MASTER'S THESIS

Fermented Coffee Selectively Increased the Abundance of *Prevotella copri* in the Human Gut

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A thesis submitted in partial fulfillment of the requirement for the degree of Master of Science

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This thesis has been examined and approved.

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ABSTRACT

Fermented foods such as kimchi and yogurt are generally known to have beneficial effects on our gut and interest in fermented foods are increasing, which increased a number of fermented food products. And, coffee is a beverage extracted from processed coffee beans, which had become one of the most widely consumed favorite drinks in the world. However, it is known that excessive consumption of coffee can cause caffeinism, such as emotional anxiety, nervousness, sleep disturbance, gastrointestinal disorders. Based on these results, the Fermentation Industry (Sunchang, Korea) developed and commercialized coffee that ferments coffee using *Lactobacillus spp.* and *Bacillus spp.* increases functionality and reduces the side effects of coffee by lowering caffeine content.

In this study, we aimed to investigate the microbial ecology of the human gut microbiota before and after drinking fermented coffee. Stool samples were collected three times a week, for 6 weeks. During the first two weeks, a total of 20 subjects kept their normal diet and had 2 cups of fermented coffee every day for the rest of 2 weeks. After drinking fermentation coffee, a total of 20 subjects again kept their normal diet and non-drinking fermentation coffee. *Prevotella copri* was increased by fermented coffee in subjects with low abundance of *Prevotella copri*, and it was confirmed that increased *Prevotella copri* was involved in various metabolism. In this study, however, the practical effect of metabolism associated with increased *Prevotella copri* by fermented coffee was not evaluated. Therefore, further experiments on the effect of *Prevotella copri*, which is increased by fermented coffee, on the human body are considered to be necessary.

Introduction

Three out of five health foods selected by Health magazines in the U.S. in 2006 are fermented foods and recently, as physiological activity by the fermentation has been known, fermented foods are recognized worldwide as health functional foods(Park 2012). Especially probiotics such as *Lactobacillus spp.* and *Bacillus spp.* have been defined as beneficial microbes to the host (Fuller 1989). These beneficial effects includes the increase of pathogenic microbial inhibition, anti-mutagenic and anti-cancer, growth-promoting factors, and immune responses (Verschuere, Rombaut et al. 2000). In addition, these microbes have long been directly and indirectly related to human life according to their characteristics, ranging from fermented dairy products to spices, kimchi, fermented sausages, medicines, and feed additives of livestock (Kim, Lee et al. 2009, Hong, Lim et al. 2013). Fermented foods have better flavor than conventional food and produces bacteriocin, a microorganisms inhibition substance, which is anti-microbial activity and produces a large amount of lactic acid, which acts as a deterrent to the growth of the bacteria in the food (Matsumura, Takeuchi et al. 1997). Previous studies have reported a reduction in mortality cardiovascular disease (CVD) and type 2 diabetes (T2D) by ingesting one of the fermented foods, yogurts (Soedamah-Muthu, Masset et al. 2013, Chen, Sun et al. 2014, Tapsell 2015). Research on anti-diabetes and anti-obesity effects of kimchi have also been reported (An, Lee et al. 2013). In fermentation of plant based food, the expression of decarboxylase, glycosyl hydrolase, phenolic acid, and esterase reductase increased by lactic acid bacteria to facilitate the conversion of phenolic compounds such as flavonoid into biologically active metabolites (Filannino, Bai et al. 2015).

Coffee is a dicotyledonous plant belonging to the genus *Rusbeaceae*, and commercially cultivated varieties can be largely divided into Coffea Arabica L. and Coffea canephora (Martın, Pablos et al. 1999). It is a beverage, made from processed coffee beans and

become one of the most widely consumed favorite foods in the world (Schilter, Cavin et al. 2001, Anderson and Smith 2002). It is known that coffee has free radical scavenging ability to prevent cell damage because of higher antioxidant contents such as polyphenols, compared to other foods (Borrelli, Visconti et al. 2002, Sánchez-González, Jiménez-Escrig et al. 2005). The ingredients of coffee contain caffeine, trigonelline, and chlorogenic acid, which are known to be effective to prevent or prevention of chronic diseases and extends the life (Van Dijk, Olthof et al. 2009, Chu 2012). Especially, caffeine stimulates the central nervous or muscles, giving a feeling of freshness or excitement, and restores energy level of the body or awareness (Corti, Binggeli et al. 2002). However, it is known that excessive consumption of coffee can cause caffeinism, such as emotional anxiety, nervousness, sleep disturbance, gastrointestinal disorders (Greden 1974). Previous studies also have reported that caffeine increases blood pressure and constricts blood vessels (Daniels, Molé et al. 1998, Mahmud and Feely 2001), and causes side effects such as bone loss in women after menopause (Rapuri, Gallagher et al. 2001). Like this, coffee has a lot of controversies about being double-sidedness due to caffeine.

Coffee cherries are fermented to enhance functionality by effectively removing the mucilage layer that covers the coffee beans before the drying process(Silva, Batista et al. 2008). Moreover, it has been reported that additional fermentation with yeast increases antioxidants such as polyphenols and flavonoids in coffee beans(Kwak, Jeong et al. 2018). Previously, the Fermentation Industry (Sunchang, Korea) developed and commercialized a coffee using *Lactobacillus plantarum* and *Bacillus amyloliquefaciens*, which increases its functionality and reduces the side effects of coffee by lowering caffeine content (Figure 1, Table 1). Therefore, this study aims to objectively evaluate the change in intestinal microbial ecology by fermented coffee, based on the previously explored biologically active compounds in fermented coffee developed by Fermentation Industry (Sunchang, Korea) to the human body in general life.

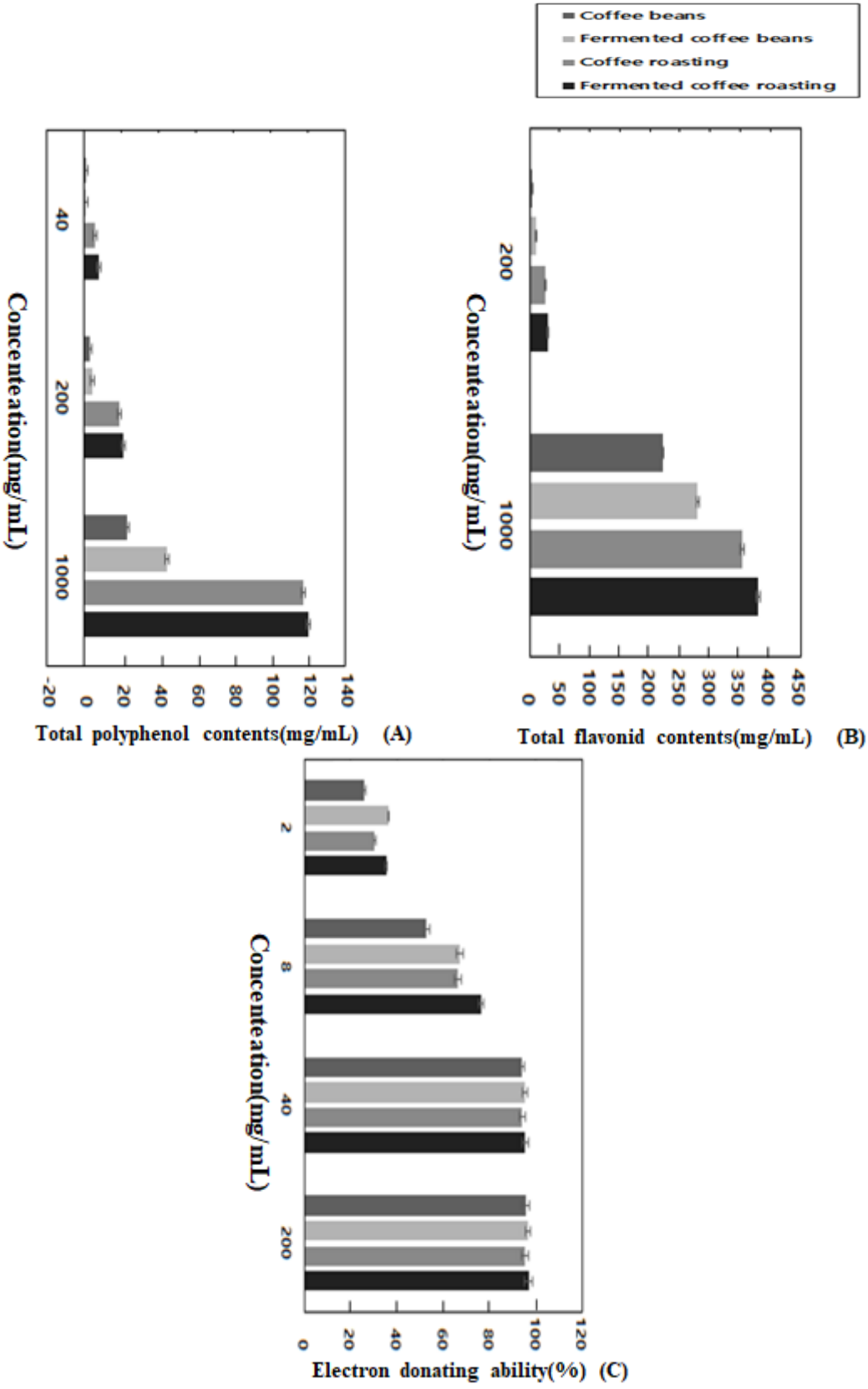


Figure 1. Results of analysis of fermented coffee ingredients: (A) Total polyphenol contents (mg/L); (B) Total flavonoid contents (mg/L); and (C) DPPH radical scavenging activity.

Table 1. Chlorogenic acid, caffeic acid and caffeine content of fermented coffee.

Samples	Caffeine	Caffeic acid	Chrologenic acid
			(mg/L)
Fermented coffee roasting	**819.69±11.49**	**24.81±1.31**	**736.57±6.67**
Coffee roasting	**875.38±29.04**	**24.55±0.17**	**640.10±22.84**
Fermented Coffee beans	**664.73±20.91**	**24.57±0.08**	**2660.17±47.48**
Coffee beans	**754.50±20.08**	**23.10±0.29**	**2332.04±21.11**

Values are presented as mean ± SD.

MATERIALS AND METHODS

Manufacturing process composition analysis of fermented coffee

Coffee beans (Brazil, Colombia, Costarica, Kenya) were purchased from woosungmf Inc. (Hwaseong, Korea). The coffee beans were soaked in water with a ratio of 1:1.5 for 1 hour and pressure-cooked at 121℃ for 30min. After that, the cooldown step was carried out, and coffee was fermented using two kinds of microbes, *Bacillus amyloliquefaciens* SRCM101368 (2%) and *Lactobacillus plantarum* SRCM100320 (2%). Brazilian coffee beans were fermented using *B. amyloliquefaciens*, while Colombian, Costarica and Kenyan coffee beans were fermented using *L. plantarum*. After fermentation, they was rinsed and then dried in a heated-air dryer at 45℃ for 24 hours. Finally, Brazilian, Colombian, Costarica and Kenyan blended coffee beans were mixed with a ratio of 4:4:2:2 (Table 2).

Table 2. Mixing ratio of fermented coffee

Samples	Use strain	Mixing ratio
Brazil	*B. amyloliquefaciens*	4
Colombia		4
Costarica	*L. plantarum*	2
kenya		2

Experiment design

In total, 20 subjects (10 men, 10 women) participated in the study and included who normally consume ordinary coffee and those who do not. But one of the subject who get acute enteritis during the experiment was excluded from the experiment. The study was approved by the Bioethics Committee (IRB) of Jeju National University (JJNU-IRB-2017-035-002).

This clinical trial was performed for a total of 6 weeks and was divided into three sessions per 2 weeks. In the first period, Subjects did not take fermented coffee for 2weeks (1week-2week). And started at 3week by consuming fermented coffee two or more cups per day till 4th week (3week-4week). During this period, when they were consuming fermented coffee, normal coffee was prohibited. However, subjects who showed side effects after consuming fermented coffee allowed to consume only one cup per day. The experiment of fermented coffee was discontinued in 5week-6week.

The subjects were not receiving any treatment for hypertension, dyslipidemia or diabetes and there was no chronic or acute enteritis, cancer, inflammatory diseases viral infections event except for one subject in the present study. Subjects were prohibited from consuming drugs, alcohol, stimulant foods, and health supplements as vitamins that could affect the intestinal microbial ecology during the experimental period. During the period when no fermented coffee was consumed, normal coffee consumption was permitted, and diet information were received from the subjects during the experiment. The subject's diet was divided into nine categories: vegetable, fruit, grains, meat, fish, seafood, flour, instant foods, and others, based on the main food in a meal. In addition, we measured BMI (Body Mass Index) by receiving information regarding the height and weight of each subject. BMI was calculated by dividing the body weight with the square of the height (kg/㎡) and, the obesity

criteria was applied in accordance with the World Health Organization of Asia-Pacific region standards (Organization 2000, Who 2004).

Fecal sampling and DNA extraction

During the course of this study, we asked each subject to collect their feces two or three times a week, and with an interval of at least one day for intestinal microbial ecology analysis. Every fecal sample from each subject was collected using the OMNIgene-Gut kit OMR-200 (DNA Genotek, Ontario, Canada), and only healthy feces were sampled as diluted or more liquid containing fecal samples were rejected. Total DNA was extracted from 200ul of feces using the MOBIO Power Fecal DNA isolation kit (MO BIO Laboratories Inc., Carlsbad, CA, USA).

Miseq preparation

V4 region of 16S rRNA gene was amplified by Polymerase Chain Reaction (PCR) for microbial community analysis, and libary was produced in accordance with Miseq platform, one of the Iluminosis Sequencing Platform through 2-step PCR. Briefly, first PCR was performed using a KAPA HiFi HotStart ReadyMix PCR kit (Kapabiosystems, USA) as follows: 95°C for 3 min, 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and 72°C for 5 min. The obtained PCR products were further purified using a HiAccuBead (Accugene, Korea). The purified PCR products were again subjected to a barcode sequence for sample

identification using PCR. The primers were removed in the same method as previously for PCR product purification, and the final PCR product concentration of each sample was measured by the Qubit assay (Invitrogen, USA). The final PCR products of all samples were collected in an e-tube with the same concentration, and sequencing methods were performed at Macrogen Inc. (Seoul, Korea).

Miseq data analysis

Sequence data obtained from MiSeq was analyzed by MOTHUR software on a server (Dell PowerEdge R920, Memory 2TB, Hard 12TB) that we have in our laboratory (Schloss, Westcott et al. 2009). Clustering was performed with 97% similarity using Opti.clust and designated as operational taxonomic units (OTU). Each OTUs was classified to the Species level according to the Green gene database (version 13.8). The distance between samples was calculated using the Bray-Curtis method, one of the statistical methods, and visualized using the MOTHUR "tree.shared" command. OTUs or Taxa with significant differences between groups were investigated using the LefSe (Linear dissociant analysis Effect Size) and community types were estimated using NMDS(Non-metric multidimensional scaling) model of the MOTHUR (Segata, Izard et al. 2011, Holmes, Harris et al. 2012). Metabolic changes correlated with bacteria were investigated using the “otu.association” command, which calculates the correlation coefficient between Otu or Taxa and metadata of the MOTHUR. At using this command, metadata used PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) data, which is used to predict the abundance of functional categories based on 16S rRNA (Langille, Zaneveld et al. 2013). Heatmap used function “heatmap.2” of R software.

Statistical analyses

Data are expressed as mean ±Standard Error of the Mean (SEM). Statistical significant differences were determined by Welch's t-test of STAMP (Statistical analysis of metagenomics profiles) (White, Nagarajan et al. 2009).

RESULTS AND DISCUSSION

Differences in the intestinal microbiota among subjects in this study

Using the first two-week samples, types of gut microbiota in each subject were investigated. Results in Figure 2 suggest that gut microbiota of the subjects were divided into three groups regardless of obesity and amount of coffee intake. Subject's sex, BMI, height, weight and age were not associated with these clusters (Table 3).

Differentially abundant genera among the three clusters were identified using LEfSe and summarized in Figure 3A. It has been reported that human gut microbiota can be largely divided into *Prevotella* and Non-*Prevotella* type(Wu, Chen et al. 2011, Gorvitovskaia, Holmes et al. 2016). Our results indicated that most of the *Prevotella* type human gut microbiota were in the Cluster III. NMDS analysis at the OTU level shows that Cluster I group was correlated with the abundance of *Coprococcus* sp., and *Sutterella* sp.; Cluster II group was correlated with the abundance of *Bacteroides* sp., and *Bacteroides uniformis*; and Cluster III group was correlated with the abundance of *Prevotella copri*, *Ruminococcus* sp., and *Oscillospira* sp. (Figure 3B). At the phylum level, Cluster III group had more Bacteroidetes and fewer Actinobacteria compared to other groups (Figure 4A). At the family level, the abundance of Prevotellaceae were most abundant in the Cluster III (Figure 4B).

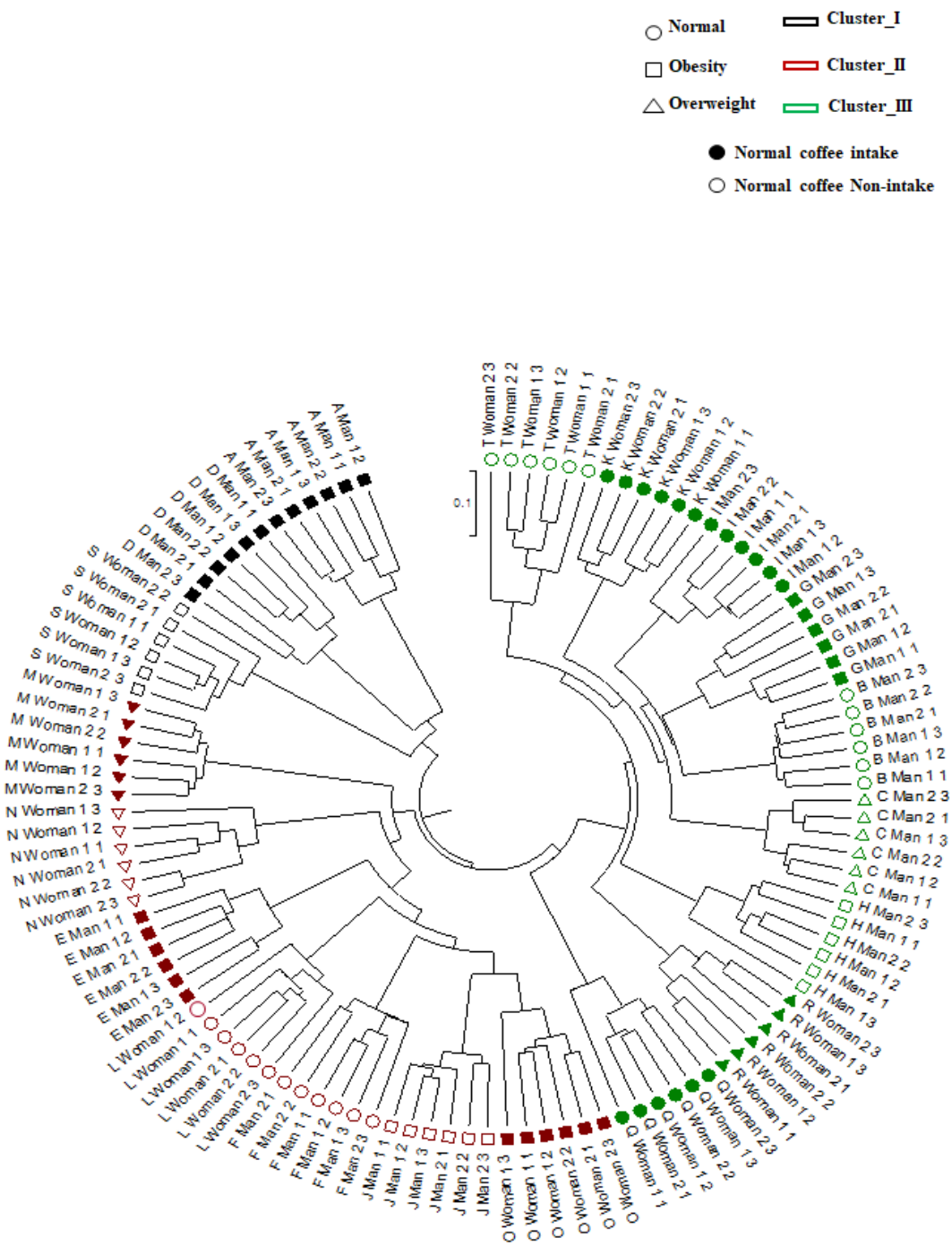
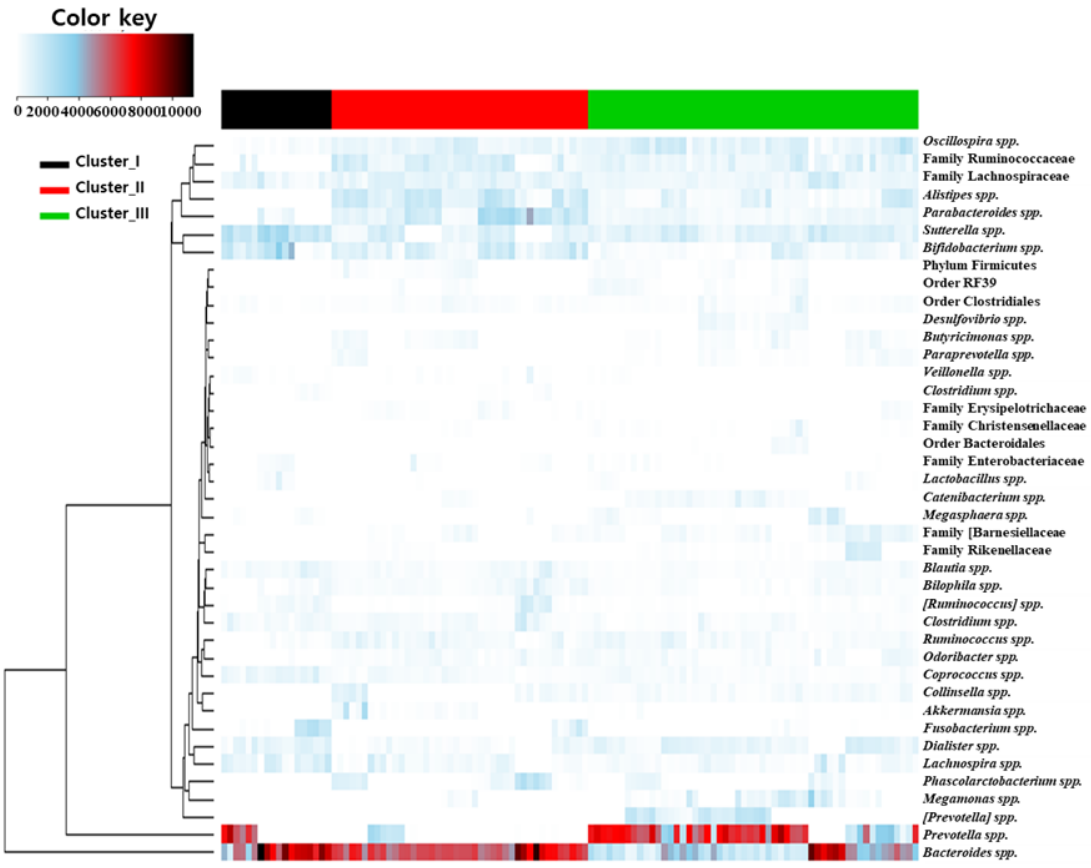


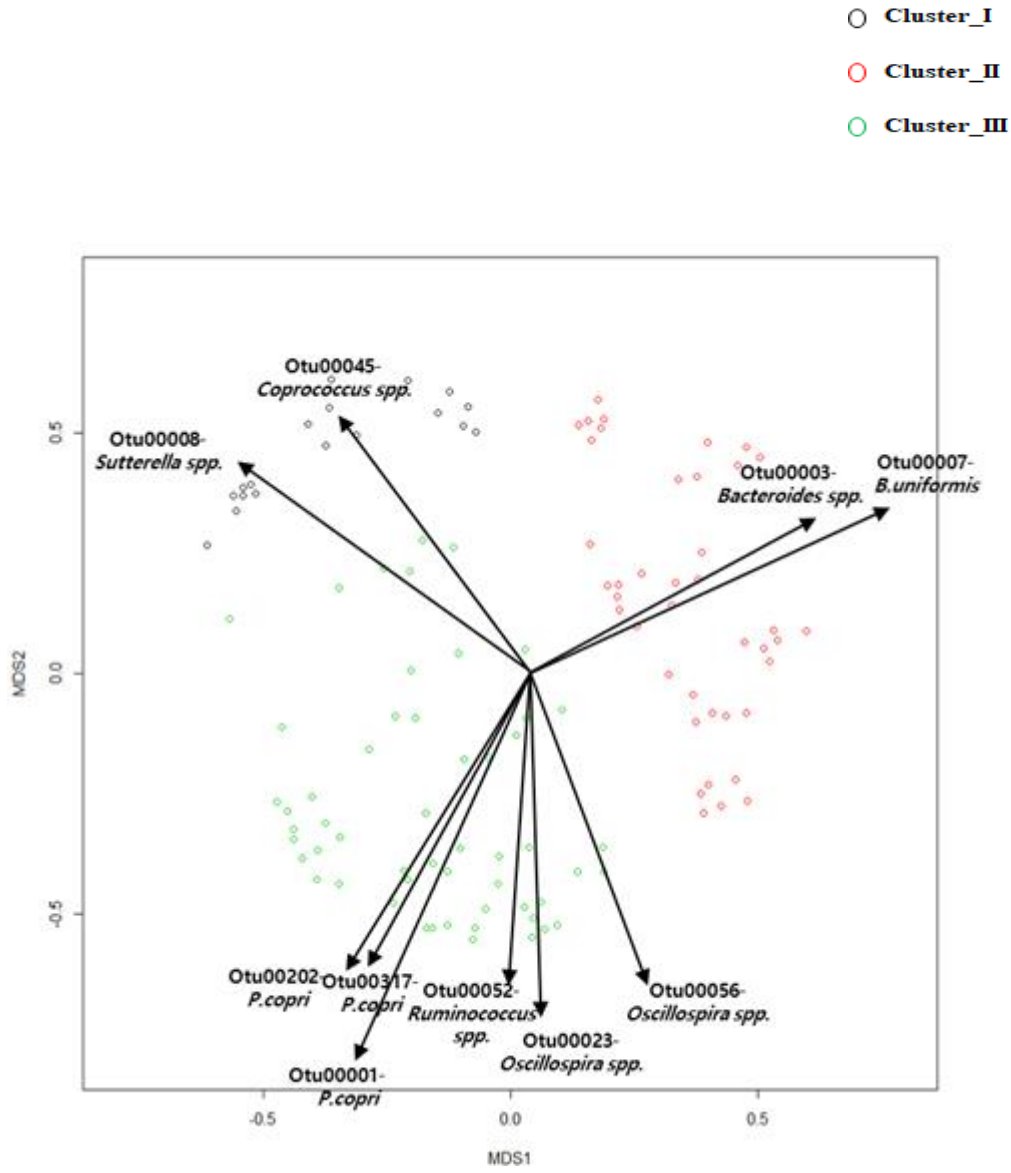
Figure 2. Tree for clustering human gut mirobial communities used in this study (1week-2week).

Table 3. Result of the subject's survey.

Subjects	Age	Sex	Height	Weight	BMI	Average daily intake of normal coffee(Cup)
A	37	M	165	80	29.4	2
B	25	M	179	68	21.2	0
C	23	M	171	73	25.0	1
D	39	M	176	81	26.2	6
E	25	M	177	88	28.1	0
F	24	M	162	51	19.4	0
G	28	M	170	83	28.7	2
H	24	M	174	86	28.4	0
I	25	M	181	68	20.8	1
J	24	M	165	80	29.4	0
K	38	W	163	55	20.7	1
L	39	W	163	50	18.8	0
M	23	W	154	55	23.2	1
N	25	W	160	60	23.4	0
O	23	W	153	60	25.6	1
Q	24	W	163	58	21.8	2
R	22	W	163	65	24.5	1
S	22	W	161	55	25.1	0
T	28	W	158	49	19.6	0

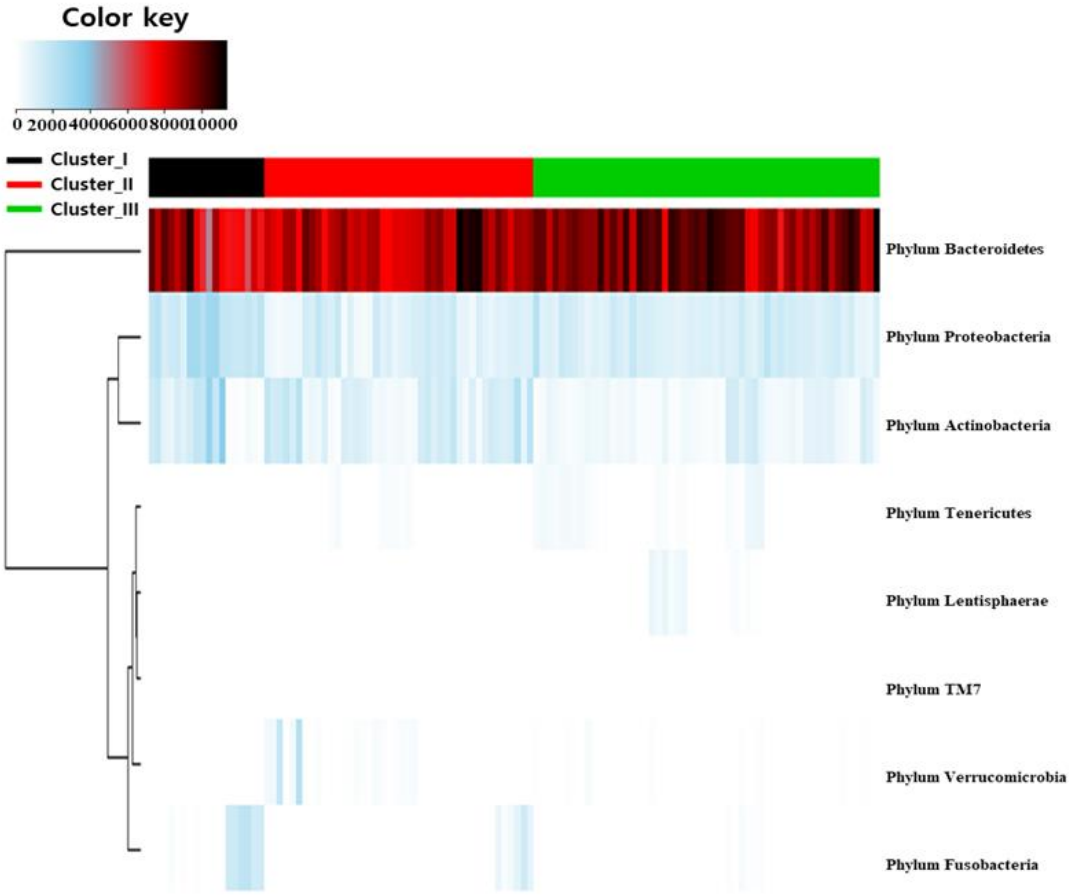


(A)

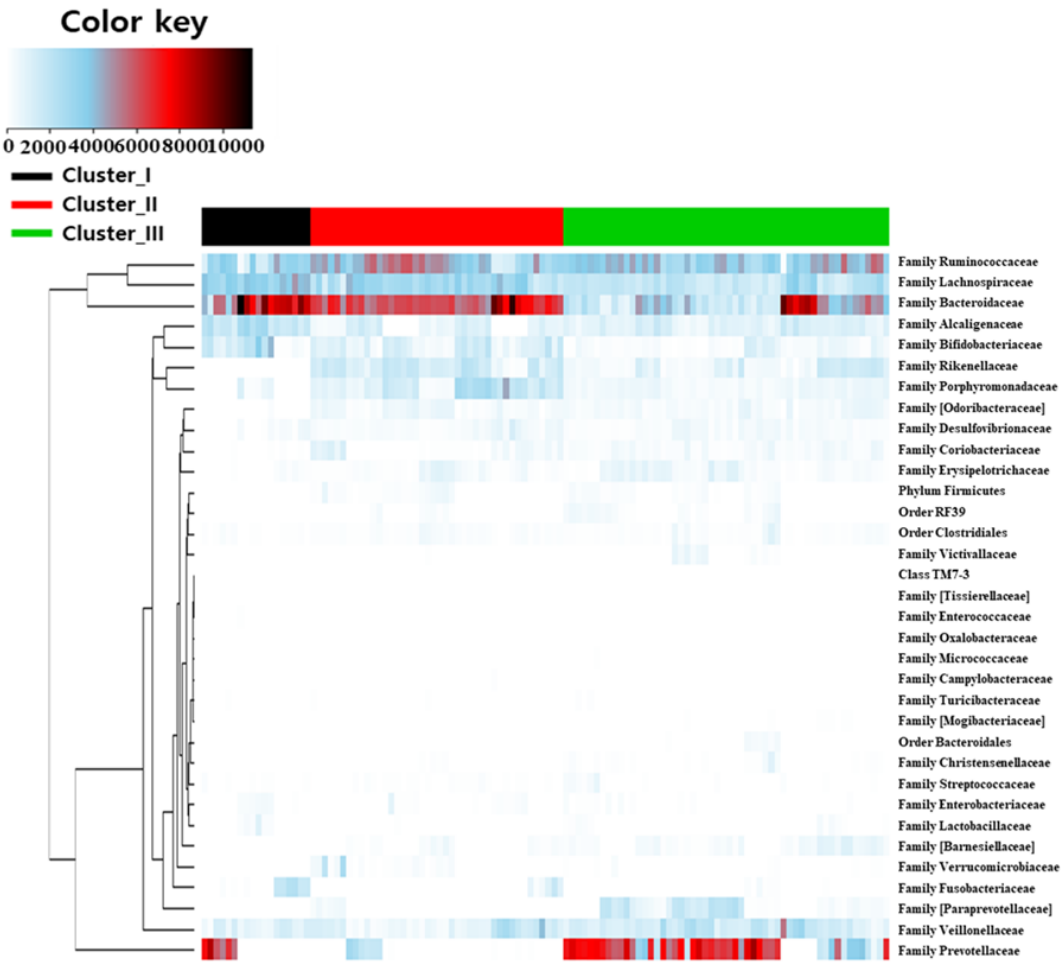


(B)

Figure 3. Result of difference by cluster (1week-2week): (A) Bacterial composition analysis at the genus level (from LEfSe data); and (B) NMDS (OTUs) with top 10 correlated OTUs.



(A)



(B)

Figure 4. Result of difference by cluster from LEfSe data (1week-2week): (A) Bacterial composition analysis at the Phylum level; and (B) Bacterial composition analysis at the Family level.

Effects of fermented coffee on the human gut microbiota

It has been suggested that different types of gut microbiota may react differently to certain substances such as fructooligosaccharides, sorghum arabinoxylan and corn arabinoxylan (Chen, Long et al. 2017). Therefore, the effects of fermented coffee on human gut microbiota may appear differently depending on the personal microbiota. OTUs that were significantly increased during taking fermented coffee (3-4 week) as well as significantly decreased after the termination of fermented coffee (5-6 week) were identified using LEfSe and organized in the Table 4. Among these OTUs, Otu00018 and Otu00001 showed significant increase more than 0.5% during taking fermented coffee ($p<0.005$).

The abundance of *Prevotella* indicates 10 subjects (A, B, C, F, G, H, I, K, R, and T) are *Prevotella* type (Figure 5), leaving other 9 subjects to be Non-*Prevotella* type. Results from Figure 6 suggest that Non-*Prevotella* type subjects had significantly increased *Prevotella*/*Bacteroides* ratio while drinking fermented coffee ($P<0.05$), whereas it did not change for *Prevotella* type subjects. Previously, higher proportion of *Prevotella/Bacteroides* was likely to get higher chance of weight-loss from dietary control(Lean, Astrup et al. 2018, Hjorth, Blædel et al. 2019). In contrast, it has been reported that obese people tend to have higher abundance of *Prevotella*(Hu, Park et al. 2015). Interestingly, two *Prevotella* type subjects (A and F) whose abundance of *Prevotella copri* was very low also showed significantly increase in the abundance of *Prevotella copri* during taking fermented coffee ($p<0.001$) (Figure 7). For these reasons, our results suggest that fermented coffee increases the abundance of *Prevotella copri* in those who have low abundance of *Prevotella copri*.

Thus we could confirm that *Prevotella copri* was increased by fermented coffee in subjects who had a low abundance of *Prevotella copri*. However, previous studies have reported that *Preovtella* is increased by normal-coffee(Jaquet, Rochat et al. 2009, Reichardt, Gniechwitz et al. 2009), so we examined the abundance of *Prevotella copri* by dividing the

subjects who drink regular normal-coffee and those who did not drink at 1week-2week periods. As a result, it was confirmed that there was no significant difference in *Prevoetella copri* in both groups (Figure 8). In addition, we could confirm that the fermented coffee contains more amount of biologically active substance than the non-fermented coffee (Figure 1, table 1). Some previous study reported that *Prevotella* was increased in humans who consume red-wine polyphenol and in the cattle fed flavonoid (Queipo-Ortuño, Boto-Ordóñez et al. 2012, Bi, Yang et al. 2017). Therefore, it was confirmed that *Prevotella copri* was increased by fermented coffee, not normal-coffee, and it is considered that biologically active substance of fermented coffee affected the abundance of *Prevotella copri*.

Table 4. Percentage of differentially abundant OTUs.

Group	OTU	Taxa	1week_2week (%)	3week_4week (%)	5week_6week (%)
Cluster I	Otu00018	*Lachnospira spp.*	3.16±0.57	5.37±0.56^{ab}	3.31±0.4
	Otu00001	*Prevotella copri*	0.01±0	0.57±0.05^{ab}	0.02±0.01
	Otu00007	*Bacteroides uniformis*	0.40±0.22	0.81±0.31	0.56±0.28
	Otu00012	*Dialister spp.*	0.97±0.35	1.19±0.37	0.81±0.3
	Otu00067	Family Rikenellaceae	0.00±0	0.35±0.03^{ab}	0±0
	Otu00032	Famly [Barnesiellaceae]	0.00±0	0.28±0.03^{ab}	0±0
	Otu00068	*Butyricimonas spp.*	0.00±0	0.23±0.02^{ab}	0±0
	Otu00021	*Sutterella spp.*	0.00±0	0.23±0.02^{ab}	0±0
	Otu00005	*Bacteroides plebeius*	0.00±0	0.21±0.02^{ab}	0±0
	Otu00059	*Haemophilus parainfluenzae*	0.21±0.07	0.43±0.13	0.21±0.07
Cluster II	Otu00001	*Prevotella copri*	0.03±0.01	0.65±0.03^{ab}	0.02±0
	Otu00012	*Dialister spp.*	0.67±0.16	0.85±0.16	0.73±0.15
	Otu00021	*Sutterella spp.*	0.31±0.07	0.62±0.11^{ab}	0.25±0.06
	Otu00067	Family Rikenellaceae	0±0	0.23±0.01^{ab}	0±0
	Otu00075	*[Eubacterium] biforme*	0.4±0.16	0.44±0.16	0.33±0.14
Cluster III	Otu00012	*Dialister spp.*	1.62±0.28	1.8±0.29	1.21±0.22
	Otu00021	*Sutterella spp.*	1.74±0.3	2.23±0.32	1.47±0.24
	Otu00024	Family Ruminococcaceae	0.53±0.18	0.57±0.21	0.25±0.1
	Otu00057	*Bacteroides coprophilus*	0.44±0.13	0.6±0.14	0.36±0.11
	Otu00067	Family Rikenellaceae	0.66±0.26	0.95±0.24^{b}	0.37±0.15

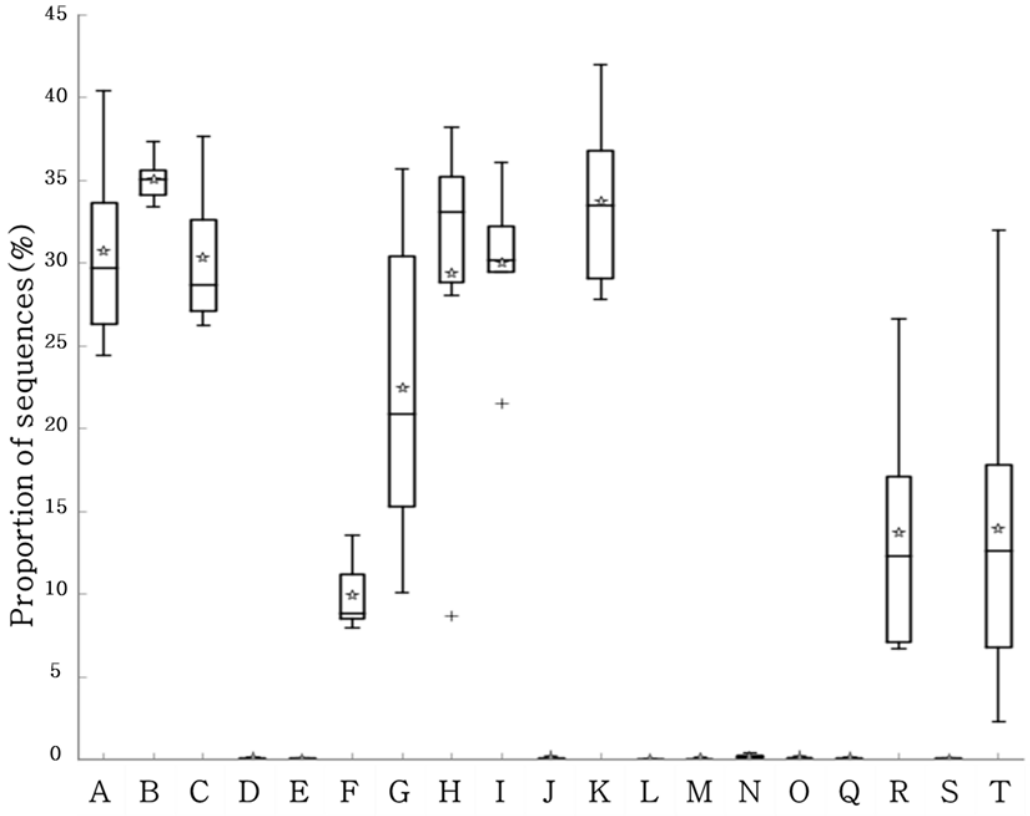
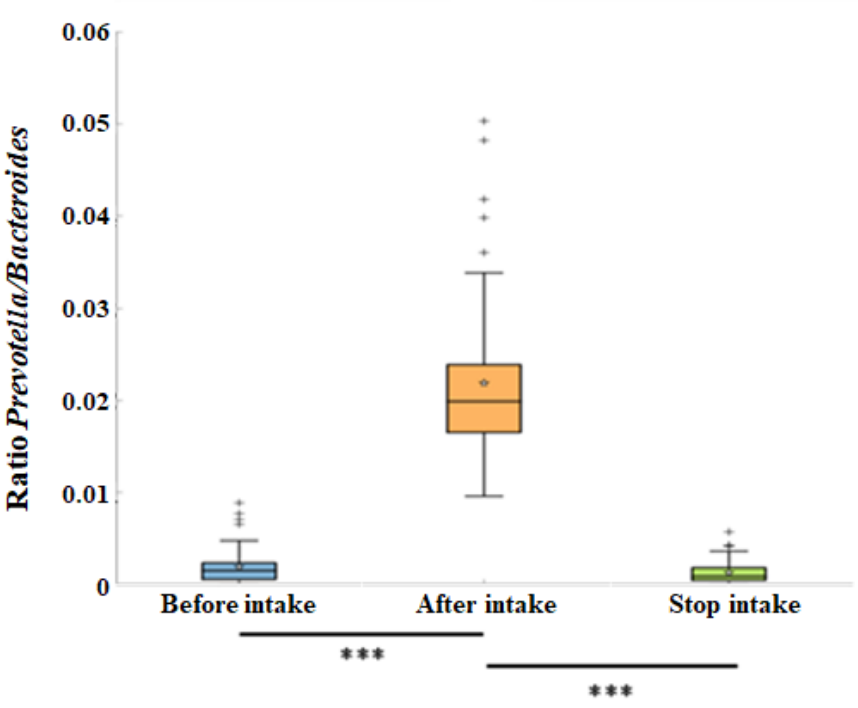
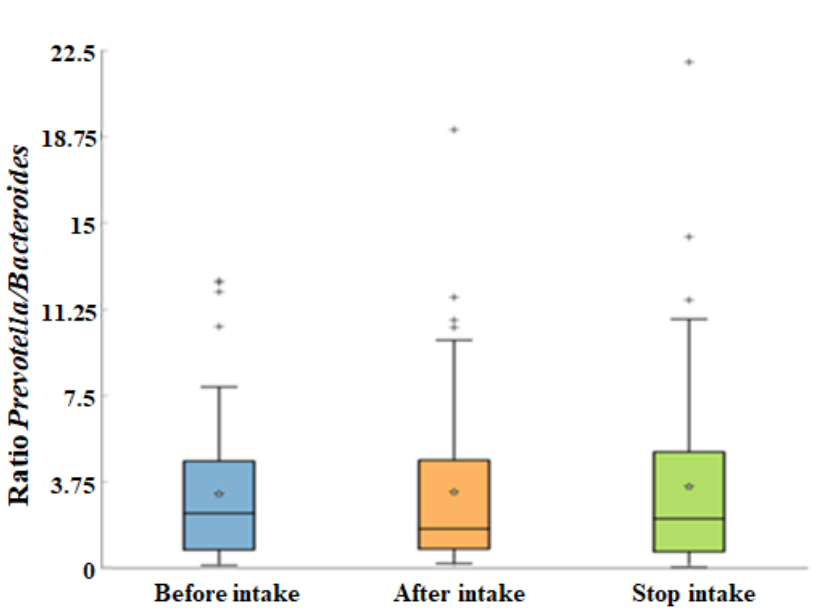


Figure 5. Differentiation of human gut microbiota type based on *Prevotalla spp.* abundance

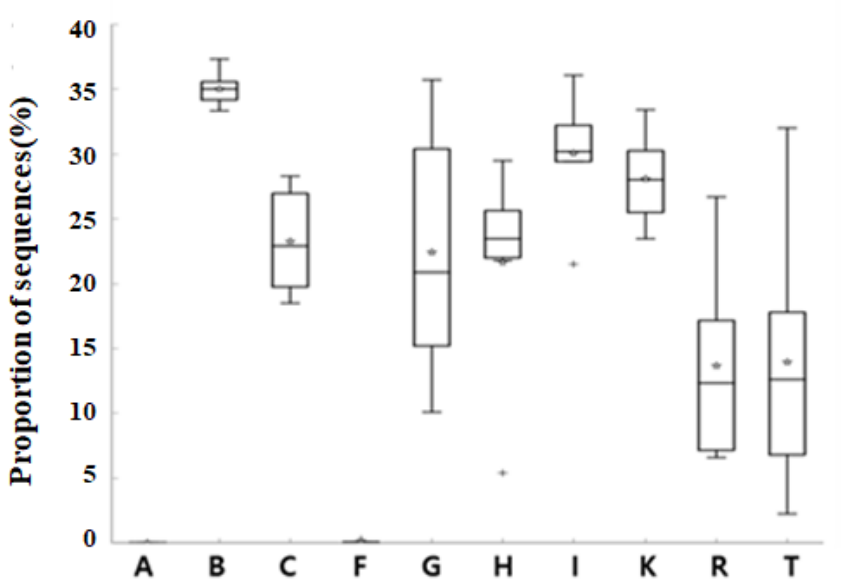


(A)

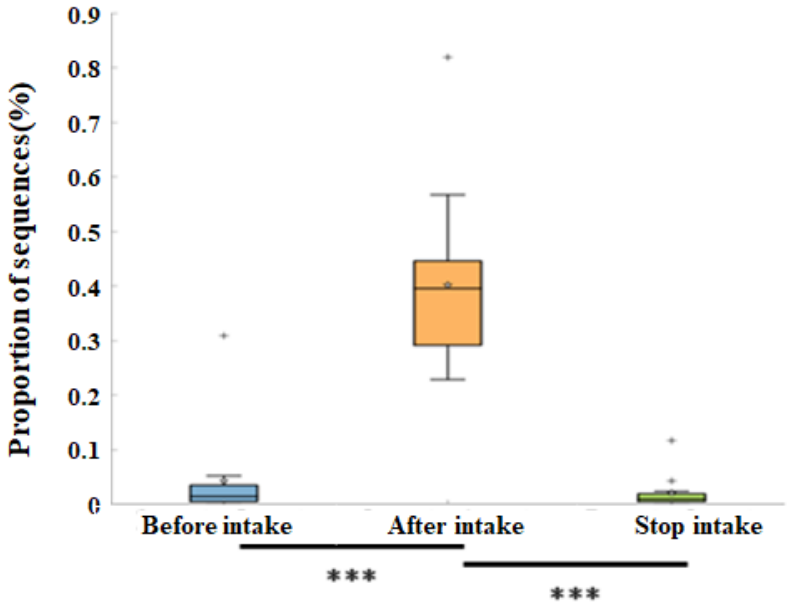


(B)

Figure 6. Ratio of *Prevotella / Bacteroides* by type: (A) Non-*Prevotella* type; (B) *Prevotella* type (*p<0.001; Welch's t-test)**

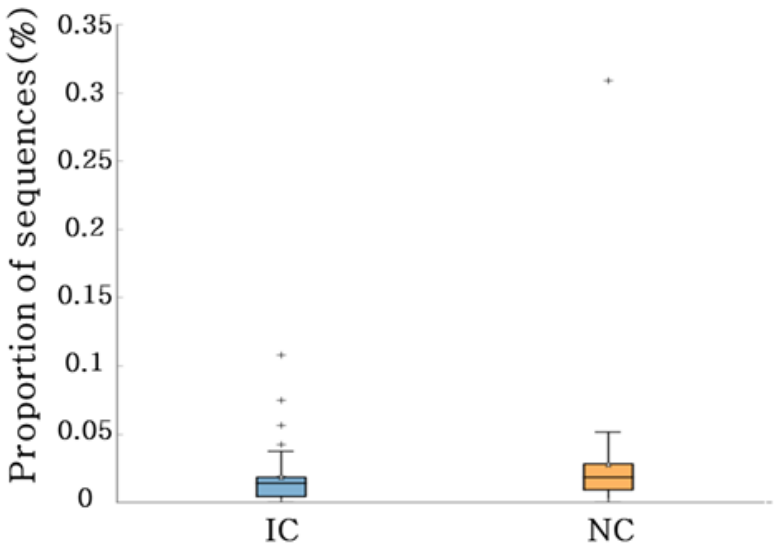


(A)

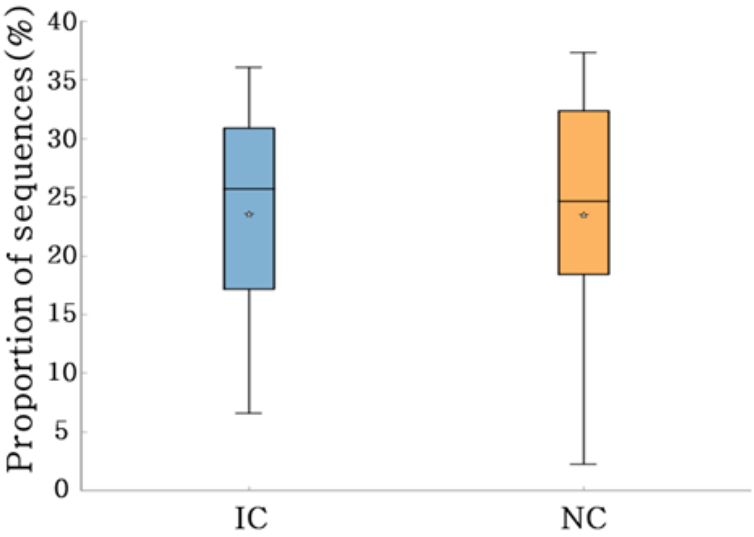


(B)

Figure 7. Relative abundance of *Prevotella copri* in among *Prevotella* type subjects: (A) All *Prevotella* type subjects; (B) Subjects A and F (*p<0.001; Welch's t-test)**



(A)



(B)

Figure 8. *Prevotella copri* comparison of relative abundance between IC (normal coffee drinkers) and NC (non-normal coffee drinkers): (A) Subjects with increased *Prevotella copri* (B) Subjects who did not change *Prevotella copri*

Metabolic changes correlated with *Prevotella copri*

Result in Table 1.show that the metabolic correlated with *Prevotella copri* increased in the 3week-4week period. The metabolic pathways were divided into 10 major metabolic pathways. Metabolic with a PearsonCoef value of 0.7 or higher was confirmed to be Betalain biosynthesis, Indole alkaloid biosynthesis, Isoflavonoid biosynthesis, Various types of N-glycan biosynthesis.

Prevotella appears in large numbers in humans who eat mainly carbohydrate and fiber(Chen, Long et al. 2017), and is reported as a microorganism that ferments carbohydrate(Zhang, DiBaise et al. 2009). Based on this, carbohydrate metabolism is considered positively correlated with *Prevotella copri*. Isoflavonoid biosynthesis uses daidzein, one of the flavonoids(Atkinson, Frankenfeld et al. 2005, Andrés-Lacueva, Medina-Remon et al. 2010). In previous studies, *Prevotella* increased in cattle ingested with daidzein(Liang, Xu et al. 2018), and it was reported that intestinal microorganisms used this substance(Rafii 2015), so it is considered that *Prevotella copri* has a positive correlation with Isoflavonoid biosynthesis. Valine, leucine and, isoleucine are amino acids. *prevotella copri* is reported to produce these substances(Pedersen, Gudmundsdottir et al. 2016), and our results also confirmed that the synthesis of this substance is positively correlated with *prevotella copri*. It is also reported that *Prevotella copri* produces succinic acid(Hayashi, Shibata et al. 2007). This substance is used in butanoate metabolism(Browser, Model et al.), which suggests that *prevotella copri* and butanoate metabolism are positively correlated.

Table 5. Metabolic changes correlated with abundance of the *Prevotella copri*

Taxa	KEGG_Pathways	Metadata	pearsonCoef	Significance
Prevotella copri	Amino Acid Metabolism	Amino acid related enzymes	0.18	0.03
	Amino Acid Metabolism	Lysine degradation	0.22	0.01
	Amino Acid Metabolism	Tryptophan metabolism	0.21	0.02
	Amino Acid Metabolism	Valine, leucine and isoleucine biosynthesis	0.17	0.04
	Amino Acid Metabolism	Valine, leucine and isoleucine degradation	0.22	0.01
	Biosynthesis of Other Secondary Metabolites	Betalain biosynthesis	0.76	0.00
	Biosynthesis of Other Secondary Metabolites	Clavulanic acid biosynthesis	0.21	0.02
	Biosynthesis of Other Secondary Metabolites	Indole alkaloid biosynthesis	0.78	0.00
	Biosynthesis of Other Secondary Metabolites	Isoflavonoid biosynthesis	0.71	0.00
	Carbohydrate Metabolism	Butanoate metabolism	0.18	0.04
	Carbohydrate Metabolism	Glycolysis / Gluconeogenesis	0.17	0.04
	Carbohydrate Metabolism	Inositol phosphate metabolism	0.19	0.02
	Carbohydrate Metabolism	Propanoate metabolism	0.22	0.01

Table 5. Metabolic changes correlated with abundance of the *Prevotella copri*

Taxa	KEGG_Pathways	Metadata	pearsonCoef	Significance
Prevotella copri	Carbohydrate Metabolism	Pyruvate metabolism	0.19	0.02
	Energy Metabolism	Oxidative phosphorylation	0.18	0.04
	Glycan Biosynthesis and Metabolism	Peptidoglycan biosynthesis	0.17	0.04
	Glycan Biosynthesis and Metabolism	Various types of N-glycan biosynthesis	0.71	0.00
	Lipid Metabolism	Ether lipid metabolism	0.39	0.00
	Lipid Metabolism	Fatty acid biosynthesis	0.20	0.02
	Lipid Metabolism	Fatty acid metabolism	0.20	0.02
	Lipid Metabolism	Glycerophospholipid metabolism	0.18	0.03
	Lipid Metabolism	Lipid biosynthesis proteins	0.20	0.02
	Lipid Metabolism	Synthesis and degradation of ketone bodies	0.20	0.02
	Metabolism of Cofactors and Vitamins	Porphyrin and chlorophyll metabolism	0.23	0.01
	Metabolism of Terpenoids and Polyketides	Biosynthesis of type II polyketide products	0.21	0.01
	Metabolism of Terpenoids and Polyketides	Carotenoid biosynthesis	0.26	0.00

Table 5. Metabolic changes correlated with abundance of the *Prevotella copri*

Taxa	KEGG_Pathways	Metadata	pearsonCoef	Significance
Prevotella copri	Metabolism of Terpenoids and Polyketides	Tetracycline biosynthesis	0.19	0.03
	Nucleotide Metabolism	Pyrimidine metabolism	0.17	0.04
	Xenobiotics Biodegradation and Metabolism	Metabolism of xenobiotics by cytochrome P450	0.18	0.03
	Unclassified Metabolism	Lipid metabolism	0.22	0.01

Prevotella copri has double-sidedness in terms of diabetes by butanoate metabolism and Valine, leucine and isoleucine biosynthesis(Cani 2018). That is, *Prevotella copri* produces succinic acid, a kind of short-chain fatty acid, to improve insulin resistance(De Vadder, Kovatcheva-Datchary et al. 2014, De Vadder, Kovatcheva-Datchary et al. 2016), on the other hand, it is produced BCAA(Valine, leucine and, isoleucine) to exacerbate insulin resistance(Pedersen, Gudmundsdottir et al. 2016). However, recent studies have shown that *prevotella copri* has a difference in correlation between carbohydrate catabolism and Valine, leucine, and isoleucine biosynthesis according to the human diet(De Filippis, Pasolli et al. 2019). That is, *Prevotella copri* associated with a fiber-diet had a higher prevalence of the carbohydrate catabolism, and associated with an omnivore diet had a higher prevalence of the Valine, leucine and isoleucine biosynthesis. Based on these results, we investigated the ratio of vegetable foods to animal foods through the diets investigated from subjects who had been increased *prevotella copri* by fermented coffee (Table S4). The subjects were classified based on the value of 1, and the Metabolic correlated with the *Prevotella copri* which was increased during the 3week-4week period between the two groups is shown in Table S5. That is, subjects with a value of 1 or higher are groups that frequently eat vegetable foods compared to animal foods, and those with a value of 1 or lower are groups that frequently eat animal foods compared to vegetable foods The average of ingestion amount of excluding vegetable foods and animal foods by group was flour: 13.5±2.8, 8.9±1.5; instance; 16.5±2.7, 13.6±2.9; others; 9.5±0.9, 15.7±2.6, and there was no significant difference in all ($p>0.05$). We cannot identify carbohydrate catabolism in our data, but in the case of Valine, leucine and isoleucine biosynthesis, similar to the findings described before, it has been confirmed that *Prevella copri* is positively correlated with groups that consume animal products more frequently than plant foods, and no other group has been identified.

Table 6. Subjects diet

subjects	vegetable	fruit	grain	meat	seafood	fish	flour	instant	others	vegetable+fruit+grain /meat+seafood+fish
J	1	0	23	15	0	3	19	24	10	1.33
O	24	1	20	32	1	3	13	13	7	1.25
F	6	0	26	22	1	5	16	17	8	1.14
S	2	2	52	46	3	7	6	12	10	1
D	0	1	21	18	7	2	4	16	19	0.81
M	0	2	26	41	0	1	12	24	9	0.67
E	0	0	11	19	1	2	7	20	10	0.5
A	1	4	9	20	6	8	7	5	24	0.41
Q	0	0	20	46	1	2	16	14	11	0.4
N	0	0	24	44	3	13	6	13	22	0.4
L	1	1	20	37	12	19	10	3	18	0.32

Table 7. Metabolic changes correlated with abundance of the *Prevotella copri* by subject's diet

Subjects with Vegetable foods / Animal foods greater than 1				
Taxa	**KEGG Pathway**	**Metadata**	**pearsonCoef**	**Significance**
Prevotella copri	Amino Acid Metabolism	Lysine degradation	0.33	0.02
	Amino Acid Metabolism	Tryptophan metabolism	0.37	0.01
	Amino Acid Metabolism	Valine, leucine and isoleucine degradation	0.32	0.02
	Biosynthesis of Other Secondary Metabolites	Betalain biosynthesis	0.81	0.00
	Biosynthesis of Other Secondary Metabolites	Clavulanic acid biosynthesis	0.31	0.03
	Biosynthesis of Other Secondary Metabolites	Indole alkaloid biosynthesis	0.83	0.00
	Biosynthesis of Other Secondary Metabolites	Isoflavonoid biosynthesis	0.59	0.00
	Carbohydrate Metabolism	Butanoate metabolism	0.29	0.04
	Carbohydrate Metabolism	Citrate cycle (TCA cycle)	0.31	0.03
	Carbohydrate Metabolism	Inositol phosphate metabolism	0.29	0.04
	Glycan Biosynthesis and Metabolism	Glycosphingolipid biosynthesis – lacto and neolacto series	0.86	0.00
	Glycan Biosynthesis and Metabolism	Various types of N-glycan biosynthesis	0.89	0.00
	Lipid Metabolism	Synthesis and degradation of ketone bodies	0.30	0.03
	Metabolism of Terpenoids and Polyketides	Carotenoid biosynthesis	0.40	0.00
	Metabolism of Terpenoids and Polyketides	Limonene and pinene degradation	0.29	0.04

Table 7. Metabolic changes correlated with abundance of the *Prevotella copri* by subject's diet

Subjects with Vegetable foods / Animal foods less than 1				
Taxa	**KEGG Pathway**	**Metadata**	**pearsonCoef**	**Significance**
Prevotella copri	Amino Acid Metabolism	Valine, leucine and isoleucine biosynthesis	0.23	0.03
	Biosynthesis of Other Secondary Metabolites	Betalain biosynthesis	0.73	0.00
	Biosynthesis of Other Secondary Metabolites	Indole alkaloid biosynthesis	0.76	0.00
	Biosynthesis of Other Secondary Metabolites	Isoflavonoid biosynthesis	0.79	0.00
	Carbohydrate Metabolism	Fructose and mannose metabolism	0.22	0.05
	Carbohydrate Metabolism	Glycolysis / Gluconeogenesis	0.22	0.04
	Carbohydrate Metabolism	Glyoxylate and dicarboxylate metabolism	0.23	0.04
	Carbohydrate Metabolism	Propanoate metabolism	0.23	0.03
	Carbohydrate Metabolism	Pyruvate metabolism	0.23	0.04
	Glycan Biosynthesis and Metabolism	Various types of N-glycan biosynthesis	0.63	0.00
	Lipid Metabolism	Ether lipid metabolism	0.49	0.00
	Lipid Metabolism	Fatty acid metabolism	0.25	0.02
	Lipid Metabolism	Glycerophospholipid metabolism	0.22	0.04
	Metabolism of Cofactors and Vitamins	Porphyrin and chlorophyll metabolism	0.27	0.01
	Metabolism of Terpenoids and Polyketides	Biosynthesis of siderophore group nonribosomal peptides	0.24	0.03

Table 7. Metabolic changes correlated with abundance of the *Prevotella copri* by subject's diet

Taxa	KEGG Pathway	Metadata	pearsonCoef	Significance
Prevotella copri	Metabolism of Terpenoids and Polyketides	Biosynthesis of type II polyketide products	0.72	0.00
	Metabolism of Terpenoids and Polyketides	Carotenoid biosynthesis	0.25	0.02
	Metabolism of Terpenoids and Polyketides	Tetracycline biosynthesis	0.25	0.02
	Xenobiotics Biodegradation and Metabolism	Dioxin degradation	0.22	0.04

CONCLUSTION

Fermentation Industry (Sunchang, Korea) has developed coffee that ferments coffee to lower the content of caffeine and increase the content of physiologically active substances, thereby reducing side effects caused by caffeine. On this, we recruited subjects to investigate intestinal microbial ecology that change by fermented coffee by comparing changing intestinal microbial ecology when fermented coffee was consumed and fermented coffee was stopped. As a result, *Prevotella copri* was increased by fermented coffee in subjects with low abundance of *Prevotella copri*, and it was confirmed that increased *Prevotella copri* was involved in various metabolism. In this study, however, the practical effect of obesity with ratio of *prevotella*/*bacteroides* and metabolism associated with increased *Prevotella copri* by fermented coffee was not evaluated. Therefore, further experiments on the effect of *Prevotella copri*, which is increased by fermented coffee, on the human body are considered to be necessary.

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