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Doenjang, a Fermented Korean Soybean Paste, Inhibits Lipopolysaccharide Production of Gut Microbiota in Mice

Se-Eun Jang,[1,2] Kyung-Ah Kim,[1] Myung Joo Han,[2] and Dong-Hyun Kim[1]

Departments of [1]Life and Nanopharmaceutical Sciences and [2]Food and Nutrition, Kyung Hee University, Dongdaemun-gu, Seoul, Korea.

ABSTRACT *Doenjang* has been reported to exhibit antioxidant, fibrinolytic, antimutagenic, anticancer, and antiobesity effects. In our preliminary study, *doenjang* decreased fecal lipopolysaccharide (LPS) levels in mice. Therefore, we investigated the effect of *doenjang* on the composition of gut microbiota in mice. Treatment with *doenjang* significantly increased the number of bifidobacteria cultured in BL media, compared with mice not treated with *doenjang*. However, *doenjang* decreased the number of Enterobacteriaceae cultured in DHL media. *Doenjang* significantly suppressed the β-glucuronidase activity, but did not influence α-/β-glucosaminidase and α-/β-glucosidase activities. When gut microbiota in mice treated with or without *doenjang* was analyzed by pyrosequencing, *doenjang* induced a significant modulation of the populations of the dominant gut microbiota. At the phylum level, *doenjang* treatment resulted in a significant decrease of Firmicutes and an increase of Bacteroidetes, which led to a decrease in the Firmicutes to Bacteroidetes ratio in gut microbiota. At the family level, the number of Ruminococcaceae and Lachnospiraceae were significantly decreased, while the number of *Odoribacter_f* was increased in *doenjang*-treated mice. Of colonic tight junction proteins, occludin, ZO-1, and claudin-1 in mice, occludin alone was significantly increased by treatment with *doenjang*. Although treatment with *doenjang* seemed to suppress NF-κB activation, it was not significant. *Doenjang* significantly suppressed tumor necrosis factor-α expression, whereas it did not influence interleukin (IL)-1β and IL-6 expression. However, *doenjang* increased IL-10 expression. Based on these findings, *doenjang* may promote gut health by regulating gut microbiota and its LPS concentrations and suppressing harmful enzyme production.

KEY WORDS: • doenjang • *gut microbiota* • *inflammation* • *lipopolysaccharide*

INTRODUCTION

HUMAN GUT MICROBIOTA consists of 10 to 100 trillion microorganisms, which is 10-fold more than the number of cells that compose the human body.[1] Although infants grow and develop in the sterile environment of the uterus of their mothers before birth, the external surfaces of infants such as gut and skin are colonized by maternal, environmental, and dietary microbes during delivery and immediately afterward. Human gut microbiota has been usually regarded as relatively stable throughout adulthood. However, gut microbiota is disturbed by exogenous and endogenous factors, such as diet, antibiotics, and stress. Gut microbiota is pivotal for the development of gastrointestinal mucosa and immune system.[2,3] Therefore, gut microbiota plays an important role in energy balance,[4,5] glucose metabolism,[6,7] low-grade inflammation[8,9] and drug metabolism.[10,11] Recently, we found that gut microbiota-derived lipopolysaccharide (LPS) involves in the onset and progression of inflammation and metabolic diseases.[6] For example, high-fat diets increase gram-negative bacteria, which induce the production of LPS in the intestine and cause inflammation, obesity, and cancer.[6,9,12]

Concentrations of LPS, a component of gram-negative bacterial cells, are increased by a high-fat diet (HFD).[6,12] We and others have confirmed that HFD could contribute to higher plasma LPS levels and low-grade inflammation.[13,14] Thus, HFD induces LPS production in the intestinal contents of humans and animals, supporting that gut microbiota initiates and perpetuates colonic inflammation. More specifically, gut bacterial LPS penetrates the epithelial barrier and stimulates mucosal immune reactions.[15,16] Subsequently, this toxin induces the production of proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, leading to inflammatory activation using distinct signaling pathways through toll-like receptor 4.[17,18] Regulating the expression of these inflammatory cytokines is therefore beneficial for treating inflammatory and metabolic diseases.

Doenjang is a traditional Korean soybean paste that has been used for centuries as a protein source and flavoring

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Address correspondence to: Dong-Hyun Kim, PhD, Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, 1 Hoegi, Dongdaemun-ku, Seoul 130-701, Korea, E-mail: dhkim@khu.ac.kr or *Myung Joo Han, PhD, Department of Food and Nutrition, Kyung Hee University, 1 Hoegi, Dongdaemun-ku, Seoul 130-701, Korea, E-mail:* mjhan@khu.ac.kr

ingredient in Korea, like that of *miso* in Japan and *tempeh* in Indonesia.[19] *Doenjang* is prepared by fermenting moldy cooked soybeans (*meju*) in brine, resulting in the degradation of soy proteins and production of organic acids, amino acids, and minerals.[20] Recently, *doenjang* research has focused on its excellent nutritional value as well as its health-promoting properties, such as its antioxidant,[21] fibrinolytic,[22] antimutagenic,[23] anticancer,[24] and antiobesity effects.[25] However, its effect on gut microbiota, particularly endotoxins, has not been studied.

Therefore, in the present study, we investigated the effect of *doenjang* on the composition of gut microbiota in mice.

MATERIALS AND METHODS

Materials

p-Nitrophenyl-β-D-glucopyranoside, 4-nitrophenyl-N-acetyl-α-D-glucosaminide, 4-nitrophenyl-N-acetyl-β-D-glucosaminide, 4-nitrophenyl-β-D-glucuronide, 4-nitrophenyl-α-D-glucopyranoside, and 4-nitrophenyl-β-D-glucopyranoside were purchased from Sigma-Aldrich (St Louis, MO, USA). The diazo-coupled limulus amoebocyte lysate (LAL) assay kit was purchased from Associates of Cape Cod, Inc. (East Falmouth, MA, USA). Antibodies for p-p65, p65, ZO-1, occludin, claudin, and β-actin were purchased from Cell Signaling Technology (Beverly, MA, USA). All antibodies were used at dilutions of 1:1000. Enzyme-linked immunosorbent assay (ELISA) kits for cytokines were purchased from R&D Systems (Minneapolis, MN, USA).

Animals

Male C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). All animals were fed standard laboratory chow (Samyang Co., Seoul, South Korea), housed in wire cages at 20–22°C and 50% ± 10% humidity, and allowed water ad libitum. All experiments were performed in accordance with the NIH and Kyung Hee University guides for Laboratory Animals Care and Use and approved by the Committee for the Care and Use of Laboratory Animals in the College of Pharmacy, Kyung Hee University (KHP-2013-07-2).

After 7 days of acclimation, mice were separated into two groups, the AIN-93G diet (Harlan Teklad Test Diets, Madison, WI, USA) with or without freeze-dried 5% *doenjang* (prepared according to the method of Hwang *et al.*[26] and freeze-dried) and treated for 7 days. The food intake and body weight were then measured daily. At the end of 7 days, mice were anesthetized and blood collected by cardiac puncture and the colonic contents removed.

Preparation of fecalase

The mouse colon contents (about 0.3 g) were prepared according to a previous method of Lee *et al.*[27] by collecting after sacrifice, carefully mixing with a spatula, and suspending in cold 2.7 mL saline. Fecal suspensions were centrifuged at 500 *g* for 5 min. The resulting supernatants were sonicated for 10 min and then centrifuged at 10,000 *g* for 20 min. The supernatants were used as fecalase suspensions.

Fecalase activity assay

Fecalase activities were assayed according to the previous reported method of Yeo *et al.*[28] Briefly, the reaction mixture (total volume of 0.5 mL) containing 0.2 mL of 1 mM *p*-nitrophenyl-β-D-glucopyranoside for β-glucosidase (or 1 mM *p*-nitrophenyl- β -D-glucuronide for β -glucuronidase, 1 mM 4-nitrophenyl-N-acetyl-α-D-glucosaminide for α-glucosaminidase, 1 mM 4-nitrophenyl-N-acetyl-β-D-glucosaminide for β-glucosaminidase, and 1 mM 4-nitrophenyl-α-D-glucopyranoside for α-glucosidase), 0.2 mL of 0.1 M phosphate buffer, pH 7.4, and 0.1 mL of the fecal suspension (wet weight, 4 mg) was incubated at 37°C for 15 min. Then, the reaction mixture was stopped by the addition of 0.5 mL of 0.5 N NaOH, centrifuged at 2000 *g* for 10 min, and the absorbance measured at 405 nm (a BioTek spectrophotometer, London, England).

Determination of LPS

Plasma endotoxin contents were determined by a LAL assay kit (Cape Cod) according to the manufacturer's protocol. Briefly, plasma was diluted 1:10 in pyrogen-free water, inactivated for 10 min at 70°C, and then incubated with LAL for 30 min at 37°C. Addition of reagents led to the formation of a magenta derivative that absorbs light at 545 nm.

Myeloperoxidase activity assay

An aliquot (50 μL) of the colon supernatant was added to a reaction mixture of 1.6 mM tetramethyl benzidine and 0.1 mM H_2O_2 and then incubated at 37°C. The absorbance was obtained at 650 nm over time. The myeloperoxidase activity was defined as the quantity of enzyme degrading 1 μmol/mL of peroxide at 37°C and expressed in unit/mg protein.[29]

ELISA and immunoblotting

For the ELISAs of IL-1β, IL-6, IL-10, and TNF-α, plasma and colon tissue homogenates were transferred to 96-well ELISA plates. IL-1β, IL-6, IL-10, and TNF-α concentrations were determined using commercial ELISA kits (Pierce Biotechnology, Inc., Rockford, IL, USA).

For immunoblot analyses of p65, p-p65, ZO-1, occludin, claudin-1, and β-actin, colon tissue homogenates were resuspended in 1 mL of RIPA lysis buffer containing 1% protease inhibitor cocktail and 1% phosphatase inhibitor cocktail. After centrifugation, the supernatant was used for the immunoblot assay. The proteins were subjected to electrophoresis on an 8–10% sodium dodecyl sulfate–polyacrylamide gel, and then transferred to a nitrocellulose membrane and immunoblotted using standard procedures.[29]

Bacterial culture of mouse stools

Fresh mouse stools (0.1 g) from each group were collected separately in sterilized plastic cups, carefully suspended in 10 volumes of peptone water, diluted 10-fold in a

Table 1. Number of Sequences Analyzed, Observed Diversity Richness, Estimated Operational Taxononic Unit Richness for ACE and Chao1, and Coverage

			Phylotype			
Group	*Number*	*Total reads*	*OTUs*	*ACE*	*Chao1*	*Goods coverage*
NC	1	4920	376	704.93	604	0.97
	2	4981	347	640.32	519.12	0.97
	3	2976	220	282.27	274.03	0.98
	4	4669	292	401.87	387.12	0.98
	5	731	106	132.48	123.4	0.96
	Mean ± SD	3655.4 ± 1830	268.2 ± 108.4	432.4 ± 240.3	381.5 ± 191.5	0.97 ± 0.01
DJ	1	4997	309	619.57	512.95	0.97
	2	2854	200	275.96	263.55	0.98
	3	1197	128	289.53	196.14	0.95
	4	1246	114	154.36	142.96	0.97
	5	4844	370	645.84	530.62	0.97
	Mean ± SD	3027.6 ± 1853	224.2 ± 112.3	397.1 ± 221.7	329.2 ± 181.0	0.97 ± 0.01

The cutoff value of phylotype is equal to or greater than 97% similarity.
OTUs, operational taxonomic units; NC, normal control group; DJ, *doenjang*-treated group.

stepwise manner, and inoculated in agar plates of blood liver medium (BL; Nissui Pharm. Co., Ltd., Tokyo, Japan) and hydrogen sulfate lactose medium (DHL; Eiken Chem. Co., Ltd., Tokyo, Japan). DHL agar plates were cultured aerobically for 1 day at 37°C and BL agar plates were cultured anaerobically for 3 days at 37°C.

DNA extraction, pyrosequencing, and data analysis

Genomic DNA was extracted from fecal samples using a commercial DNA isolation kit (QIAamp DNA stool mini kit; Qiagen, Hilden, Germany) by following the manufacturer's protocol. For pyrosequencing, amplification of genomic DNA was performed using barcoded primers, which targeted the V1 to V3 region of the bacterial 16S rRNA gene. The amplification and sequencing were performed according to the methods described by Chun *et al.*[30] and completed by Chunlab, Inc. (Seoul, Korea) using a 454 GS FLX Titanium Sequencing System (Roche, Branford, CT, USA). Sequence reads were identified using EzTaxon-e database (http://eztaxon-e.ezbiocloud.net)[31] on the basis of 16S rRNA sequence data. Number of sequences analyzed, observed diversity richness (operational taxonomic units [OTUs]), estimated OTU richness (ACE and Chao1), and coverage in the present pyrosequencing are indicated in Table 1.

Statistics

The data are expressed as the mean ± standard deviation. Statistical analysis of the data was performed with the Student's *t*-test. Differences with a $P < .05$ were considered to be statistically significant.

RESULTS

Effect of doenjang *on fecal and plasma LPS levels in mice*

To understand the possible role of gut microbiota in the antiobesity properties of *doenjang*, we orally administered *doenjang* with diet in mice for 7 days and measured fecal and plasma LPS levels (Fig. 1). Treatment with *doenjang* reduced body weight and fecal and plasma LPS levels, compared with those of untreated normal control mice. The fecal LPS level alone was significantly decreased by treatment with *doenjang*.

Effect of doenjang *on gut microbiota composition in mice*

To understand the fecal LPS production inhibitory mechanism of *doenjang*, we analyzed the gut microbiota in mice treated with or without *doenjang* by the culture method (Fig. 2A). Treatment with *doenjang* significantly increased the number of *Bifidobacteria* cultured in BL media, compared with that of mice untreated with *doenjang*, however, fewer Enterobacteriaceae were cultured in DHL media. Then, we analyzed the activities of harmful enzymes β-glucuronidase and α-/β-glucosaminidases, which caused inflammation or cancer,[32,33] in the gut content of mice treated with or without *doenjang* (Fig. 2B). Treatment with *doenjang* significantly decreased the β-glucuronidase

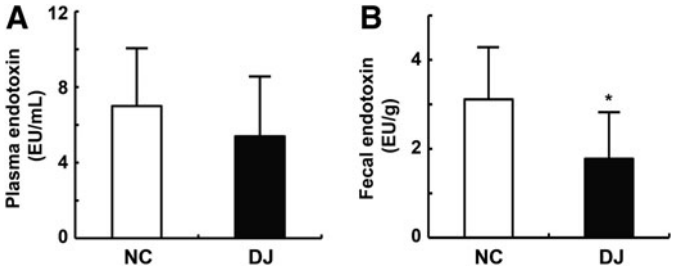

FIG. 1. Effect of *doenjang* on fecal and plasmatic lipopolysaccharide levels in mice. **(A)** Limulus amoebocyte lysate assay was used to measure the plasma **(A)** and fecal endotoxin concentrations **(B)**. NC, normal control group; DJ, *doenjang*-treated group. All values are indicated as the mean ± S.D. ($n = 8$). *$P < .05$ compared with NC.

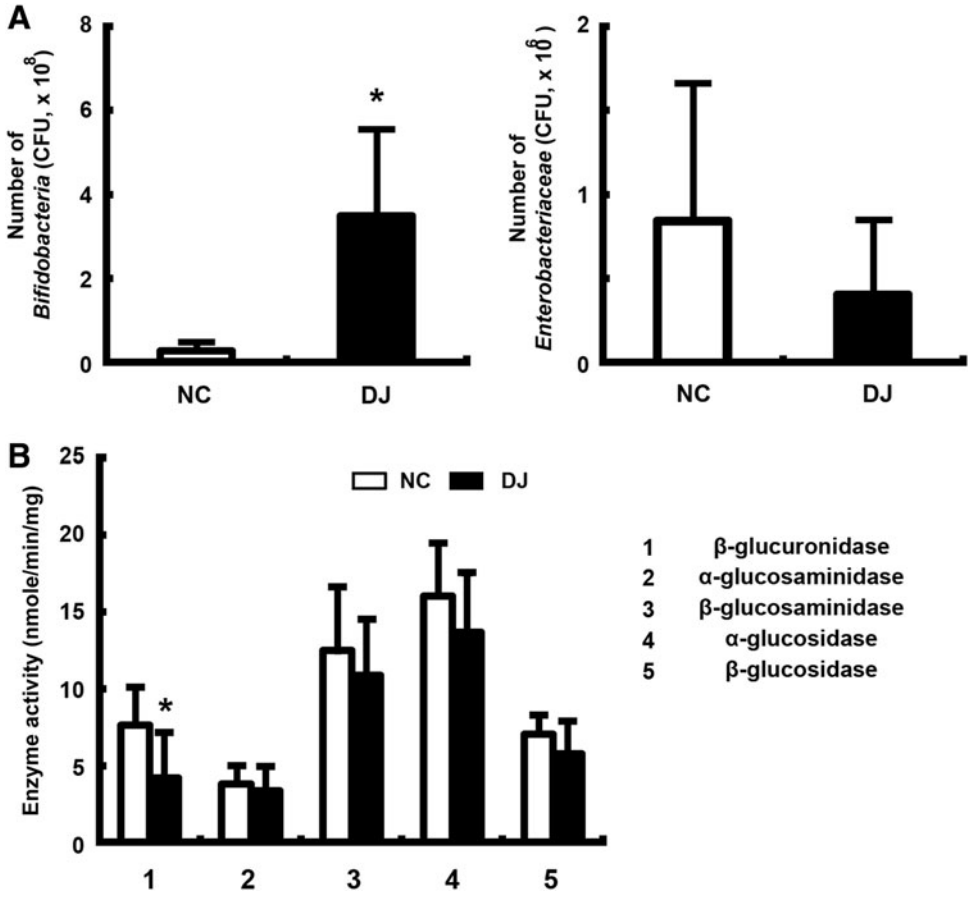

FIG. 2. Effect of *doenjang* on the number of *Bifidobacteria* and Enterobacteriaceae **(A)** and harmful enzyme activities **(B)** in fecal samples from mice treated with or without *doenjang*. The fresh feces were suspended in 9 volumes of dilution media, inoculated in BL agar plates and DHL agar plates. DHL agar plates were aerobically cultured for 1 day at 37°C, and BL agar plates were anaerobically cultured for 3 days at 37°C. The fecal enzyme activities were measured as described in **Materials and Methods.** All values were indicated as the mean ± S.D. ($n = 5$). $^*P < .05$ compared with NC.

activity, but did not influence α-/β-glucosaminidase and α-/β-glucosidase activities.

Next, we analyzed the gut microbiota in mice treated with or without *doenjang* by a 454 pyrosequencing method. As demonstrated by the rarefaction curves (Fig. 3) and the number of sequences analyzed, estimated OUT richness, coverage (Table 1), bacterial richness, and diversity were not different between normal control and *doenjang*-treated mice. Taxomomy-based analysis showed that treatment with *doenjang* induced a significant modulation in the populations of dominant gut microbiota as compared with vehicle treatment. At the phylum level, *doenjang* treatment resulted in a significant decrease of Firmicutes and an increase of Bacteroidetes, which led to a decrease in the Firmicutes to Bacteroidetes ratio in the gut microbiota (Fig. 4A and 4C). At the family level, the number of Ruminococcaceae and Lachnospiraceae was significantly decreased, while the number of *Odoribacter_f* was increased in *doenjang*-treated mice (Fig. 4B). Interestingly, EU845084_f (Bacteroidetes phylum) was only observed in *doenjang*-treated mice. In addition, treatment with *doenjang* lowered the number of Enterobacteriaceae, *Clostridium_g7_f*, and Rikenellaceae, whereas it expanded the populations of Porphyromonadaceae and Lactobacillaceae. At the genus level, *doenjang* significantly increased the number of *EF603735_g*, *DQ815871_g* (Bacteroidales, order), *and Odoribacter*, whereas decreased the number of *Pseudoflavonifractor*, *DQ789121*_g, and *Clostridium_g9* (Clostridiales, order) (Table 2). At the species level, *doenjang* increased the number of *EF406456_s*, *4P003085_s*, and *EF406459_s* (Bacteroidales, order), but decreased the number of *EF406686_s*, *EF603419_s* (Bacteroidales, order), and EF604627_g (Clostridiales, order) significantly (Table 3). We also processed all these sequences at the same length and position to match the length and position of the gut microbiota 16S rRNA gene sequences, computed all pairwise distances among normal control and *doenjang*-treated groups, and performed principal coordinate analysis to cluster these communities along axes of maximal variance (Fig. 5). Gut microbial communities of each group member were clustered and the maximum variations were 48.64% (PC1) and 16.84% (PC2).

Effect of doenjang *on the expression of colonic tight junction proteins and the activation of NF-κB in mice*

Next, we measured the effect of *doenjang* on expression of colonic tight junction proteins—occludin, ZO-1, and claudin-1—in mice (Fig. 6A). Generally, treatment with *doenjang* increased expressions of tight junction proteins. Especially, occludin alone was increased most potently by treatment with *doenjang*. We also measured its effect on

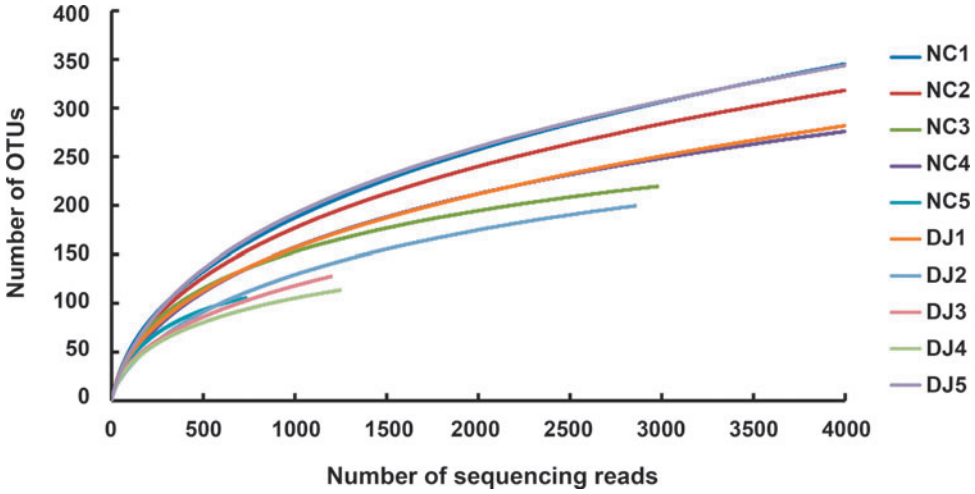

FIG. 3. Rarefaction curves. Rarefaction analysis of V1–V3 pyrosequencing tags of the 16S rRNA gene in fecal microbiota from normal control group (NC1–5) and *doenjang*-treated group (DJ1–5).

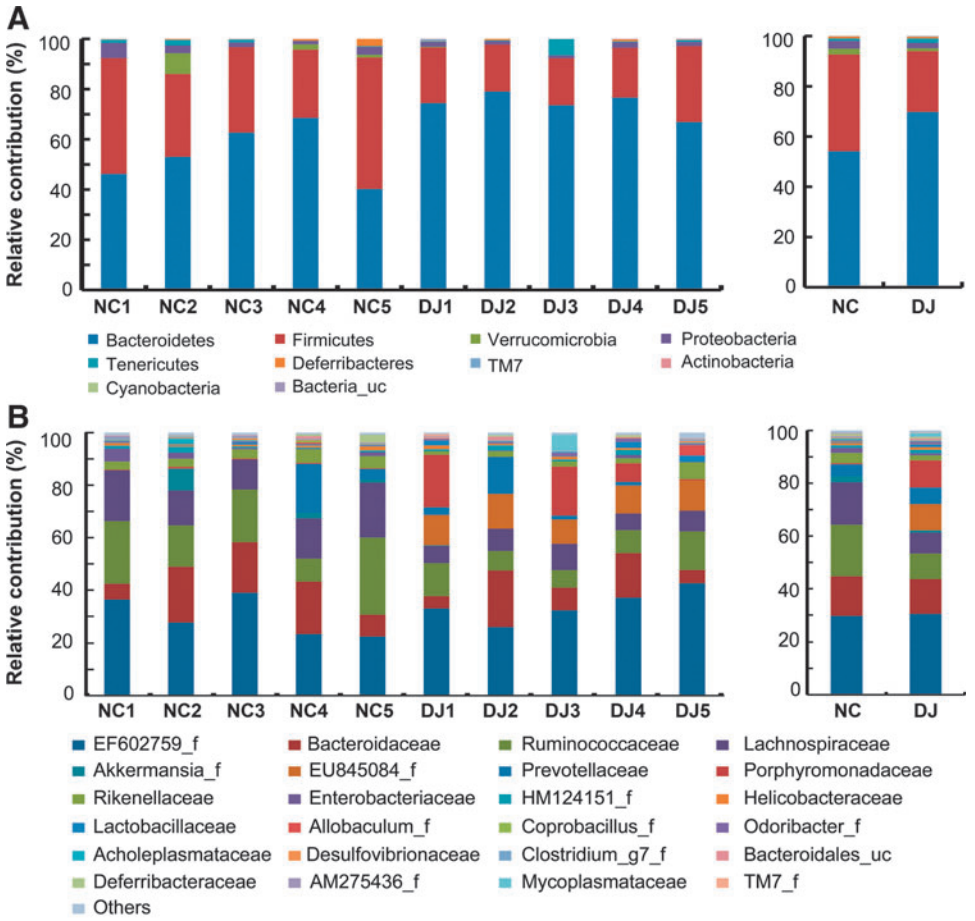

FIG. 4. The composition of gut microbiota in phylum and family levels. Taxonomy compositions: **(A)** phylum and **(B)** family levels are shown (individual samples are on the left panels and pooled samples are on the right panels). Genomic DNA was extracted from the cecal samples taken from mice maintained for 7 days on diets with or without *doenjang*. Samples were analyzed for the bacterial composition by pyrosequencing of the bacterial 16S rRNA fragments. **(C)** The Firmicutes to Bacteroidetes ratio ($n = 5$). $^*P < .05$ compared with NC.

Table 2. The Difference Between Normal Control and *Doenjang*-Treated Groups in the Composition of Fecal Bacterial Genera

Genus	*Composition*[a] *(%)*		P *value*
	NC	*DJ*	
Bacteroides	16.24 ± 6.93	11.53 ± 6.88	.321
Akkemansia	2.60 ± 3.92	0.84 ± 1.73	.183
E603735_g (Bacteroidales)	nd	8.95 ± 5.20	< .001
Pseudoflavonifractor	6.04 ± 3.14	2.11 ± 1.04	.047
Oscillibacter	4.57 ± 1.21	2.23 ± 0.87	.480
Prevotellaceae_uc	4.69 ± 9.37	5.84 ± 5.67	.891
Parabacteroides	0.41 ± 0.21	9.21 ± 9.69	.114
Alistipes	3.72 ± 0.98	1.70 ± 0.51	.424
DQ815871_g (Bacteroidales)	0.48 ± 0.17	5.4 ± 3.5	.006
DQ789121_g (Clostridiales)	3.70 ± 1.34	1.59 ± 0.95	.031
Clostridium_g9	2.02 ± 0.41	0.81 ± 0.29	.035
Escherichia	2.00 ± 2.13	0.71 ± 0.96	.136
Lactobacillus	0.43 ± 0.29	1.02 ± 1.07	.129
Clostridium_g6	0.26 ± 0.33	0.07 ± 0.15	.125
Odoribacter	0.33 ± 0.21	0.70 ± 0.42	.049
Clostridium_g7	0.50 ± 0.20	0.17 ± 0.12	.084

[a]Mean ± SD ($n = 5$).
nd, not detected.

NF-κB activation. Although treatment with *doenjang* suppressed NF-κB activation, it was not significant. Next, we measured TNF-α, IL-1β, IL-6, and IL-10 levels and myeloperoxidase activity in the colon of mice treated with or without *doenjang* (Fig. 6B). Although IL-1β and IL-6 expression was not influenced by treatment with *doenjang*, a proinflammatory cytokine TNF-α expression was significantly suppressed, but an anti-inflammatory cytokine IL-10 expression was significantly increased. However, the colonic inflammation marker myeloperoxidase activity was not influenced by treatment with *doenjang*. Furthermore, treatment with *doenjang* did not influence plasma TNF-α, IL-1β, IL-6, and IL-10 levels.

Table 3. The Difference Between Normal Control and *Doenjang*-Treated Groups in the Composition of Fecal Bacterial Species

Species	*Composition*[a] *(%)*		P *value*
	NC	*DJ*	
Bacteroides acidifaciens	14.51 ± 6.93	10.97 ± 7.16	.178
EF406456_s (Bacteroidales)	5.24 ± 3.87	9.17 ± 2.65	.047
4P003085_s (Bacteroidales)	nd	11.11 ± 1.36	.005
EF406686_s (Bacteroidales)	3.35 ± 1.63	1.75 ± 1.25	.042
EU504499_s (Bacteroidales)	3.56 ± 4.63	0.09 ± 0.20	.068
EF603419_s (Bacteroidales)	2.79 ± 1.33	1.60 ± 0.68	.009
EF406459_s (Bacteroidales)	0.12 ± 0.07	3.84 ± 2.36	.005
Escherichia fergusonii	1.39 ± 1.64	0.05 ± 0.11	.157
Clostridium cocleatum	0.24 ± 0.29	0.01 ± 0.03	.568
Lactobacillus reuteri	0.04 ± 0.06	0.42 ± 0.47	.104
Blautia_uc	0.31 ± 0.39	0.06 ± 0.08	.073
Lactobacillus johnsonii	0.08 ± 0.13	0.57 ± 0.82	.444
Pseudoflavonifractor_uc	0.27 ± 0.22	0.09 ± 0.11	.42
EF604627_g (Clostridiales)	0.32 ± 0.17	0.02 ± 0.05	.026

[a]Mean ± SD ($n = 5$).

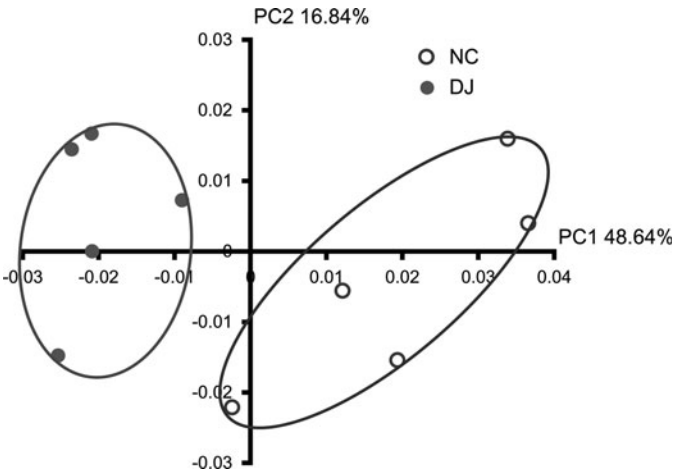

FIG. 5. Principal coordinate analysis (PCoA) plot. The plot showed the clustering pattern between normal control and *doenjang*-treated groups based on weighted pairwise Fast UniFrac analysis. ($n = 5$).

DISCUSSION

Doenjang has been documented as having antimutagenic,[23] anticancer,[24] antioxidative,[21] and anti-inflammatory properties.[34] Recently, antiobesity effects of *doenjang* have been observed in animals,[35,36] which may have been due to constituents such as genistein activating the transcription of carnitine palmitoyltransferase-I, a rate regulating enzyme for fatty acid oxidation, and peroxisome proliferator-activated receptor-alpha target genes involved in fatty acid beta oxidation.[25,34,35] However, *doenjang* is a unique soybean paste fermented by diverse microorganisms, which uses *Bacillus subtilis* and *B. licheniformis* and molds such as *Rhizopus*, *Mucor*, and *Aspergillus oryzae* from rice straw and local environments instead of inoculation.[37,38] Recently, 16S rRNA cloning and sequencing, PCR-DGGE, and 454 pyrosequencing were used to analyze the bacterial community of finally fermented *doenjang* and revealed that its dominant microbes are *Staphylococcus equorum*, *Enterococcus faecium*, *Tetragenococcus halophilus*, *Leuconostoc mesenteroides*, and *Staphylococcus gallinarum.*[39,40] Most of these bacteria belong to phylum Firmicutes. Thus, the composition of microbiota and consituents of *doenjang* was not significantly different according to rice straw and local environments. Therefore, of *doenjang* constituents, microbes may play an important role in expressing their biological activities.

Endotoxins such as LPS of gut microbiota constantly promote the aging process and metabolic diseases such as obesity, diabetes, and colitis.[6,10] Therefore, to maintain a healthy condition, LPS production of gut microbiota should be decreased.

In the present study, we found that *doenjang* decreased plasma and gut bacterial LPS levels as well as a harmful gut bacterial enzyme β-glucuronidase activity, which is increased in colitis,[31,32,41] in normal mice. Furthermore,

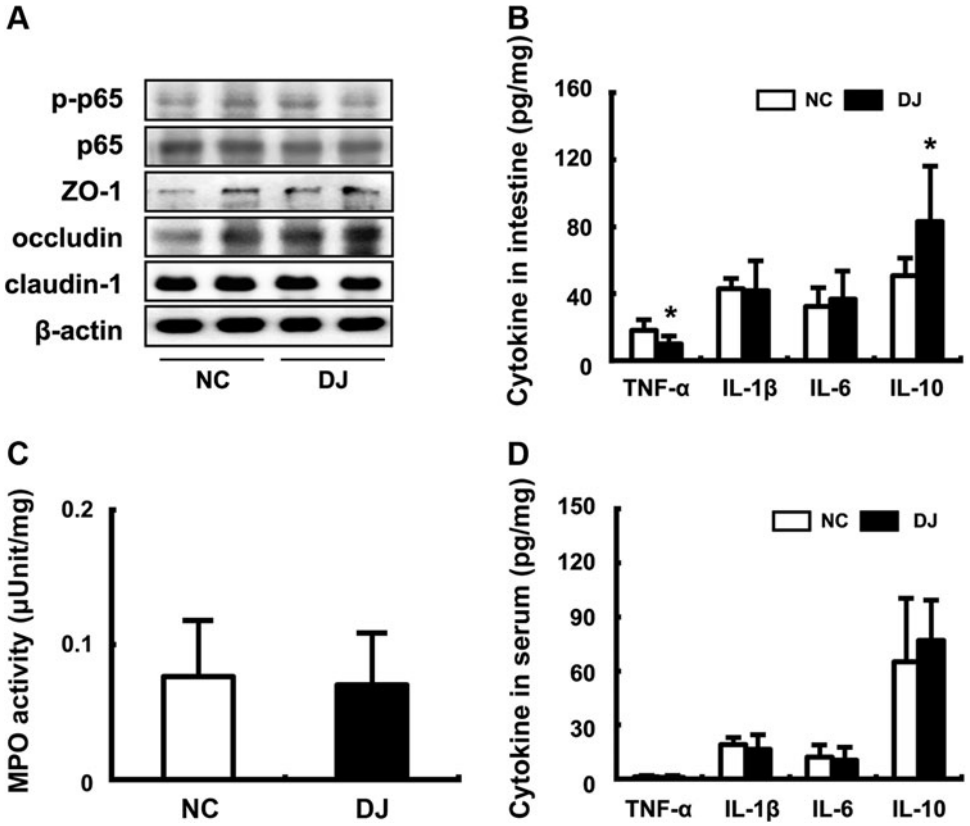

FIG. 6. Effect of *doenjang* on colonic p65, p-p65, ZO-1, occludin, claudin-1, β-actin **(A)**, cytokine expression levels **(B)**, myeloperoxidase activities **(C)**, and plasma cytokine levels **(D)** in mice. p65, p-p65, ZO-1, occludin, claudin-1, and β-actin were measured by Western blot analysis. Proinflammatory cytokine levels were analyzed by ELISA. All values were indicated as the mean ± S.D. ($n = 5$). $^*P < .05$ compared with NC. ELISA, enzyme-linked immunosorbent assay.

doenjang stimulated the growth of LAB, particularly *Bifidobacteria*. However, *doenjang* inhibited the number of Enterobacteriaceae, which potently produces the endotoxin, LPS.[42] These results suggest that the stimulation of significant bifidobacterial growth by *doenjang* may suppress the growth of Enterobacteriaceae, such as *Escherichia coli* and LPS production.

We also found that treatment with *doenjang* inhibited proinflammatory cytokine TNF-α expression, which significantly activates inflammatory reactions, such as NF-κB activation, and increased anti-inflammatory cytokine IL-10 expression, which inhibits inflammatory responses. Therefore, these effects may also be due to the decrease in Enterobacteriaceae in the intestine by treatment with *doenjang*.

In addition, Ley *et al.* reported that the decrease in the Bacteroidetes phylum and the increase in Firmicutes were observed in gut microbiota of *ob/ob* mice as compared with those in lean littermates, as well as in those of obese human as compared with those in healthy humans.[5,43] We and others also observed that HFD resulted in an increase in Firmicutes and a decrease in Bacteroidetes, which resulted in an increase in the Firmicutes/Bacteroidetes ratio in the gut microbiota according to the pyrosequencing method.[6,12] Although *doenjang* exhibits antiobesity and body weight reduction effects as Cha *et al*.[35] reported, it did not significantly reduce body weight in the present study. The difference may be due to the duration of *doenjang* administration. *Doenjang* induced growth of Porphyromonadaceae and Lactobacillaceae, which is related to obesity. Nevertheless, *doenjang* decreased fecal LPS levels, which are absorbed into the blood and cause obesity and body weight increase.[6,10] Furthermore, *doenjang* may be a potent prebiotic and ameliorates disturbed gut microbiota.

Based on these findings, *doenjang* may promote gut health by regulating gut microbiota and its LPS and harmful enzyme productions.

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AUTHOR DISCLOSURE STATEMENT

The authors state no conflict of interests.

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