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Analysis of the Intestinal Lactobacillus and Bifidobacterium among Mongolian Adults, and Their Associated Host Factors

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Objectives: To characterize gut Lactobacillus and Bifidobacterium and identify the factors shaping its composition, we collected fecal samples from healthy Mongolians residing in various regions of Mongolia. **Methods:** This was a population-based cross-sectional study involving 256 relatively healthy adults between July 2018 and February 2019. Fecal samples were collected for bacterial analysis using culture method and the species of *Bifidobacterium spp*. and *Lactobacillus spp*. were determined by PCR. **Results:** The participants had a mean age of 38.9 ± 12.8 years. The abundance of Lactobacillus was weakly negatively correlated with grams of fats, potato, cereals foods consumed per day (r = -.20; r = -.16 and r = -.18 respectively). Significant differences in the counts of Lactobacillus were identified depending on the quantity of milk products consumed per day. In adult intestinal tracts, *B. bifidum* was the most common Bifidobacterium taxon at 29% (31 participants) followed by *B. angulatum* 13.1% (14), *B. adolescentis* 9.3% (10) *B. catenulatum group* 9.3% (10), *B. longum* 8.4% (9). *B. lactis, B. breve, B. dentium* and *B. gallicum* were subdominant species. **Conclusion:** The Lactobacillus abundance in healthy adults was higher in the region of Khangai, Eastern and Western Mongolia than other locations. The composition of Lactobacillus species varied. Significant but modest correlations were found between amounts of fiber, fats, potatoes consumed, and the amount of Lactobacillus.

Keywords: Bifidobacterium, Colony-forming unit, Gut microbiota, Lactobacillus, Mongolia

Introduction

The microbiota of the human gut constitutes a complex micro-ecology that is beneficial to the host under hemostasis[1]. Gut microbiota (GM) or microflora play essential roles in producing energy, absorption of micronutrients, enhancing the trophic effect on the gut epithelium, mediating maturation, and the development of the human immune system. Gut microflora also plays an essential role in protecting against colonization by pathobionts, such as *Clostridium Difficile*[2-4]. However, gut

infection or administration of antibiotics or xenobiotics perturbs the GM composition and has been linked to a variety of problems from bowel function symptoms to many severe diseases. Also, the gut microbiota influences a variety of gastroenterological diseases, such as non-alcoholic fatty liver disease, environmental enteropathy, inflammatory bowel disease, and primary sclerosing cholangitis. It also influences chronic diseases such as obesity, chronic periodontitis, and atherosclerosis, as well as neurodegenerative disorders[2,5-13]. Hence, prevention of dysbiosis and modulation of the gut microbiota may be an important tool for developing personalized therapeutic strategies.

Age, gender, and body weight have been linked to the diversity and abundance of gut microbiota and differences in dietary habits, daily routines, and geographical regions[14]. Arumugam and his colleagues studied the fecal microbiota of humans from several countries and reported three strong enterotypes enriched in Bacteroides, Prevotella, and Ruminococcus that were independent of ethnicity or location of residence[15].

The GM of Mongolians and its composition is of great interest to researchers, but few studies have been done on this topic. Mongolians live in a uniquely broad range of environmental conditions, ethnos geographical cohorts, and historically have lived a traditional nomadic lifestyle[16]. In contrast, nowadays, 60% of the populations live an urban style, and others in the nomadic way. The typical Mongolian diet is characterized by the consumption of dairy products and meat[16].

Previous studies of Mongolian's GM have not considered potential differences in intestinal microbiota between urbanized and rural people or the effect of their diet on their GM's composition[14,16]. Furthermore, these have not been studied at a national level. The purpose of this study was to characterize gut *Lactobacillus spp* and *Bifidobacterium spp* in Mongolians and identify the factors influencing its composition. We chose to study these species because they play an essential role in the digestion of in dairy products which are prominent in Mongolian cuisine.

Materials and Methods

Study design and subjects

This population-based cross-sectional study examined the gut microbiome of healthy adult volunteers with no history of gastrointestinal diseases from seven regions of Mongolia. A total of 256 randomly selected people were enrolled between July 2018 and February 2019. Among these participants, 116 lived a typical urban lifestyle in Ulaanbaatar, and 140 participants lived a rural lifestyle in the countryside in six provinces (Orkhon, Umnugovi, Tuv, Khuvsgul, Sukhbator, Uvs) (Table 1). Local assistants recruited by the study team taught the participants the stool collection procedure. Study participants had to be 18 to 70 years of age, have a good general health status, no history of antibiotics usage or active gastrointestinal disease, no prior gastrointestinal surgery and no history of chronic constipation or diarrhea. The anthropometric measurements of height (cm), weight (kg), waist circumference (cm), and body fat (%) were measured for each participant while standing.

Lifestyle questionnaire

Each participant was asked to complete a survey containing 164 questions about regarding their demographics, physical activity, and dietary habits. To assess the participants' dietary habits, we used the EPIC-Norfolk food frequency questionnaire (FFQ) to measure a participant's usual food intake[17]. The FFQ consists of two parts, Part 1 and 2. Part 1 contained a list of common and less commonly consumed foods. For each item on the list, participants are asked to indicate their usual frequency of consumption. Part 2 included a set of additional questions on type and brand of cereal and fat and further questions regarding the variety and quantity of dairy foods they consumed. The FFQ data were entered into a database (Oracle Corporation, USA), and processed using the FETA program (CAMB/PQ/6/1205)[17].

Stool sampling and fecal analysis methodology

Of the 288 stool samples requested, 32 samples could not be analyzed for technical reasons, leaving 256 for the microbiota analysis. The participants collected fresh feces in stool sample collection kits (Ridascreen, R-Biopharm Ag, Darmstadt, Germany, and Omnigene-GUT OM-200, DNA Genotek, Ottawa, Canada) and refrigerated them until they were transported to the laboratory within 12h of defecation. One milliliter of stool from each collection kit was added to 9 ml of sterile selective broth. The ten times diluted suspension sample was serially diluted 10^1 – 10^6 and 100 µml from each dilution was plated on Lactobacillus selection broth agar base and Lactobacillus selection bile agar base with glacial acetic acid to enhance the selective growth of Lactobacillus. All diluted stool samples

were also plated on Bifidobacterium selective agar base to facilitate designed to favor the growth of Bifidobacterium. After incubation, the number of bacterial colonies cultured in the MRS selective media was counted by a colony counter machine for each dilution (ProtoCol3, Synbiosis, Division of Synoptics Ltd., USA). The results were expressed as $\log_{10}$ of the number of bacteria per milliliter of feces (colony forming units/ml, CFU/ml). All these procedures were done using standard laboratory methods and certified assays in the Biomedical Laboratory of School of Bio Medicine at the Mongolian National University of Medical Sciences (MNUMS).

Species-specific polymerase chain reaction (PCR) analysis of *Bifidobacterium spp*.

Bifidobacterium spp DNA from cultivated samples was extracted with a standardized protocol using a PCR methodology. The PCR was performed using 25 µL PCR master mix (Choros Onosh company, Mongolia). A reaction mixture (25 µL) consisted of 2x reaction buffer, a 500 µM concentration of each deoxynucleic triphosphate, 0.2 µM of each primer, 50 ng of bacterial DNA (extracted from cultivated samples) and 1.5 U of Taq Plus DNA polymerase. The PCR was performed with PeqStar, and thermal cyclers 96x universal (PeqLab VWR brand, VWR International, Lutterworth, UK) using optical grade ninety-six well plates. The conditions were set as follows: 5 min at 94°C, followed by thirty-five cycles consisting of denaturation at 94°C for 20s, annealing 55°C for 20s, elongation at 72°C for 30s, and finally at 72°C for 5 min. Amplification products were detected by agarose gel electrophoresis on 1.5% agarose gel, using ethidium bromide staining, and UV transillumination (320 nm).

Species-specific PCR analysis of Lactobacillus spp.

Lactobacillus spp DNA from cultivated samples was likewise extracted with a standard protocol. The PCR was performed on a total volume of 25 µL of using PCR master mix (Choros Onosh LLC, Mongolia). Each reaction included 50 ng of template DNA, 25 µL of PCR master mix, and 2µM of each primer. The amplification program consisted pre-denaturation at 94°C for 4min, followed by thirty-five cycles of 94°C for 30s, 30s at the appropriate annealing temperature of 59°C, and 72°C for 30s. A cycle of 72°C for 5min concluded the program. Amplification products were detected by agarose gel electrophoresis on 1.5% agarose gel, ethidium bromide staining, and UV transillumination (320 nm).

References strains

The Bifidobacterium reference strains used in the study were: *B. adolescentis, B. angulatum, B. bifidum, B. breve, B.*

Table 1. Primers used for the detection of *Bifidobacterium spp*. and *Lactobacillus spp* in this study.

Target	Primers	Sequence (5'-3')	Amplicon length (bp)
B. adolescentis	BiADO-1 BiADO-2	`CTCCAGTTGGATGCATGTC CGAAGGCTTGCTCCCAGT	279
B. angulatum	BiANG-1 BiANG-2	CAGTCCATCGCATGGTGGT AAGGCTTGCTCCCCAAC	275
B. bifidum	BiBIF-1 BiBIF-2	CCACATGATCGCATGTGATTG CCGAAGGCTTGCTCCAAA	278
B. breve	BiBRE-1 BiBRE-2	CCGGATGCTCCATCACAC ACAAAGTGCCTTGCTCCCT	288
B. catenulatum group	BiCATg-1 BiCATg-2	CGCATGCTCCGAACTCCT CGAAGGCTTGCTCCCGAT	285
B. longum	BiLON-1 BiLON-2	TTCCAGTTGATCGCATGGTC GGGAAGCCGTATCTCTACGA	831
B. infantis	BiNF-1 BiNF-2	TTCCAGTTGATCGCATGGTC GGAAACCCCATCTCTGGGAT	828
B. dentium	BiDEN-1 BiDEN-2	ATCCCGGGGGTTCGCCT GAAGGGCTTGCTCCCGA	387
B. gallicum	BiGAL-1 BiGAL-2	TAATACCGGAGTTCCGCTC ACATCCCCGAAAGGACGC	303
B. lactis	Bflac2 Bflac5	GTGGAGACACGGTTTCCC CACACCACACAATCCAATAC	680

catenulatum group, B. longum, B. infatis, B. dentium, B. gallicum, and B. lactis. The Lactobacillus reference strains used in the study were *L. acidophilus, L. gasseri, L. fermentum and, L. casei*, and their detection primers are found in Table 1.

Statistical analysis

Descriptive statistics were used to describe the study participants age, education status, smoking status, alcohol consumption, antibiotic usage, and defecation frequency. Continuous numerical values were expressed as the mean ± SD. Normally distributed data were analyzed using the Student's t-test or ANOVA. To facilitate the ANOVA, participants were stratified into three age groups, and four groups based on the quantity of dairy products consumed per day. When ANOVA results were statistically significant, multiple post-hoc comparisons were

Table 2. Demographic characteristics of study participants stratified by where they lived.

Variables	Total % (n = 256)	Urban % (n = 116)	Rural % (n = 140)
Age group			
<35 years	40.7 (104)	44.0 (51)	37.8 (53)
35 - 54 years	44.9 (115)	34.5 (40)	53.6 (75)
>54 years	14.5 (37)	21.6 (25)	8.6 (12)
Education level			
Primary education	4.6 (12)	0.8 (1)	7.9 (11)
Secondary education	23.4 (60)	20.0 (22)	27.1 (38)
Higher education	71.9 (184)	80.2 (93)	65.0 (91)
Tobacco use			
Yes	19.1 (49)	22.4 (26)	16.4 (23)
No	80.9 (207)	77.6 (90)	83.6 (117)
Alcohol use			
Yes	75.8 (194)	80.2 (93)	72.1 (101)
No	24.2 (62)	19.8 (23)	27.9 (39)
Antibiotics use (most recent)			
More than >24weeks	1.6 (4)	1.7 (2)	1.4 (2)
Between 12 and 24 weeks	5.1 (13)	5.2 (6)	5.0 (7)
Less than 12 weeks	14.8 (38)	16.4 (19)	13.6 (19)
No	78.5 (201)	76.7 (89)	80.0 (112)
Defecation frequency			
>3/day	2.3 (6)	17.4 (2)	2.9 (4)
1 - 2/day	57.4 (147)	50.0 (58)	63.5 (89)
4 - 6/week	21.1 (54)	29.3 (34)	14.3 (20)
2 - 3/week	16.5 (42)	17.2 (20)	15.7 (22)
~1/week	2.7 (7)	1.7 (2)	3.6 (5)
Region	Number of Subjects	Mean Age	Gender ratio
Total	256	38.9	94:162
Ulaanbaatar	116	38.5	44:72
Orkhon	23	37.4	6:17
Umnugovi	13	36.2	4:9
Tuv	25	40.8	9:16
Sukhbator	15	39.7	4:11
Uvs	35	42.4	17:18
Khuvsgul	29	36.7	10:19

performed using the Tukey test. The amounts of Lactobacillus and Bifidobacterium were found to be non-normally distributed and consequently underwent log transformation. Correlations between independent variables were determined using Pearson's method. Multiple linear regression analyses were used to determine the associations between Lactobacillus cell counts and continuous variables. To guard against Type II statistical error, the number of participants required for this study was determined using a power analysis. A study error of 5% was chosen with the CI 1.96% leading to a minimum sample size of 245 to have a 90 percent probability of detecting a statistically significant difference if present. All statistical analyses were performed using SPSS (IBM Inc., USA). A p-value < .05 was considered statistically significant.

Ethical statements

The research methodology and recruitment procedure were reviewed by Department of Gastroenterology and Hepatology (№18/18, March 06, 2018) and the Academic Senate of School Medicine at the Mongolian National University of Medical Sciences (March 30, 2018). Institutional Review Board approval was obtained from the Medical Ethics Committee (2018/03-7) at the MNUMS. Written Informed consent was obtained from all study participants.

Results

Subjects

A total of 256 healthy participants (162 females, 94 males, 18 - 70 years of age) were enrolled in the study. There were participants from seven regions in Mongolia with most of them being from the capital city of Ulaanbaatar since most of the population is centered there. The demographic characteristics of the cohort stratified by urban versus rural living location are shown in Table 2.

Participants had a mean age of 38.9 ± 12.8 years. Their anthropometric characteristics are provided in Table 3.

Determination of intestinal Lactobacillus and Bifidobacterium abundance

For all participants, the amounts of the Lactobacillus and Bifidobacterium were measured from cultured samples. The mean amount of Bifidobacterium and Lactobacillus for all participants were 6.24 ± 0.94 and 5.9 ± 1.28 $\log_{10}$ CFU/ml (4.66×10^6 and 4.67×10^6 CFU/ml) respectively. The amount of Lactobacillus for participants Ulaanbaatar, Orkhon, Umnugovi, Tuv, Sukhbator, Uvs and Huvsgul were 5.73 ± 1.33, 5.55 ± 1.34, 4.76 ± 1.30, 6.17 ± 0.99, 6.55 ± 0.56, 6.55 ± 0.72 and 6.89 ± 0.38 $\log_{10}$ CFU in log-transformed colony number per ml of samples, respectively (p = .001) (Figure 1A). The amounts of Bifidobacterium in participants from Ulaanbaatar, Orkhon, Umnugovi, Tuv, Sukhbator, Uvs and Huvsgul were 6.40 ± 0.69, 5.64 ± 1.39, 4.84 ± 1.03, 6.54 ± 0.91, 5.62 ± 1.17, 6.25 ± 0.76 and 6.58 ± 0.80 $\log_{10}$ CFU/ml in log-transformed colony number per ml of samples, respectively (p < .0001) (Figure 1B). Of the 173 participants, the Lactobacillus cell count was significantly lower in the less than 34 years of age group (5.67 ± 1.27 $\log_{10}$ CFU/ml) than the two older groups (p = .047, p = .022) (Table 4). The was no difference in Bifidobacterium cell count between age groups (p = .414).

Table 5 shows the average daily nutrient intake from the food frequency questionnaire.

Association between dietary habits and intestinal microbiota

In Table 6, the amount of Lactobacillus had a moderate positive correlation with the grams of fiber consumed per day

Table 3. Anthropometric variables of the study population, stratified by where they lived.

Variables	Total	Urban		Rural		p-value*
	Mean ± SD	Mean ± SD	95% CI	Mean ± SD	95% CI	
Age (years)	38.9 ± 12.8	38.5 ± 14.8	37.8 - 41.2	39.2 ± 10.8	37.4 - 41.0	.653
Height (cm)	163.6 ± 7.8	164.2 ± 7.7	162.8 - 165.6	163.1 ± 8.0	161.7 - 164.4	.259
Weight (kg)	70.6 ± 14.5	68.3 ± 13.8	65.8 - 70.9	72.4 ± 14.9	69.9 - 74.9	.024
BMI (kg/m²)	26.3 ± 5.0	25.3 ± 4.4	24.4 - 26.1	27.2 ± 5.3	26.3 - 28.1	.001
Waist circumference (cm)	87.2 ± 16.0	86.0 ± 17.9	82.7 - 89.3	88.1 ± 14.3	85.7 - 90.5	.313
Fat (%)	28.5 ± 8.0	27.7 ± 7.1	26.4 - 29.0	29.1 ± 8.6	27.7 - 30.6	.138

*p-value comparing the urban and rural groups using the Student t-test.

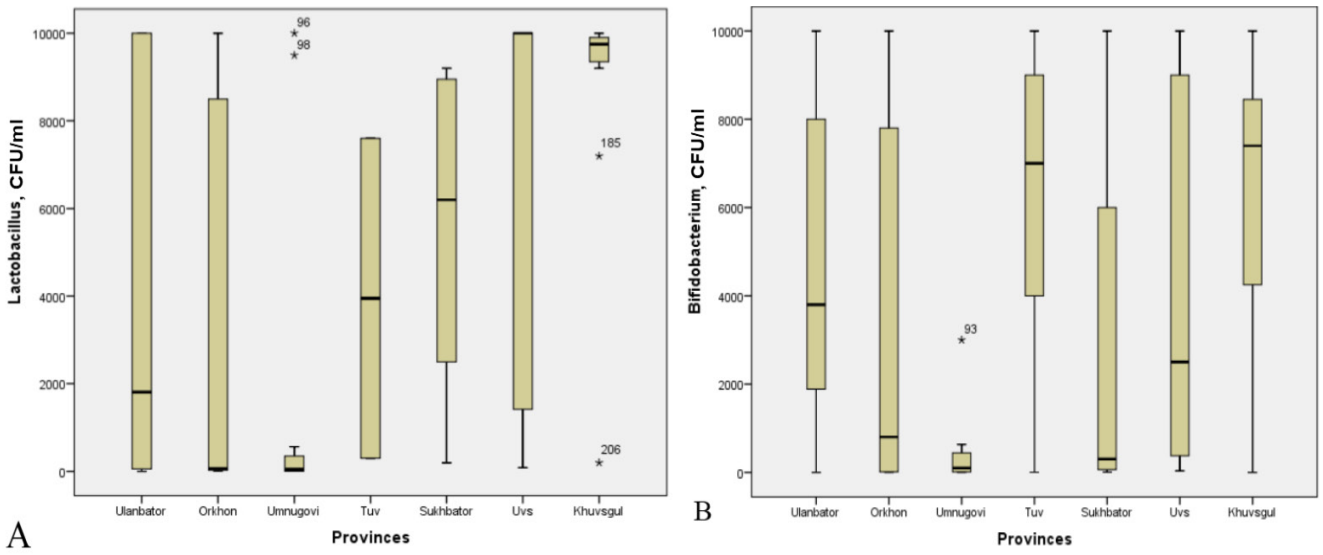

Figure 1. The amounts of Lactobacillus (A) and Bifidobacterium (B) stratified by where they lived.

Table 4. The amount of Lactobacillus, by age group.

	Participants n = 173 (%)	Lactobacillus Mean ± SD	*p-value	Variable interval	**p-value
Age groups					
<35 years	73 (45)	5.67 ± 1.27	.011	<35 x 35-54 years	.047
1-54 years	72 (23.75)	6.09 ± 1.20		35 - 54 x >54 years	.998
>54 years	28 (31.25)	6.07 ± 1.42		<35 x >54 years	.022

*p-value using comparing age groups using ANOVA.
**p-value comparing age groups using Tukey test.

Table 5. Mean daily nutrient intake estimated by the printed EPIC-Norfolk food frequency questionnaire.

Variables	Total (n = 256) Mean ± SD	Urban (n = 116) Mean ± SD	Rural (n = 140) Mean ± SD	p value*
Meat (g/day)	179.4 ± 152.7	192.0 ± 175.2	169.1 ± 131.0	.431
Fats (g/day)	30.8 ± 29.0	32.3 ± 29.5	29.5 ± 28.6	.179
Fish (g/day)	11.5 ± 56.5	14.2 ± 60.9	9.3 ± 52.7	.067
Fruit (g/day)	103.5 ± 160.5	122.8 ± 202.8	87.4 ± 112.5	.031
Cereal (g/day)	342.2 ± 384.0	359.1 ± 299.0	328.1 ± 442.9	.019
Vegetables (g/day)	289.5 ± 300.8	322.4 ± 373.0	262.4 ± 221.9	.484
Milk (g/day)	344.6 ± 245.8	313.3 ± 232.6	370.5 ± 254.2	.078
Potato (g/day)	102.3 ± 143.1	110.7 ± 145.2	95.3 ± 141.5	.020
Soup and sausage (g/day)	156.3 ± 182.6	183.4 ± 216.4	133.8 ± 146.1	.76
Sugar (g/day)	53.5 ± 60.0	61.9 ± 63.3	46.6 ± 56.5	.010

*p-value comparing urban and rural groups using Student's t-test.

(r = .451, p = .04). It also had weakly positive effect on the abundance of Bifidobacterium (r = .210, p = .04). However, the number of lactobacilli had a weak negative correlation with the number of grams consumed per day of fats, potatoes and cereal grain foods (r = -.20, p < .0001; r = -.16, p = .040; r = -.18, p = .01).

Table 6. Pearson correlation matrix between food type, anthropometric measurements, and intestinal microbiota.

	Lacto-baciilus	Bifido-bacterium	Cereal grain	Fiber	Fat	Fruit	Meat	Potato	Vegetable	WC	BMI	Body fat
Lactobacillus	1											
Bifidobacterium	.21*	1										
Cereal grain	-.18*	.03	1									
Fiber	.45*	.256	-.09	1								
Fat	-.20**	-.01	.49**	.19	1							
Fruit	-.01	-.15	.28**	.12	.26**	1						
Meat	.03	-.04	.28**	-.03	.25**	.23**	1					
Potato	-.16*	-.08	.57**	-.11	.31**	.05*	.18**	1				
Vegetable	-.04	.04	.34**	.45*	.43**	.49**	.29**	.24*	1			
WC	.08	.08	-.02	.43	-.01	.01	-.03	-.11*	.10	1		
BMI	.19*	.15*	-.07	.45*	-.09	-.05	-.03	-.16*	-.02	.60**	1	
Body fat	.08	.10	.04	.24	.04	.02	-.05	-.01**	.00	.48**	.53**	1

*- *p* value <.05, **- *p* value <.01
WC – waist circumference, BMI – body mass index.

All ethnic Mongolians consume a similar diet consisting of traditional dairy products and red meat as their primary foods. This includes frequent consumption of fermented and non-fermented dairy products, red meat with animal fat, and liquor from other nationalities. Hence, we determined the quantity of dairy consumed per day using the modified questionnaire. Based on dairy foods frequency questionnaire, 117 (45.7%) reported less than 50ml intake of milk per day, 92 (32.9%) reported 50-300ml intake of milk per day and 34 (13.3%) reported above 300ml intake of milk per day, while only 13 (5.1%) reported no daily consumption of milk products. Statistically significant

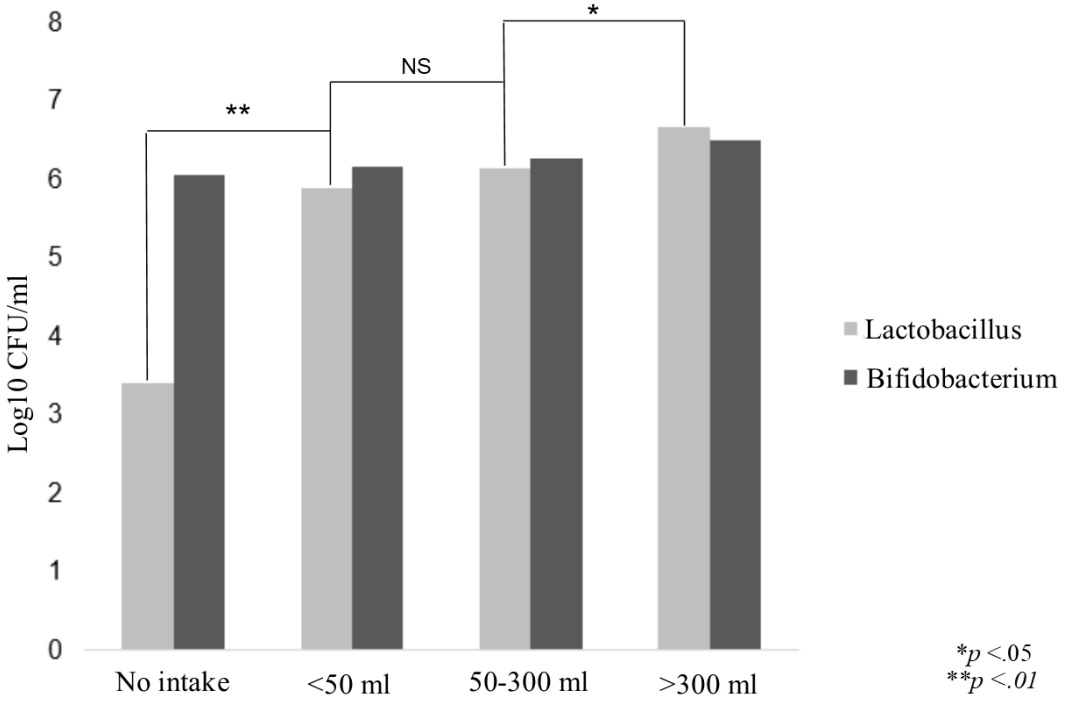

Figure 2. The quantity of Lactobacillium based on milk intake.

differences in the amounts of Lactobacillus were observed between all groups of milk quantities per day (Figure 2). But the amount of Bifidobacterium was not influenced by the intake frequency of foods (Table 6).

Correlations between body measurements and intestinal microbiota

Higher BMIs were associated with more frequent defecation ($p = .002$) and the amounts of Lactobacillus and Bifidobacterium were very weakly positively associated with BMI ($r = .192$, $p = .01$; $r = .148$, $p = .023$, respectively) (Table 6). But the amounts of Lactobacillus and Bifidobacterium were not associated with body fat or waist circumferences ($p > .05$).

Regression of some factors affecting Lactobacillus counts

With Lactobacillus counts as the dependent variable, and Bifidobacterium counts, BMI, and the quantity of milk, cereal grains, fats and potatoes consumed per day as independent variables, multiple regression analysis revealed that the amount of milk, fiber, and fats consumed per day were associated with counts of cultivable Lactobacillus (Table 7).

Identifying Intestinal *Bifidobacterium spp*. and *Lactobacillus spp*.

Bifidobacterium species-specific PCR analysis was performed with 107 cultivable samples. Interestingly, the prevalence of *Bifidobacterium spp*. was 30% (n = 32) as shown in Figure 3. The species *B. bifidum, B. angulatum, B. adolescentis* were the most commonly identified at 31 (29%), 4 (13.1%) and 10 (9.3%) followed by *B. catenulatum group, B. longum, B. lactis* at 10 (9.3%), 9 (8.4%) and 7 (6.5%), respectively. Less common were *B. breve, B. dentium and B. gallicum* at 6 (5.6%), 5 (4.7%), and 3 (2.8%) respectively. But *B. infantis* was the least common and not was detected in any participant.

Lactobacillus species-specific PCR analysis was performed with 104 cultivable samples. But none of the reference strains (*L. acidophilus, L. gasseri, L. fermentum, L. casei*) were identified in any of the study participants.

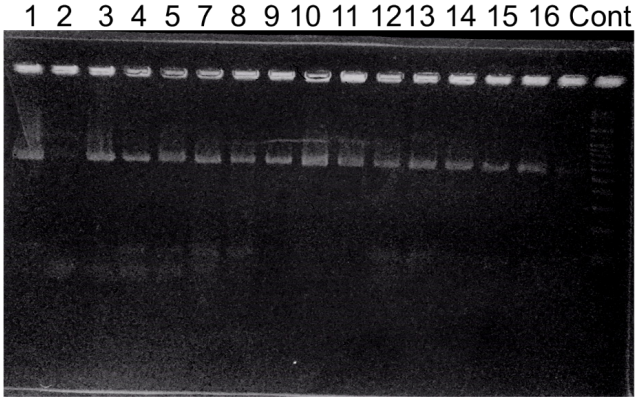

Figure 3. PCR results for the *Bifidobacterium spp*.

Discussion

Gut microbiota plays a vital role in the health and wellbeing of humans. The gut microbiota of a healthy individual contains a

Table 7. Regression results of dietary factors, BMI, and Bifidobacterium on Lactobacillus counts.

Variables	Lactobacilli, log_{10} CFU/ml			
	β	95% CI	p-value	VIF
Bifidobacterium	0.17	[-0.03 ; 0.36]	.109	1.81
BMI	0.03	[-0.01 ; 0.07]	.158	2.42
Milk intake	0.74	[0.49 ; 0.99]	.000	1.98
Cereal grains	-0.001	[-0.001 ; 0.001]	.436	2.18
Fiber	0.01	[0.001 ; 0.02]	.045	1.60
Fats	-0.01	[-0.02 ; - 0.002]	.012	1.94
Potato	0.001	[-0.001 ; 0.002]	.399	3.12
R^2	.27	Mean VIF	2.15	
F value	8.79			

hundred trillion bacteria[3,18,19]. The richness and diversity of the human microbiota are affected by many factors, such as mode of birth, diet, medications, age, and ethno-geographic area[3,15,20]. In this study, we aimed to determine the amount of Lactobacillus and Bifidobacterium in the fecal samples of relative healthy Mongolian adults residing in various regions of Mongolia using the conventional culture method. We then aimed to determine if there were differences in fecal microbiota associated with the individual's rural or urban lifestyle, age, and their demographic information combined with a dietary questionnaire.

Our study demonstrated several important findings regarding Mongolian adults. Previous researchers have identified Lactobacillus in more than 90% of people using real-time qPCR with a sensitivity of 10^2 - 10^4 cells/g feces using the non-culture method. Using this method, they found Lactobacillus in 98% of feces from healthy adults[21,22]. The true Lactobacillus colonization rate may be closer to 100%. In this study, we found the mean population count of Lactobacillus in adults was 4.67 x 10^6 CFU/ml ($\log_{10}$ 5.9 ± 1.28 CFU/ml) feces. This result is similar to those commonly reported in the past using culture-based methodology[22]. In previous studies, data about the ecosystem of the Bifidobacterium in the gut of individuals were rare and incomplete because there may exist of up to four or even five different Bifidobacterium species in humans[22]. Our study indicates that the counts of most Bifidobacterium species may be above the normal the detection level (≥6.0 $\log_{10}$ CFU/ml)[22]. We found that mean population count of Bifidobacterium in adults was near 4.66 x 10^6 CFU/ml ($\log_{10}$ 6.24 ± 0.94 CFU/ml) feces. This result is similar to the results commonly reported in the past using culture-based methods[22,23]. These results may be useful in the future to establish a diagnosis of dysbiosis in Mongolian adults.

An interesting finding of our study was differences in the amount the Lactobacillus between age groups. Lactobacillus counts were highest in the youngest group, adults less than 35 years of age (Table 5). This result is similar to the study by Mitsuoka and colleagues[24]. But the counts of Bifidobacterium did not differ with age. Previous studies have suggested that the composition of gut microbiota is relatively stable during maturity[25]. But the community composition of gut microbiota could be affected by genetics, morbidity, dietary habits, aging, and treatment choices[10,26]. In culture studies of Lactobacillus and Bifidobacterium related to aging, Mitsuoka found that Bifidobacterium decreased as adults aged, whereas *C. perfringens*, Lactobacillius and Enterococci increased[24]. On the other hand, Hopkin and colleagues reported that Lactobacillus and Bifidobacterium were lower in younger adults[27]. A limitation of our study is that we did not study younger age groups.

An important finding our study was that we found that amounts of Lactobacillus and Bifidobacterium varied significantly between provinces. These geographic differences in the profile of the Lactobacillus and Bifidobacterium have to be considered as we consider what a healthy microbiome is.

Some studies have shown that gut microbiota fluctuates in response to a variety of host parameters and environmental factors, including genetic makeup, age, diet, and stress[28-31]. Of all these factors, age and diet have been suggested to be the components that exert the most significant effect on homeostasis of gastrointestinal micro-ecosystem. In this study, the quantity of Lactobacillus had a moderately positive correlation with the number of grams of fiber consumed per day ($r = .451$, $p = .04$). The abundance of Lactobacillus had a weakly negative correlation with the daily quantity of fats, potatoes and cereal grain foods consumed ($r = -.20$, $p < .0001$; $r = -.16$, $p = .040$, $r = -.18$, $p = .01$ respectively). Also, significant differences in the amount of Lactobacillus were observed depending on the amount of dairy products consumed per day. We also found that the abundance of Lactobacillus was weakly but positively correlated with the amount of Bifidobacterium ($r = .210$, $p = .04$). In all, these findings were similar results to the study of Wenjun Liu and colleagues[16]. Previous researchers have established that Lactobacillus stimulated the colonization of Bifidobacterium[32]. Thus, this relation between Lactobacillus and Bifidobacterium may be linked to the consumption of fermented foods. Changes in diet composition have been associated with changes in the composition and metabolism of gut microbial populations. Our study showed a dietary link with the count of Lactobacillus, but the connection between nutritional factors and gut lactobacillus counts have not fully elucidated among Mongolians.

In one recent study, dietary amylase trypsin inhibitors (ATIs) of wheat grain were negatively correlated with intestinal Lactobacillus[33,34]. This might be caused by the degrading of ATIs by the lactobacilli, and this function may be important in the homeostasis of gut microbiota[34]. In general, the Lactobacillus of the gastrointestinal tract may be an immunomodulatory bacterium. Additionally, the ATIs are known to trigger

intestinal inflammation via overexpression of pro-inflammatory cytokines[34]. Our study suggested that long term high fiber intake may synergistically influence the composition of Lactobacillus and Bifidobacterium. But long-term fat or wheat/carbohydrate intake may be negatively affecting the balance of Lactobacillus, but it is unclear how by what mechanisms these influence the human gut microbiota in Mongolians.

In our study, we performed species-specific PCR analyses for *Bifidobacterium spp*. and *Lactobacillus spp*. We identified all ten Bifidobacterium species in our study that have been previously described as members of the fecal microbiota of humans. Our results showed that *B. bifidum, B. angulatum, B. adolescentis, B. catenulatum group, B. longum* were the more common species in Mongolians which agrees with previous studies. Previous studies have reported that in the adult intestinal tract, the *B. cateculatum group* was the most common taxon, followed by *B .longum, B. adolescentis* and *B. bifidum*[35]. In our study, *B. infantis* was not identified in any of the samples, which is similar to previous studies in adults. *B. infantis* and *B. breve* are predominant species in the intestinal tract of human infants[36]. Although fecal samples from infants are dominated by a relatively small number of different Bifidobacterium species and strains, they do reveal a high between-individual variation in taxonomic composition. In contrast, the Bifidobacterium populations isolated from adults seems more complex, even though they exhibit remarkable conservation of Bifidobacterium species and strains[37].

The four lactobacillus species we identified in the present study were previously described as members of the fecal microbiota of humans. But these members of *Lactobacillus spp*. were not detected in all samples. The composition of intestinal Lactobacillis varies widely between persons and location in the gastrointestinal tract. In another study, *L. acidophilus, L. salivarius, L. paracasei, L. casei, L. plantarum, L. brevis* and *L. fermentum* were the most common species in both age groups[38]. Perhaps the frequent consumption fermented dairy products and protein-rich foods such as red meat are the reason why uncommon species of Lactobacillus colonize in the gastrointestinal tract of Mongolian adults. The species *L. sakei* is one of the predominant food-associated Lactobacillus species that occurs in human feces[39,40]. *L. sakei* has been isolated from meat, sausages and sauerkrauts, also used for the production of fermented meat products[41]. This may be why uncommon Lactobacillus species like a *L. sakei* are the predominant colonizers in adult Mongolians.

While our study identified several novel findings, we do note some limitations. First, we studied the only subjects 18 - 70 years of age, and we are unable to draw any conclusions in people or younger than this. Our sample size was small, prohibiting us from drawing conclusions about people living in other areas of Mongolia. We studied only the effect of diet, age, and where one lives on the microbiota of only healthy adults and are unable to define what constitutes dysbiosis in Mongolian adults. Finally, our analysis involved frozen stool samples and was performed using a culture method in contrast to the non-culture techniques using real-time qPCR, which likely are more sensitive.

It will be essential to the field of gastrointestinal research in Mongolia to further understand the Lactobacillus and Bifidobacterium strains for their use as probiotics with gastrointestinal or liver diseases and dysbiosis in Mongolian adults.

In conclusion, the quantity of Lactobacillus in healthy adults was higher in the region of Khangai, Eastern, and Western Mongolia than in other areas. We found significant correlations between the consumption of fiber, fats, potatoes, and the amount of stool Lactobacillus. The composition of Lactobacillus was also altered. The most prevalent Bifidobacterium genera were *Bifidobacterium spp., B. bifidum, B. angulatum, B. adolescentis*.

Conflict of Interest

The authors declare that there are no competing interests related to this study.

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