m^2, mean ± SD)	25.64 ± 0.92	20.66^{a,b} ± 0.43	27.38^{a,c} ± 0.55	33.56^{b,c} ± 0.97	$p < 0.000$
Waist (inch, mean ± SD)	34.60 ± 0.87	30.12^{a,b} ± 0.60	36.85^{a,c} ± 0.86	41.09 ^{b,c} ± 1.22	$p < 0.000$

^{a}Lean < Overweight ($p < 0.05$), ^{b}Lean < Obese ($p < 0.05$), ^{c}Overweight < Obese ($p < 0.05$).

Stool collection and DNA extraction

Fecal samples were collected from the volunteers using sterile containers, divided in aliquots, weighed (g) and stored at −80℃. Total DNA was isolated from the samples by using the innuPREP Stool DNA Kit (Analytik Jena AG, Germany) according to manufacturer's specifications. The quantity and quality of the DNA was assessed by spectrophotometry (NanoDrop, Thermo Scientific™, USA) and gel electrophoresis. The extracted DNA samples were standardized to a final concentration of 2 ng/μl. Total genomic DNA was also extracted from pure bacterial cultures for subsequent use as qPCR standards.

Primer information

The *Staphylococcus*, *Prevotella* and *Christensenella minuta* sets of primers were newly designed for this study (Table 2). Primers used in previous studies to detect *Staphylococcus* amplified the *nuc* gene rather than the 16S rDNA [25]. Similarly, *Prevotella* primers used in previous studies also amplified *Bacteroides* and *Porphyromonas*. The *Staphylococcus* and *Prevotella* pairs of primers were designed based on multiple sequence alignments of available sequences from GenBank. The species-specific *C. minuta* primer set was designed based on the available 16S rDNA gene (Accession number NR_112900.1). Primer specificity was checked against pooled fecal samples. Identification of amplicons was confirmed by sequencing and by performing BLAST searches against the NCBI database.

In total, the 16S rRNA gene of 17 prokaryotic taxa was amplified (Table 2). Primers covered conserved regions for various taxon levels ranging from the highest (total bacteria) to the lower (genus and species).

Absolute quantitative real time PCR

Gradient analysis was performed in order to optimize temperature of PCR reactions. Temperatures with specific amplification were deemed appropriate for further experiments. Gel electrophoresis was used to check size and purity of amplicons. Standard curves were established for each pair of primers by using the sequenced plasmid templates of known quantities. A set of standards was generated by making a series of five 10-fold dilutions covering the range of concentrations corresponding to the observed cycles threshold (Ct) of the samples. All the recombinant plasmid templates were bidirectionally sequenced (First Base, Malaysia). The concentration of the digested qPCR plasmid template was quantified by using gel electrophoresis and NanoDrop spectrophotometer.

Reactions for each template were carried out in triplicate. The total reaction mix for each sample was 10.0 μl containing 1X SensiFAST™ SYBR No-ROX Kit (BIOLINE, UK) Reagent mix, 100–200 nM of each forward and reverse primer and 4 ng of genomic DNA template. Reactions were run in 96-well plates in a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, USA). Cycling conditions were as follows: a polymerase activation cycle at 95.0℃ for 2.0 min, 40 cycles of denaturation at 95.0℃ for 5 sec, annealing at the indicated Ta (Table 2) for 10 sec and extension at 72.0℃ for 20 sec. At the end of every run, the product specificity was assessed by melting-curve analysis after denaturation at 95.0℃ for 10 sec and slowly heating the mixture from 65.0℃ to 95.0℃ with plate readings every 0.5℃ for 5 sec. A negative control was used for every run in which 2.0 μl of dH_2O were used instead of DNA template. For the absolute quantification, the obtained Ct

Table 2. List of primers used in this study.

Targeted prokaryotic taxa	Primer set	Primer sequence (5'→3')	Amplicon size (bp)	Ta (℃)	Reference/ Importance
Total bacteria	Uni926F	F: AAACTCAAAKGAATTGACGG	180	60	[41]
	Uni1062R	R: CTCACRRCACGAGCTGAC			
Phylum Bacteroidetes					
Bacteroidetes (P)	798cfbactF	F: CRAACAGGATTAGATACCCT	240	64	[41]/[a]
	cfbact967R	R: GGTAAGGTTCCTCGCGTAT			
Bacteroides spp. (G)	Bac303F	F: GAAGGTCCCCCACATTG	103	64	[42]/[a]
	Bfr-Fmrev	R: CGCKACTTGGCTGGTTCAG			[43]/[a]
Prevotella spp. (G)	Prov_F1	F: GCCGCGGTAATACGGAAGG	271	56	This study/[a]
	Prov_R1	R: CTAATCCTGTTYGATACCCGCAC			
Phylum Firmicutes					
Firmicutes (P)	928F-Firm	F: TGAAACTYAAAGGAATTGACG	200	64	[41]/[d]
	1040FirmR	R: ACCATGCACCACCTGTC			
Roseburia spp. &	RrecF	F: GCGGTRCGGCAAGTCTGA	80	55	[44]/[d]
Eubacterium rectale (G)	Rrec630mR	R: CCTCCGACACTCTAGTMCGAC			[43]/[d]
Ruminococcus spp. (G)	Rflbr730F	F: GGCGGCYTRCTGGGCTTT			
	Clep866mR	R:CCAGGTGGATWACTTATTGTGTTAA	156	63	[43]/[d]
Lactobacillus spp. (G)	Rin_LactF	F: AGCAGTAGGGAATCTTCCA	341	56	[45]/[d]
	Rin_LactR	R: CACCGCTACACATGGAG			[46]/[d]
Enterococcus spp. (G)	Rin_EntF	F: CCCTTATTGTTAGTTGCCATCATT	144	56	[47]/[d]
	Rin_EntR	R: ACTCGTTGTACTTCCCATTGT			
Staphylococcus spp. (G)	N-StaphF2	F: CGTCTTGACGGTACCTAATC	237	60	This study/[d]
	N-StaphR2	R: CTCCATATCTCTGCGCATTTC			
Oscillospira spp. (G)	OSCI-RV-Fmod	F: ACGGTACCCCTTGAATAAGCC	359	64	[48][d]
	OSC-808mod	R: TCCCCGCACACCTAGTATTG			[49][d]
Faecalibacterium prausnitzii (S)	FPR-2F	F: GGAGGAAGAAGGTCTTCGG	247	63	[50][d]
	Fprau645R	R: AATTCCGCCTACCTCTGCACT			[43]/[d]
Christensenella minuta (S)	ChrisM_F1	F: GTAATACGTAGGGAGCAAGC	145	55	This study/[d]
	ChrisM_R1	R: CCCTCTCCTGTACTCAAGTC			
Actinobacteria phylum					
Actinobacteria (P)	Act920F3	F: TACGGCCGCAAGGCTA	170	64	[41]/[b]
	Act1200R	R: TCRTCCCCACCTTCCTCCG			
Proteobacteria phylum					
γ-Proteobacteria (G)	1080γF	F: TCGTCAGCTCGTGTYGTGA	170	53	[41]/[c]
	γ1202R	R: CGTAAGGGCCATGATG			
Fusobacteria phylum					
Fusobacterium spp. (G)	FussoF1	F: CGGGTGAGTAACGCGTAAAG	204	55	[51]/[c]
	FussoR1	R: GCCGTGTCTCAGTCCCCT			
Verrucomicrobia phylum					
Akkermansia muciniphila (S)	AkMF1	F: CAGCACGTGAAGGTGGGGAC	349	60	[52]/[e]
	AkMR1	R: CCTTGCGGTTGGCTTCAGAT			
Archaea					
Methanobacteria	Met630F	F: GGATTAGATACCCSGGTAGT	190	56	[53]/[f]
(Methanogens)	Met803R	R: GTTGARTCCAATTAAACCGCA			

P = Targeted bacterial phyla, G = Targeted bacterial genus/group, S = Targeted bacterial species, [a] = Associated to obesity. [b] = Beneficial microbe, [c] = Pathogenic bacteria/associated to obesity, [d] = Associated to short chain fatty acid (SCFAs) production/obesity, [e] = Mucin degrading/associated to obesity, [f] = methane producing/associated to obesity.

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were subsequently quantified against the corresponding standard curve and converted to the unit of copy number per ng of DNA template (copy/ng DNA). For the comparison among the volunteers, copy/ng DNA was multiplied by the concentration of bacterial DNA per wet weight of fecal sample (ng DNA/g fecal). The final concentration is reported as copy/g of fecal sample.

Data normalization and statistical analyses

Statistical analyses were performed with the statistical software package version 21.0 (SPSS Inc., Chicago, IL, USA). Initially, we demonstrated the subject characteristics (independent variables) with descriptive statistics (mean, standard deviation – SD, median and range). Distribution of the data for each variable was evaluated by using the Kolmogorov-Smirnov test. Anthropometric and blood profile variables were normally distributed thus they were analyzed by parametric statistics. The exception was gender, which had non-parametric distribution and was analyzed using χ^2 test. Differences among groups were calculated using One Way ANOVA, whereas for differences between two groups Independent Sample *t*-test was employed. The rest of the variables were analyzed by nonparametric statistics. Difference of median values was compared by Kruskal Wallis H (among BMI categories) and Mann-Whitney U (between 2 categories) tests. Significance level (α) was set at 0.05 in all cases. Correlation between variables was analyzed by Pearson's correlation (r) at a minimum of 95% confidence interval.

Results

Quantification of prokaryotic taxa in fecal samples of Thai volunteers

Waist circumference, BMI, SBP and FBS differed significantly among the three groups (Table 3). Levels of Tg and HDL-c were higher in the obese subjects when compared to the overweight and lean volunteers respectively.

Prevalence and quantification of prokaryotic groups in lean, obese and overweight groups as determined by qPCR are depicted in Table 4 and Fig. 1. Bacteroidetes, Firmicutes, *Staphylococcus* spp., *Akkermansia muciniphila* and methanogens were significantly higher in lean volunteers compared to the obese, whereas *Ruminococcus* spp., *Christensenella minuta*, γ-Proteobacteria and *A. muciniphila* were more abundant in overweight as opposed to obese individuals.

Correlation of prokaryotic microbiota to anthropometric and metabolic parameters

Significant correlations between BMI and prokaryotic components of gut microbiota are found in Fig. 2. A detailed account of all correlations can be seen in supplementary Table S1. Bacteroidetes, Firmicutes, *Staphylococcus* spp., *A. muciniphila* and methanogens had a significant negative correlation with BMI and waist circumference. *Oscillospira* and Actinobacteria were negatively correlated to cholesterol and LDL-c, both of which are indicators of obesity. In contrast, Bacteroidetes, *Staphylococcus* and methanogens had a positive correla-

Table 3. Blood pressure and biochemical parameters of 42 Thai volunteers. The difference of mean for each characteristic among groups was calculated using ANOVA, while the mean between groups was calculated by using Independent Sample *t*-test.

Blood profile	Total (*n* = 42)	Lean (*n* = 21)	Overweight (*n* = 10)	Obese (*n* = 11)	*P* value
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
SBP (mmHg)	116.76 ± 2.70	109.00[b,c] ± 3.59	121.80[b,d] ± 4.15	127.00[c,d] ± 5.18	0.010
DBP (mmHg)	73.29 ± 1.71	70.62 ± 2.28	75.80 ± 3.65	76.09 ± 3.49	0.302
FBS (mg/dl)	87.31 ± 1.16	84.38[b,c] ± 1.28	89.60[b,d] ± 2.36	90.82[c,d] ± 2.61	0.034
Triglyceride (mg/dl)	102.51 ± 7.45	86.62[c] ± 9.79	113.8 ± 19.90	122.58[c] ± 8.88	0.092
Cholesterol (mg/dl)	204.48 ± 4.85	199.05 ± 7.38	205.70 ± 4.74	213.75 ± 11.28	0.460
LDL-c (mg/dl)	131.82 ± 4.96	125.67 ± 7.47	135.50 ± 5.38	140.24 ± 11.53	0.448
HDL-c (mg/dl)	59.08 ± 2.26	64.43[a] ± 3.58	55.20 ± 4.04	52.38[a] ± 2.55	0.051

[a]Lean < Overweight ($p < 0.05$), [b]Lean < Obese ($p < 0.05$), [c]Overweight < Obese ($p < 0.05$).
SBP; Systolic blood pressure, DBP; Diastolic blood pressure, FBS; Fasting blood sugar, LDL-c; low-density lipoprotein cholesterol, HDL-c; High-density lipoprotein cholesterol.

Table 4. Prevalence and microbial abundance of gut microbiota investigated by quantitative PCR. *P* values among BMI groups were calculated using Kruskal Wallis Test. Comparisons between groups were calculated using Mann-Whitney U test. Asterisks denote significance at 0.05.

Bacterial groups		All data (*n* = 42)	LN (*n* = 21)	OV (*n* = 10)	OB (*n* = 11)	*P* value	Mann-Whitney U		
		Median	Median	Median	Median	Kruskal Wallis Test	LN-OV	LN-OB	OV-OB
Total bacteria	Universal-180	9.73	9.77	9.65	9.08	0.201	0.291	0.092	0.526
Bacteroidetes	Bacteroidetes (P)	9.12	9.42	8.9	8.18	0.016*	0.009*	0.037*	0.778
	Bacteroides spp. (G)	9.68	9.67	9.69	9.19	0.772	0.735	0.463	0.833
	Prevotella spp. (G)	9.44	9.6	9.95	9.25	0.725	0.422	0.953	0.573
Firmicutes	Firmicutes (P)	7.97	8.09	7.98	7.68	0.127	0.447	0.041*	0.324
	Roseburia spp.-E. *rectale* (G)	9.03	9.03	9.24	8.9	0.583	0.447	0.736	0.231
	Ruminococcus spp.	8.89	8.92	9.08	8.63	0.19	0.237	0.677	0.035*
	Lactobacillus spp. (G)	6.13	6.18	6.19	6.02	0.827	0.642	0.592	0.944
	Enterococcus spp. (G)	7.07	6.86	7.14	7.17	0.595	0.447	0.372	0.944
	Staphylococcus spp. (G)	1.97	2.73	2.25	1.44	0.069	0.527	0.034*	0.078
	Oscillospira spp. (G)	5.33	5.67	5.36	4.39	0.258	0.554	0.137	0.205
	Faecalibacterium prausnitzii (S)	8.5	8.5	8.72	7.94	0.451	0.375	0.487	0.26
	Christensenella minuta (S)	8.01	7.92	8.36	7.8	0.037*	0.091	0.351	0.006*
Actinobacteria	Actinobacteria (P)	7.03	7.1	6.72	8.09	0.358	0.473	0.439	0.121
Proteobacteria	gamma-Proteobacteria (G)	6.04	6.04	6.27	5.85	0.040*	0.016*	0.62	0.049*
Fusobacteria	*Fusobacterium* spp.	6	6.04	6.1	5.67	0.128	0.8	0.065	0.091
Verrucomicrobiaceae	*Akkermansia muciniphila* (S)	5.4	5.6	6.15	4.19	0.007*	0.447	0.007*	0.005*
Archaea	Methanogens (G)	8.31	8.46	8.3	7.5	0.036*	0.526	0.012*	0.091

P = Targeted bacterial phyla, G = Targeted bacterial genus/group, S = Targeted bacterial species.

tion to HDL-c, a cardioprotective effect indicator.

Waist circumference, BMI, SBP and FBS differed significantly among the three groups (Table 3). In some cases significant differences were noted only between two groups. Specifically, levels of Tg and LDL-c were higher in the obese group when compared to the lean group. Body mass index was positively correlated to waist circumference, SBP, FBS and Tg level, but had a negative correlation to HDL-c level. Our findings corroborate previous reports in Thai [26], and other Asian populations [27].

Discussion

Research on the human gut microbiota has increased exponentially in the last few years and new roles in health and disease are continuously being uncovered. A consensus is now emerging that alterations of the gut microbiota are linked with several diseases including obesity [9]. Herein, we examined specific components of the prokaryotic gut microbiota from lean, overweight and obese Thai adults.

In the course of our study, the issue of the categorization of volunteers according to BMI emerged. Asian populations, including Thais, are considered lean when compared to their western counterparts. Much discussion has centered on whether a lower BMI threshold than the one officially recommended by the world health organization (WHO) should be used to define obesity in Asians [28]. This stems from reports indicating propensity of Asians to accumulate abdominal fat more than non-Asians [29]. Accumulation of this type of fat is especially concerning, as it accounts for insulin sensitivity and subsequent development of diabetes [3]. Nonetheless, an investigation on the matter of BMI led by the WHO unearthed a large degree of variation among

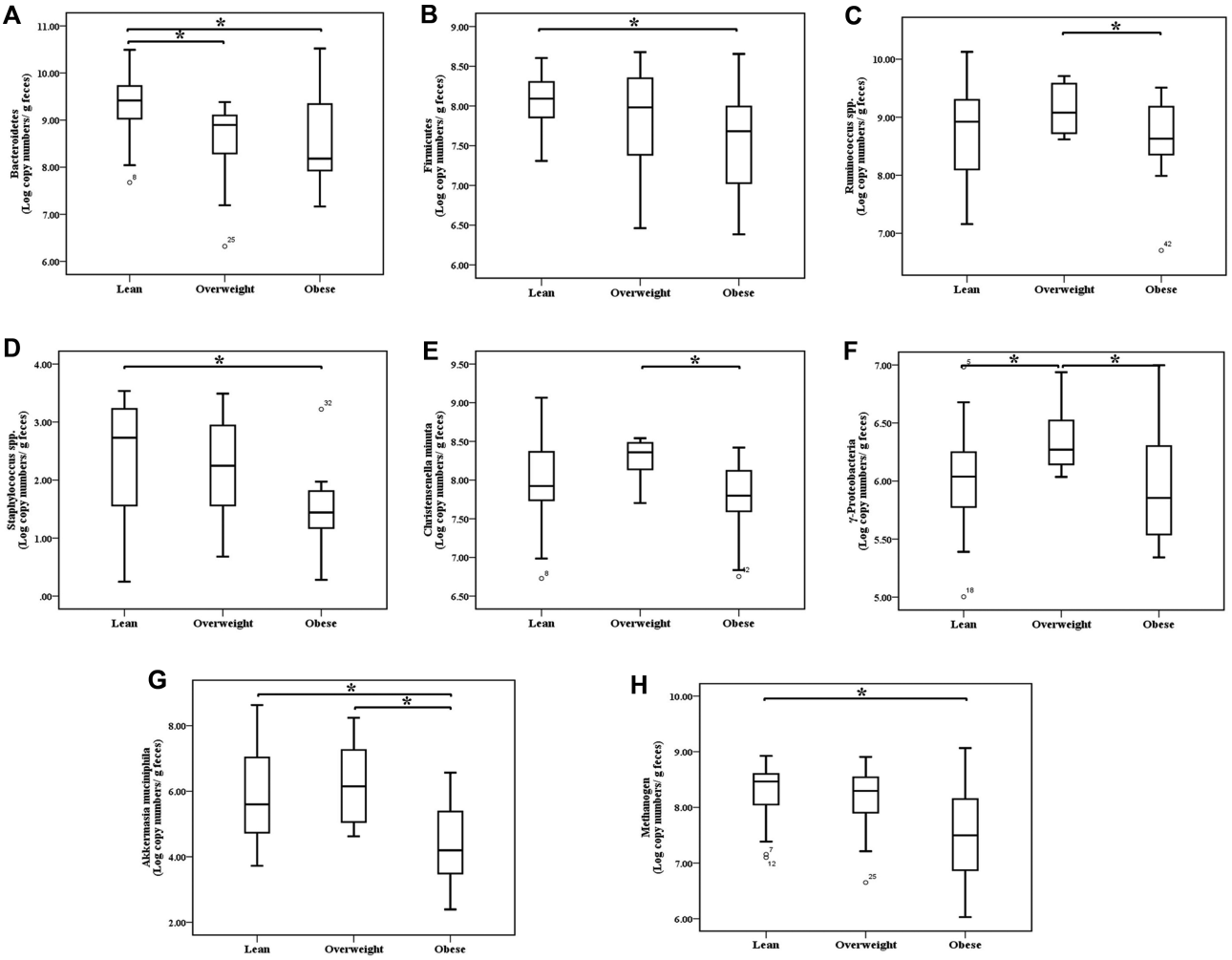

Fig. 1. Box-and-whisker plot distribution of bacteria in fecal samples of lean, overweight, and obese subjects: Bacteroidetes (A), Firmicutes (B), *Ruminococcus* spp. (C), *Staphylococcus* spp. (D), *Christensenella minuta* (E), γ-Proteobacteria (F), *Akkermansia muciniphila* (G), and Methanogen (H). *indicates significance at 0.05 level (two-tailed) usingMann-Whitney U test.

Asians from different countries, suggesting that lowering the BMI cut-off is not warranted [30]. Interestingly, variation was noted even among populations within the same country. In the case of Thailand, for instance, urban Thais had lower BMIs than rural Thais, the latter being very similar to non-Asian values [30]. Thus, in this study, we used the international WHO standard to ensure that our data is directly comparable to data from the rest of the world. Using a lower BMI cut-off precludes comparisons with non-Asian countries making global level conclusions impossible [21].

In our study, Bacteroidetes, Firmicutes, methanogens and *Staphylococcus* spp., were associated with the lean phenotype. Bacteroidetes and Firmicutes dominate the gut comprising more than 90% of its microbiota [13, 31]. A shift in the abundance of these two taxa represents the most commonly reported alteration in obesity in European and North American populations [9, 10], though this is not always the case [14, 15]. Bacteroidetes abundance has been linked with leanness and weight loss in both humans and animals [9, 10, 21, 32, 33]. Previous studies have reported that reduced levels of Bacteroidetes in obese individuals are concomitant with aberrant amounts of Firmicutes, as well as, an increase in the amount of the latter taxon during weight gain, which is in direct contrast with our finding. A few factors could account for this discrepancy. The different results could potentially reflect the use of diverse methodologies

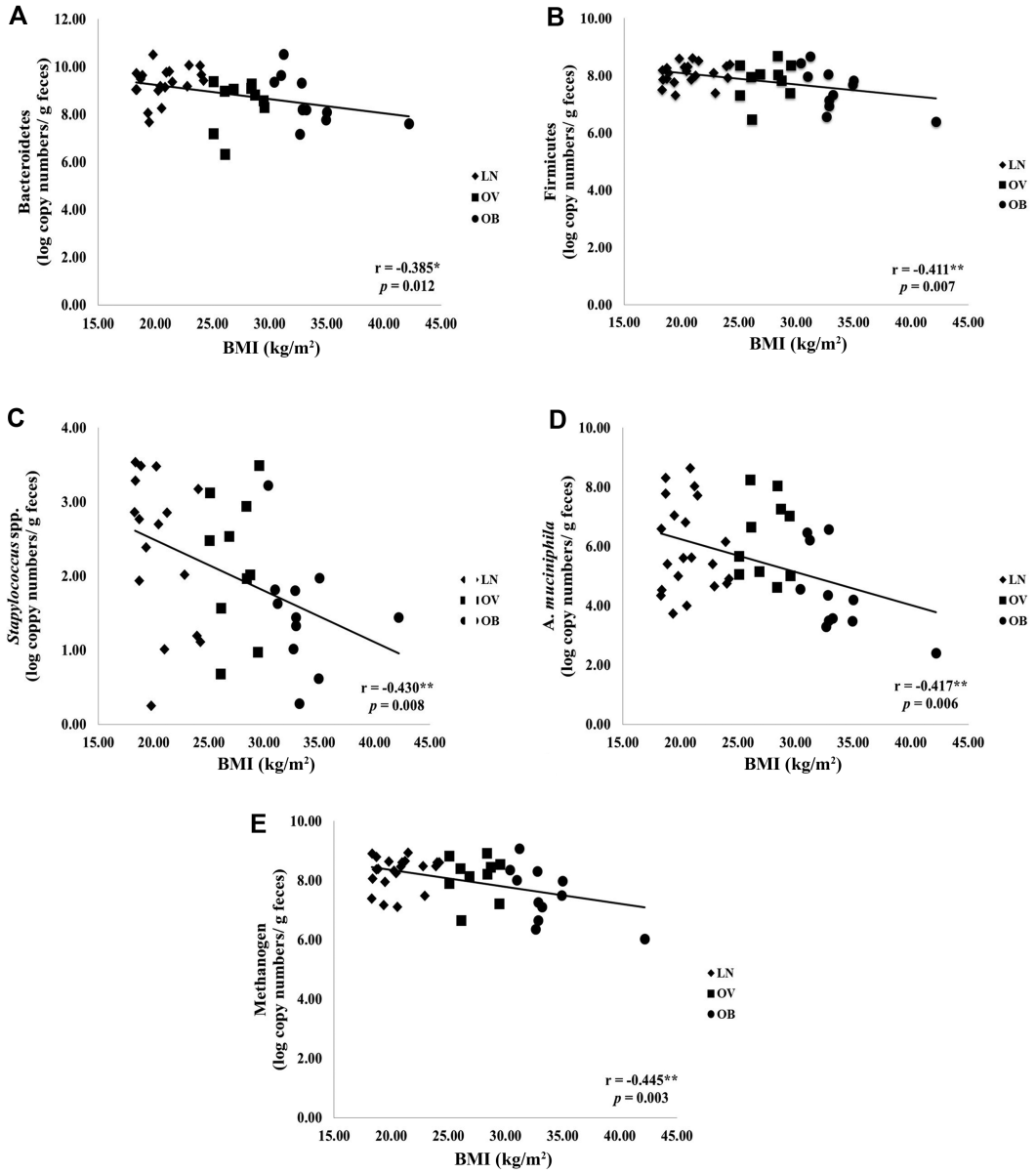

Fig. 2. Significant correlations between BMI and copy number of targeted bacteria of 42 Northern Thai volunteers. (A) BMI and Bacteroidetes; (B) BMI and Firmitues; (C) BMI and *Staphylococcus* spp.; (D) BMI and *A. muciniphila*; (E) BMI and Methanogens *Correlation significant (P) at 0.05 level (two-tailed). **Correlation significant (P) at 0.01 level (two-tailed). r, Correlation coefficient

and protocols across studies. For instance, while some-studies employ next generation sequencing, others use terminal restriction fragment length polymorphism (T-RFLP) or qPCR [21, 34]. A second explanation is that there are some as yet unidentified members of Firmicutes in the Thai gut that contribute to the observed over-abundance.

Contrary to many studies, we did not find any taxa that are associated exclusively with the obese phenotype, but a few were more abundant in overweight individuals. Many microbiota-related studies either do not include overweight individuals or lump them with obese [35]. Nonetheless, overweight and obesity represent two separate physiological states, whereby obesity is considered a disease and overweight is not. With this proviso in mind, we designated overweight as a separate group

in our study. We found that γ-Proteobacteria, *Ruminococcus* spp., *Christensenella minuta* and *Akkermansia muciniphila* were significantly more abundant in overweight individuals. Though the role of γ-Proteobacteria and *Ruminococcus* spp. in obesity remains unclear, *Christensenella minuta* and *Akkermansia muciniphila* have both been linked to leanness and weight loss [20, 36]. *Akkermansia muciniphila* has also been associated with thickness of intestinal mucus and overall intestinal and metabolic health [37, 38]. The presence of a significantly higher amount of some taxa in the intermediate state (overweight) signifies perhaps the transition point between healthy and unhealthy metabolism. Therefore, these taxa could potentially be used as microbial signatures of such metabolic switches so that intervention measures can be taken. Regrettably, definitive conclusions regarding abundance of these taxa in overweight cannot be drawn since many studies do not include overweight individuals. Thus, any findings similar to ours would go unnoticed.

In recent years, studies have started to explore links between microbiota and metabolic markers [39, 40]. Here, we investigated whether the taxa used in this study associate with adiposity and glucose and lipid homeostasis. Some taxa were associated with both adiposity and lipid homeostasis markers. For instance, Bacteroidetes, *Staphylococcus* and methanogens had a significant negative correlation to BMI and waist circumference, but were positively correlated to HDL-c level, a cardio protective effect indicator. Other taxa, such as Actinobacteria and *Oscillospira* showed negative correlations to total cholesterol and LDL levels, but no relationship to any of the other parameters. It is tempting to speculate that taxa exhibiting negative correlations with adiposity and lipid homeostasis markers are suitable metabolic health indicators. However, more studies with increased sample size are needed to determine such relationships in a conclusive manner.

Our pilot study represents the first investigation of the gut microbiota of Thai adults and sets a rough baseline. We found that some bacterial groups are associated with the lean phenotype, others with the overweight, while none was linked to the obese individuals. While some of our findings are similar to previous studies, we have also identified some notable differences. These alterations could reflect the uniqueness of the Thai diet and warrant further investigation, whereby nutrition will be considered. Future studies should focus on larger sample sizes from northern Thailand, as well as, the central, northeast and south. Upcoming work should also expand to include next generation sequencing and culturomics approaches to reveal the full extent of diversity of Thai gut microbiota.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Barlow GM, Yu A, Mathur R. 2015. Role of the gut microbiome in obesity and diabetes Mellitus. *Nutr. Clin. Pract.* **30**: 787-797.
2. Patterson E, Ryan PM, Cryan JF, Dinan TG, Ross RP, Fitzgerald GF. *et al.* 2016. Gut microbiota, obesity and diabetes. *Postgrad. Med. J.* **92**: 286-300.
3. World Health Organization. 2000. Obesity: preventing and managing the global epidemic. *World Health Organ. Tech. Rep. Ser.* **894**: 1-253.
4. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D. *et al.* 2007. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* **56**: 1761-1772.
5. World Health Organization. 2014. Global Status Report on noncommunicable diseases. http://apps.who.int/iris/bitstream/10665/148114/1/9789241564854_eng. pdf
6. Hall KD, Sacks G, Chandramohan D, Chow CC, Wang YC, Gortmaker SL. *et al.* 2011. Quantification of the effect of energy imbalance on bodyweight. *Lancet* **378**: 826-837.
7. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M. *et al.* 2012. Human gut microbiome viewed across age and geography. *Nature* **486**: 222-227.
8. Koh A, De Vadder F, Kovatcheva-Datchary P, Backhed F. 2016. From dietary fiber to host Physiology: short-chain fatty acids as key bacterial metabolites. *Cell* **165**: 1332-1345.
9. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. 2006. An obesity associated gut microbiome with increased capacity for energy harvest. *Nature* **444**: 1027-1031.

10. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE. *et al.* 2009. A core gut microbiome in obese and lean twins. *Nature* **457**: 480-487.
11. Cani PD, Bibiloni R, Knauf C, Neyrinck AM, Delzenne NM, Burcelin R. 2008. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet induced obesity and diabetes in mice. *Diabetes* **57**: 1470-1481.
12. Chassaing B, Gewirtz AT. 2016. Has provoking microbiota aggression driven the obesity epidemic?. *Bioessays* **38**: 122-128.
13. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. 2005. Obesity alters gut microbial ecology. *Proc. Natl. Acad. Sci. USA* **102**: 11070-11075.
14. Duncan SH, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P. *et al.* 2008. Human colonic microbiota associated with diet, obesity and weight loss. *Int. J. Obes (Lond)* **32**: 1720.
15. Schwiertz A, Taras D, Schäfer K, Beijer S, Bos NA, Donus C. *et al.* 2010. Microbiota and SCFA in lean and overweight healthy subjects. *Obesity* **18**: 190-195.
16. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. 2006. Microbial ecology: human gut microbes associated with obesity. *Nature* **444**: 1022-1023.
17. Balamurugan R, George G, Kabeerdoss J, Hepsiba J, Chandragunasekaran AM, Ramakrishna BS. 2010. Quantitative differences in intestinal *Faecalibacterium prausnitzii* in obese Indian children. *Br. J. Nutr.* **103**: 335-338.
18. Mai V, McCrary QM, Sinha R, Glei, M. 2009. Associations between dietary habits and body mass index with gut microbiota composition and fecal water genotoxicity: an observational study in African American and Caucasian American volunteers. *Nutr. J.* **8**: 49.
19. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB. *et al.* 2013. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proc. Natl. Acad. Sci. USA* **110**: 9066-9071.
20. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R. *et al.* 2014. Human genetics shape the gut microbiome. *Cell* **159**: 789-799.
21. Kasai C, Sugimoto K, Moritani I, Tanaka J, Oya Y, Inoue H. *et al.* 2015. Comparison of the gut microbiota composition between obese and non-obese individuals in a Japanese population, as analyzed by terminal restriction fragment length polymorphism and next-generation sequencing. *BMC Gastroenterol.* **15**: 100.
22. Tyakht AV, Kostryukova ES, Popenko AS, Belenikin MS, Pavlenko AV, Larin AK. *et al.* 2013. Human gut microbiota community structures in urban and rural populations in Russia. *Nat. Commun.* **4**: 2469.
23. Zhang J, Guo Z, Xue Z, Sun Z, Zhang M, Wang L. *et al.* 2015. A phylo-functional core of gut microbiota in healthy young Chinese cohorts across lifestyles, geography and ethnicities. *ISME J.* **9**: 1979-1990.
24. World Health Organization. 2011. *Noncommunicable diseases in the South-East Asia region*, 1st Ed., New Delhi, India.
25. Brakstad OG, Aasbakk K, Maeland JA. 1992. Detection of *staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. *J. Clin. Microbiol.* **30**: 1654-1660.
26. Yamborisut U, Riabroy N, Phonrat B, Tungtrongchitr R. 2009. Serum leptin levels and body composition in obese Thai children. *Southeast Asian J. Trop. Med. Public. Health*. **40**: 544-552.
27. Shiwaku K, Nogi A, Kitajima K, Anuurad E, Enkhmaa B, Yamasaki M. *et al.* 2005. Prevalence of the metabolic syndrome using the modified ATP III definitions for workers in Japan, Korea and Mongolia. *J. Occup. Health*. **47**: 126-135.
28. Ramachandran A, Ma RCW, Snehalatha C. 2010. Diabetes in Asia. *Lancet* **375**: 408-418.
29. Ramachandran A, Chamukuttan S, Shetty SA, Arun N, Susairaj P. 2012. Obesity in Asia–is it different from rest of the world. *Diabetes Metab. Res. Rev.* **28**: 47-51.
30. World Health Organization Expert Consultation. 2004. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* **363**: 157-163.
31. Sekirov I, Russell SL, Antunes LC, Finlay BB. 2010. Gut microbiota in health and disease. *Physiol. Rev.* **90**: 859-904.
32. Armougom F, Henry M, Vialettes B, Raccah D, Raoult D. 2009. Monitoring bacterial community of human gut microbiota reveals an increase in *Lactobacillus* in obese patients and Methanogens in anorexic patients. *PLoS One* **4**: https://doi.org/10.1371/journal.pone.0007125.
33. Furet JP, Kong LC, Tap J, Poitou C, Basdevant A, Bouillot JL. *et al.* 2010. Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. *Diabetes* **59**: 3049-3057.
34. Karlsson CL, Onnerfält J, Xu J, Molin G, Ahrné S, Thorngren-Jerneck K. 2012. The microbiota of the gut in preschool children with normal and excessive body weight. *Obesity* **20**: 2257-2261.
35. Bervoets L, Van Hoorenbeeck K, Kortleven I, Van Noten C, Hens N, Vael C. *et al.* 2013. Differences in gut microbiota composition between obese and lean children: a cross-sectional study. *Gut. Pathog.* **5**: 10.
36. Yan M, Song M-M, Bai R-X, Cheng S, Yan W-M. 2016. Effect of Roux-en-Y gastric bypass surgery on intestinal *Akkermansia muciniphila*. *World J. Gastrointest. Surg.* **8**: 301-307.
37. Dao MC, Everard A, Aron-Wisnewsky J, Sokolovska N, Prifti E, Verger EO. *et al.* 2016. *Akkermansia muciniphila* and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. *Gut*. **65**: 426-436.
38. Ottman N, Huuskonen L, Reunanen J, Boeren S, Klievink J, Smidt S. *et al.* 2016. Characterization of outer membrane proteome of *Akkermansia muciniphila* reveals sets of novel proteins exposed to the human intestine. *Front. Microbiol.* **7**: 1157.
39. Brahe LK, Le Chatelier E, Prifti E, Pons N, Kennedy S, Blædel T. *et al.* 2015. Dietary modulation of the gut microbiota a randomised controlled trial in obese postmenopausal women. *Br. J .Nutr.* **114**: 406-417.
40. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G. *et al.* 2013. Richness of human gut microbiome correlates with

metabolic markers. *Nature* **500**: 541.
41. De Gregoris TB, Aldred N, Clare AS, Burgess JG. 2011. Improvement of phylum and class-specific primers for real-time PCR quantification of bacterial taxa. *J. Microbiol. Methods*. **86**: 351-356.
42. Bartosch S, Fite A, Macfarlane GT, McMurdo MET. 2004. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Appl. Environ. Microbiol*. **70**: 3575-3581.
43. Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Holtrop G, Louis P. 2009. Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. *Br. J. Nutr*. **101**: 541-550.
44. Walker AW, Duncan SH, Leitch ECM, Child MW, Flint HJ. 2005. pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Appl. Environ. Microbiol*. **71**: 3692-3700.
45. Walter J, Hertel C, Tannock GW, Lis CM, Munro K, Hammes WP. 2001. Detection of *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella* species in human feces by using group-specific PCR primers and denaturing gradient gel electrophoresis. *Appl. Environ. Microbiol*. **67**: 2578-2585.
46. Heilig H, Zoetendal EG, Vaughan EE, Marteau P, Akkermans AD, de Vos WM. 2002. Molecular diversity of *Lactobacillus* spp. and other lactic acid bacteria in the human intestine as determined by specific amplification of 16S ribosomal DNA. *Appl. Environ. Microbiol*. **68**: 114-123.
47. Rinttila T, Kassinen A, Malinen E, Krogius L, Palva A. 2004. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *J. Appl. Microbiol*. **97**: 1166-1177.
48. Mackie RI, Aminov RI, Hu WP, Klieve AV, Ouwerkerk D, Sundset MA. *et al.* 2003. Ecology of uncultivated *Oscillospira* species in the rumen of cattle, sheep, and reindeer as assessed by microscopy and molecular approaches. *Appl. Environ. Microbiol*. **69**: 6808-6815.
49. Yanagita K, Manome A, Meng XY, Hanada S, Kanagawa T, Tsuchida T. *et al.* 2003. Flow cytometric sorting, phylogenetic analysis and in situ detection of *Oscillospira guillermondii*, a large, morphologically conspicuous but uncultured ruminal bacterium. *Int. J. Syst. Evol. Microbiol*. **53**: 1609-1614.
50. Wang RF, Cao WW, Cerniglia CE. 1996. PCR detection and quantitation of predominant anaerobic bacteria in human and animal fecal samples. *Appl. Environ. Microbiol*. **62**: 1242-1247.
51. Walter J, Margosch D, Hammes WP, Hertel C. 2002. Detection of *Fusobacterium* species in human feces using genus-specific PCR primers and denaturing gradient gel electrophoresis. *Microb. Ecol. Health Dis*. **14**: 129-132.
52. Collado MC, Derrien M, Isolauri E. 2007. Intestinal integrity and *Akkermansia muciniphila*, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. *Appl. Environ. Microbiol*. **73**: 7767-7770.
53. Hook SE, Northwood KS, Wright ADG, McBride BW. 2009. Long-term monensin supplementation does not significantly affect the quantity or diversity of methanogens in the rumen of the lactating dairy cow. *Appl. Environ. Microbiol*. **75**: 374-380.