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Comparative study of gut microbiota Mongolian and Asian people

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Abstract

Mongolia has the unique dietary habit having a great deal of animal products especially among rural resident.To capture the status of Mongolian gut microbiome, we characterized bacterial community of 98 healthy Mongolian adults and compared with that of adults in five Asian countries, including Korea, China, Japan, Thailand and Indonesia. Principal component analysis (PCA) was performed based on genus composition of each sample. As a result, three microbiome-type cluster, the so-called "enterotype", driven by the three taxonomic groups, Prevotella (P-type), Bacteroides and Bifidobacterium (BB-type), and Ruminococcaceae (R-type), were observed. Most of Mongolian subjects harbored P-type, which is known to strongly depend on carbohydrate-based diets. Further, the metagenomic analysis indicated that Catenibacterium and Lactobacillus were enriched in Mongolian subjects which may be concerned with intake of animal -based and dairy products-based diets, respectively. These results suggest that gut microbiome status of Mongolian people associates with the traditional unique dietary habit.

Keywords: Gut microbiota, Lactobacillus, Enterotypes, Mongolian people

Introduction

Several hundred microbial species inhabit the human intestine where all of them compose the so-called as gut microbiota, including bacteria, archaea, eukarya, as well as viruses [1]. The gut microbiota play an important role in health and diseases of the host via co-metabolism bio-synthesizing beneficial or non-beneficial products [2]. Particularly bacteria that reflect over 90% of commensal community in the gut have a great and sustainable influenced on the host health [3,4]. The accumulated data of human gut microbiome has revealed three particular types of gut microbiota structures, called "enterotype", although it remains controversial in the aspect of consistency [5]. The enterotypes represent classification of gut microbiota, firstly introduced by Arumugam *et al.*[6]. Three enterotypes are clustered by *Bacteroides*, *Prevotella*, and *Ruminococcus*, which exist over ethnicity, gender, age, as well as body mass index (BMI). A number of studies have indicated that the enterotypes are associated with dietary patterns. Notably, *Prevotella* enterotype strongly depends on carbohydrates in diets whereas *Bacteroides* enterotype depends on a high fat and low fiber diets[7,8].Indeed, the key industry in Mongolia is stock farming. According to the report of National Statistical Office of Mongolia in 2013, livestock in Mongolia accounted for 14.6% of total GDP, and 35% of the population was relied on livestock industry as the source of food and income[9]. Mongolian people have maintained their diet characterized by high consumption of livestock products since ancient times. Their traditional dietary habit is characterized by the distinct seasonality. Variety of dairy products, called white food "tsagaan idee", are normally consumed during summer-to-autumn period. On the other hand, meat, called red food "ulaan idee", is normally consumed during winter-to-spring period[11].

The International Center for Tropical Agriculture in 2009 stated that consumption of animal products in Mongolian people is 875 kcal/capita/day that is much higher than the global standard (508 kcal/capita/day)[10]. Gut microbiota of Mongolian people has been reported in few years[12,13], in which *Prevotella* and *Bacteroides* are mainly predominant bacteria, accounting for 47.11% and 6.33%, respectively[12]. Moreover, by comparison of gut microbiome between Mongolian, Han, and European people, it was found that Mongolian people have higher abundance of *Prevotella*, *Faecalibacterium, Collinsella*, and *Bifidobacterium* than that of other countries[13]. To investigate the links of different traditional diets, gut microbiome, and health of Asian people, so far we have established the Asian microbiome project (AMP) with ten Asian countries since 2009 (http://www.agr.kyushu-u.ac.jp/lab/microbt/ AMP/). However, gut microbiome of Mongolian people has not been clarified in more details in comparison of other Asian countries. To accomplish this knowledge, here we investigated gut microbiome of Mongolian adults in comparison with other five Asian countries, namely Japan, China, Indonesia, Korea, and Thailand.

Materials and methods

Ethics declaration

This study was approved by the Ethics Committees of the Faculty of Agriculture in Kyushu University (No. 17-55) and Ethical clearance from the Ethics Committee under the Ministry of Health of Mongolia (No. 78) All methods were carried out in accordance with the relevant guidelines and regulations. Written informed consent was obtained from all subjects participating this study. We entered and analyzed all samples and questionnaire data anonymously and will publish all data anonymously using personnel numbers.

Sample information

In this study, we combined the dataset of Asian adult through AMP phase Ⅱ and Ⅳ. In AMP phase Ⅱ study, we recruited 218 adults from Mongolia (n=29), China (n=28), Japan (n=79), Indonesia (n=29), Korea (n=6), and Thailand (n=27). As for AMP phase Ⅳ study, samples were collected from 69 Mongolian subjects.

Fecal sample collection and transportation process

All subjects provided two parts of fresh feces by using small spoon equipped stool collection tube (76 × 20 mm, Sarstedt, Germany) containig 2ml of RNAlater (Invitrogen, Thermo Fisher Scientific, Lithuania) and five zirconia balls, YTZ®-2.5 mm (Nikkato, Japan). After collecting, the feces were shaken several times to be suspended. The samples were transferred to the laboratory, briefly homogenized by vortexing, and stored at -20℃ until transporting to Japan. The samples were transported within 24 h by air transportation to Kyushu University in Japan under the temperature control (< 8°C) and stored at -20°C until DNA extraction.

DNA extraction

DNA were extracted from stool samples by using the bead-beating method as previously described[14].

16S rRNA gene amplicon sequencing and sequence data process

We performed high-throughput 16S rRNA gene sequence analysis was followed from our previous work[15]. The V3-V4 region of the bacterial 16SrRNA gene was PCR amplified by using TaKaRa Ex Taq® HS (Takara Bio, Japan) and the barcode-tag universal primer sests including Bakt_341F (5′-CGCTCTTCCGATCTCTGCCTACGGGNGGCWGCAG-3′) and Bakt_805R (5′-TGCTCTTCCGATCTGACGACTACHVGGGTATCTAATCC-3′)[16]. The sequence data were processed by using the UPARSE pipeline in USEARCH v9.2.64 software (http://drive5.com/usearch/download.html)[17], following by the UCLUST algorithm (assign_taxonomy.py) in QIIME pipeline software version 1.9.1 (http://qiime.org/)[18] to assign taxonomy. EZBioCloud was used as reference sequence database (https://www.ezbiocloud.net/mtp/view_myMTPList).

Statistical analysis

Statistical analyses and graphics were accomplished by using RStudio software, version 1.0.153 (https://rstudio.com/) under R software, version 3.5.1 (http://www.R-project.org) and Stata/SE, version 12.0. To compare physiological indices and bacterial relative abundance. Wilcoxon rank-sum test was used to compare between two groups.

Beta diversity analysis

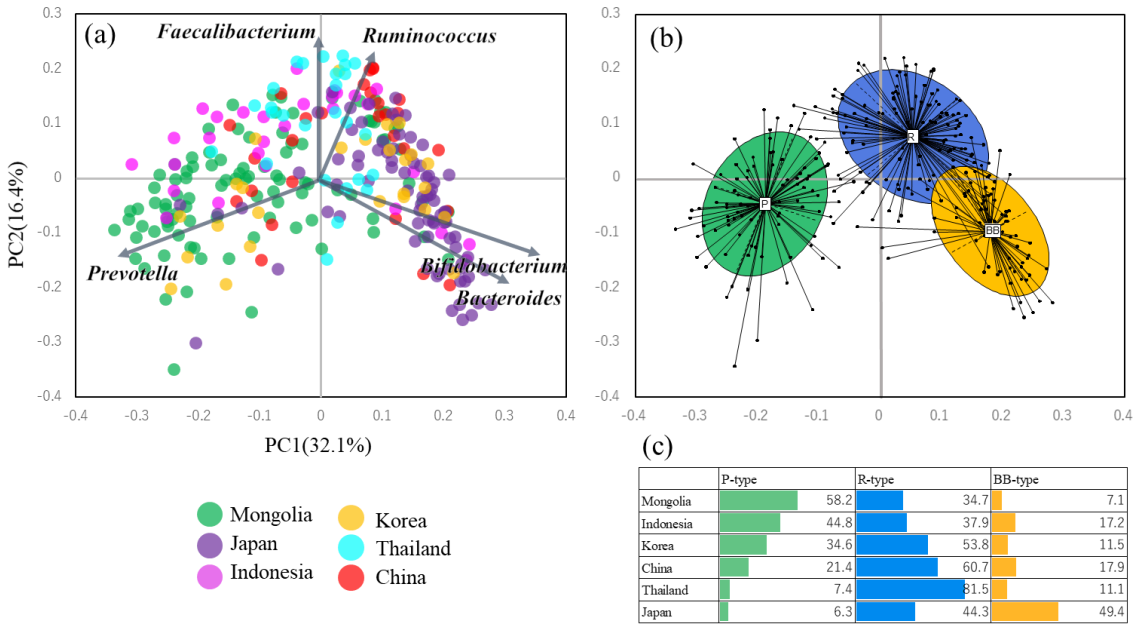
PCA was performed based on the genus composition by using "rda" function in the R Vegan package (https://CRAN.R-project.org/package=vegan).

Clustering analysis

Clustering was performed based on the genus composition by using "Enterotyping: R tutorials" provided in the R environment by EMBL (https://enterotype.embl.de/enterotypes.html). The JSD was calculated according to the relative genus abundance of each sample by using the "dist.JSD" function coded in R (http://enterotype.embl.de/enterotypes.html). All samples were then clustered into three clusters using PAM clustering by using the ''pam'' function in the R library ''cluster'' (https://cran.r-project.org/web/packages/ cluster/cluster.pdf). The result of clustering was visualized by using ade4 packages (https://cran.r-project.org/web/ packages /ade4/index.html).

Results

To explore the gut microbiota feature of Mongolian people, we compared gut bacterial composition of Mongolian subjects with the subjects from five Asian countries, including China, Indonesia, Japan, Korea, and Thailand. Using Principal component analysis (PCA), bacterial composition based on genus-level of all samples were decomposed into two factors (PC1 and PC2) that explained 48.5% of the variance (Fig. 2a). Principal component 1(PC1) was negatively loaded with *Prevotella* and was positively loaded with *Bacteroides* and *Bifidobacterium*. Principal components 2(PC2) was positively loaded with *Faecalibacterium* and *Ruminoccoccus*, both genera of which are known to be the members of the family Ruminococcaceae (Figure 1a). To classify gut bacterial composition based on enterotypes, we performed clustering analysis by using genus composition data. The result showed enterotype clusters on the PCA (Figure 1b). The three clusters were divided into PC1- positive, PC-1 negative, and PC-2 positive regions, which indicated the strong reflection of PC1 in the cluster as observed in the PC1 and PC2 projection, shown in Figure 1a. Henceforth, the *Prevotella*-defined microbiota of PC1-negative group is referred as "P-type", whereas the *Ruminococcus* and *Faecalibacterium*, which are classified in Ruminococcaceae family-defined microbiota of PC2- positive group, and the *Bacteroides* and *Bifidobacterium* -defined microbiota of PC-1 positive group, are termed as "R-type" and "BB-type", respectively. The ratio of P-type, R-type, and BB-type in each country is shown in Figure 1c. Notably, 58.2% of the samples from Mongolia fell into P-type, which was the highest ratio compared with the other Asian countries. On the other hand, only 6.3% of the samples from Japan fell into P-type, while most of them fell into R- type or BB-type. Comparison was made for bacteria with an average abundance higher 1% in all subjects (Figure 2). As a result, Mongolian subjects showed significantly higher abundance of *Prevotella*, *Lactobacillus*, *Catenibacterium* than five Asian countries ($p < 0.001$, Wilcoxon rank-sum test). On the other hand, abundance of *Bacteroides*, *Faecalibacterium* were significantly low in Mongolian subjects ($p < 0.001$, Wilcoxon rank-sum test). Operational taxonomic units (OTUs) representing species level were observed. Dominant and predominant OTUs were shown in Table1. As a result, OTU 15 and OTU230 closely related to *Prevotella sp*., OTU 36 closely related to *Clostridium celatum*, OTU38 closely related to *Catenibacterium sp.*, OTU79 closely related to *Ligilactobacillus ruminis* (formerly *Lactobacillus ruminis*)were high in Mongolian subjects ($p < 0.001$, Wilcoxon rank-sum test). Conversely, OTU 2 closely related to *Faecalibacterium prausnitzii*, OTU1 closely related to *Blautia wexlerae*, OTU8 closely related to *Phocaeicola dorei*, OTU4 closely related to *Fusicatenibacter saccharivorans*, OTU16 closely related to *Blautia massiliensis*, OTU7 closely related to *Romboutsia timonensis*, OTU9 closely related to *Anaerostipes hadrus*, OTU13 closely related to *Anaerobutyricum hallii*, OTU 23 closely related to *Bacteroides uniformis* were lower in Mongolian subjects ($p < 0.001$, Wilcoxon rank-sum test).



	P-type	R-type	BB-type
Mongolia	58.2	34.7	7.1
Indonesia	44.8	37.9	17.2
Korea	34.6	53.8	11.5
China	21.4	60.7	17.9
Thailand	7.4	81.5	11.1
Japan	6.3	44.3	49.4

Figure1. PCA and clustering of adult samples of Mongolian and five Asian countries by using species-level composition data. (a) We performed PCA by using the relative abundance data of all bacterial genus; in this figure, we have plotted the results with specific colors being used to indicate countries. The four dominant bacterial genera are indicated by arrows together with their genus names. (b) Clustering of participants based on genus composition data. The center of gravity of each cluster is indicated by a rectangle filled with the name of microbiota type. (c) The ratio of the P-, R- and BB-type subjects in each country

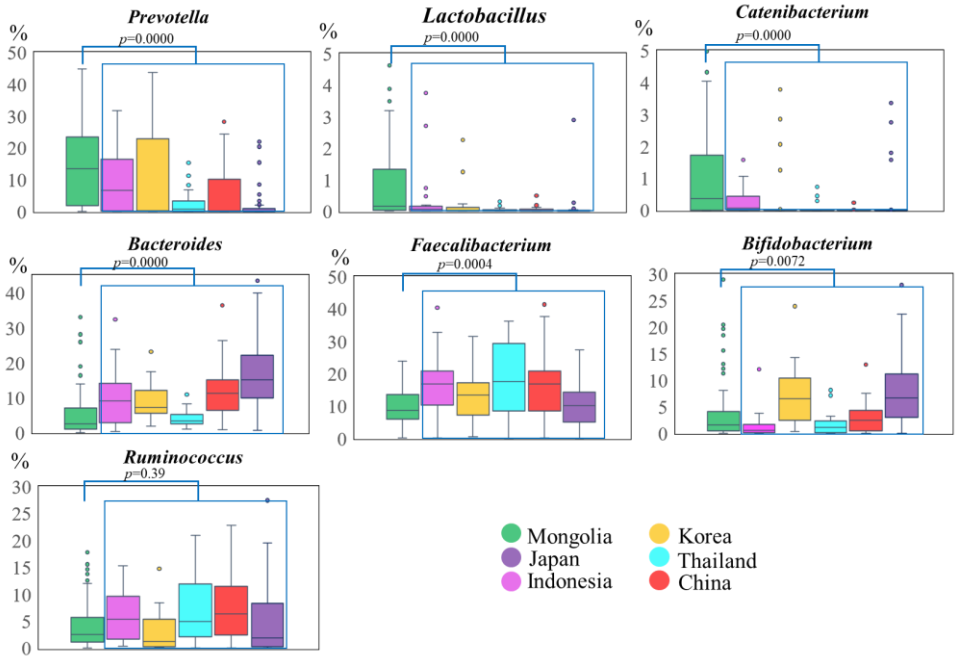


Figure 2. Genus abundance of *Prevotella*, *Lactobacillus*, *Catenibacterium*, *Bacteroides*, *Faecalibacterium*, *Bifidobacterium* and *Ruminococcus* of microbiota of Mongolia and five Asian countries. Statistical significance was assessed by Wilcoxon rank-sum test between Mongolia and five Asian countries.

Table 1
Taxonomic information of dominant phylotypes detected in the stool samples of Mongolian and Asian populations

OTUID	EzBioCloud[a]	Similarity[a]	statistical analysis[b]	Relative abundance (%)							carrier(%)[d]					
				MN	IN	KR	TL	CH	JP	Average of five countries[c]	MN	IN	KR	TL	CH	JP
Otu2	***Faecalibacterium prausnitzii***	97.76	<<	10.1	16.7	13.4	17.9	16.6	10.2	15.0	100	100	100	100	100	100
Otu3	***Agathobacter rectalis***	97.98	<	4.7	5.3	4.6	5.2	4.1	4.7	4.8	100	100	100	100	100	95
Otu1	***Blautia wexlerae***	97.98	<<	3.4	2.5	4.3	4.6	4.3	6.5	4.5	100	100	100	100	100	100
Otu8	***Phocaeicola dorei***	98.07	<<	2.3	2.6	4.5	1.4	3.6	6.6	3.7	97	100	100	100	100	100
Otu15	***VZCB_s (Prevotella sp.)***	97.85	>>	6.5	4.2	4.9	1.2	3.1	0.6	2.8	87	90	81	63	50	28
Otu230	***PAC001304_s (Prevotella sp.)***	96.78	>>	3.7	3.4	5.2	0.9	2.0	1.1	2.5	88	90	69	59	46	24
Otu12	***Ruminococcus bromii***	97.99		2.2	2.5	1.5	4.3	4.8	2.1	3.1	72	93	77	81	89	68
Otu4	***Fusicatenibacter saccharivorans***	97.98	<<	1.6	1.2	4.4	1.3	2.1	3.3	2.5	97	100	100	96	93	95
Otu16	***Blautia massiliensis***	98.21	<<	1.8	0.9	2.3	2.3	2.0	2.6	2.0	99	100	100	100	100	100
Otu7	***Romboutsia timonensis***	97.98	<<	1.1	2.0	1.5	4.0	5.7	1.2	2.9	94	100	100	96	100	94
Otu11	***Mediterraneibacter faecis***	97.98	>	2.6	1.4	1.4	1.8	2.1	1.5	1.6	100	93	100	96	100	98
Otu9	***Anaerostipes hadrus***	97.98	<<	1.2	0.8	2.8	1.5	2.8	2.6	2.1	100	97	100	96	100	91
Otu6	***Gemmiger formicilis***	97.76	<	1.7	2.0	2.4	2.1	2.7	1.6	2.2	97	97	100	85	86	85
Otu13	***Anaerobutyricum hallii***	97.98	<<	1.3	1.1	1.8	2.0	2.3	2.5	1.9	98	97	100	96	100	93
Otu5	***Bifidobacterium pseudocatenulatum***	98.01		1.3	0.4	1.7	0.5	1.5	3.4	1.5	89	76	88	59	86	93
Otu237	***Bifidobacterium adolescentis***	98.68	<	1.2	0.6	3.4	0.4	0.6	2.5	1.5	85	83	100	67	61	92
Otu10	***Dorea longicatena***	97.98		1.6	0.9	1.5	2.3	1.0	1.1	1.4	99	97	100	93	100	76
Otu36	***Clostridium celatum***	98.21	>>	1.7	1.1	0.3	3.2	0.8	0.5	1.2	97	97	85	81	96	72
Otu23	***Bacteroides uniformis***	98.28	<<	0.5	0.4	0.7	0.4	1.1	2.5	1.0	76	97	96	93	96	95
Otu38	***PAC002523_s (Catenibacterium sp.)***	98.3	>>	1.2	0.3	0.4	0.1	0.0	0.4	0.2	64	62	27	22	7	17
Otu79	***Ligilactobacillus ruminis (formerly Lactobacillus ruminis)***	98.09	>>	0.7	0.2	0.2	0.0	0.0	0.0	0.1	51	31	27	4	7	1

a The values in parentheses represent sequence identity to the 16S rRNA of the indicated species in EzBioCloud 16S Database.

b > and >> represent significantly higher ($P < 0.05$ and $P < 0.001$, respectively) in subjects of Mongolian than in those of five Asian countries in Mann Witney U-test.
< and << represent significantly lower ($P < 0.05$ and $P < 0.001$, respectively) in subjects of Mongolian than in those of five Asian countries in Mann Witney U-test.

c Average abundance of five Asian countries including Indonesia, Korea, China, Thailand, and Japan.

d The percentage of carriers is indicated.

MN: Mongolia, IN: Indonesia, KR: Korea, TL: Thailand, CH: China, JP: Japan

Discussion

Mongolian gut microbiota has been studied so far, but little has been reported on Mongolian gut microbiota in Asia. In this study, we compared gut microbiota of Mongolian people with five Asian countries. Among those countries, Mongolian had high abundance of *Prevotella*. This result is similar with a study of Zhang *et al.*[12]. It is known that *Prevotella* is increased by consumption of carbohydrate while *Bacteroides* is positively associated with consumption of animal protein and fat[8]. It seems to be contracted result because Mongolian have high amount of meat however, Mongolian have grain such as wheat and millet in their daily life[19]. The intake of grain may contribute the high abundance of *Prevotella* in Mongolian. International Center for Tropical Agriculture also showed that wheat has been found to be the main grain source in Mongolia. The average intake of wheat in Mongolia is 984 kcal/capita/day and that is twice as much as global standard (498 kcal/capita/day) in 2009[10].

Also, *Lactobacillus* and *Catenibacterium* were high in Mongolian. *Lactobacillus* is widely distributed in fermented dairy products such as fermented horse milk “airag” and yogurt “tarag” which are habitually consumed by Mongolian[11]. It is not surprising that the fecal samples of Mongolian consisted of high amounts of *Lactobacillus*.

It is known that *Catenibacterium* had positive correlation with the dietary level of animal fat[20]. High consumption of meat may increase *Catenibacterium* in the gut microbiota of Mongolian. In this study, we found that Mongolian population has unique gut microbiota in Asian countries. Further analysis will be expected to confirm that how Mongolian unique dietary habit correlate with gut microbiota.

Conclusion

Mongolian have *Prevotella* dominat gut microbiota which is opposite to the *Bacteroides* dominant gut microbiota in Japanese people. Also, Mongolian have unique tendency having higher abundance of *Lactobacillus* and *Catenibacterium* than other countries. The high abundance of *Lactobacillus* and *Catenibacterium* in Mongolian may be due to their high consumption of dairy food and meat.

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ACCESSION CODES

AMP Ⅳ Mongolian Sequence data from this article were deposited in the DNA Data Bank of Japan (DDBJ) database under BioProject no. PRJDB5860, which contains links and access to stool sampling data under BioSample SAMD00320633 to SAMD00320659 and 16S rRNA amplicon sequence data designated in the DDBJ sequence read archive (DRA011968).

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