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Title: *Bacillus coagulans* SANK 70258 suppresses *Enterobacteriaceae* in the microbiota of ulcerative colitis *in vitro* and enhances butyrogenesis in healthy microbiota

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Author Contributions

KS, TM, RY, and AK contributed to the major design of this study. KS, DS, JI, and NH contributed to data acquisition and analysis related to the model culture system. KS, DS, TM, and RY contributed to microbiota analysis. KS, NH, TM, RY and AK drafted and revised the manuscript. All authors approved the final version of the manuscript.

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Abstract

The aim of this study was to clarify the effect of the spore-forming and lactic acid-producing probiotic strain, *Bacillus coagulans* SANK 70258 on human colonic microbiota of healthy subjects and ulcerative colitis patients. A model culture system was employed to construct the *in vitro* human colonic microbiota, to retain the bacterial species richness and simulate the patient's disordered composition, from the fecal inoculum. Bacterial 16S rRNA gene sequencing confirmed that administration of *B. coagulans* SANK 70258 (at an initial concentration of 4×10^7 - total cells/mL) suppressed bacteria related to the family *Enterobacteriaceae* in the microbiota models for both healthy subjects ($P = 0.016$) and ulcerative colitis patients ($P = 0.023$). In addition, administration of *B. coagulans* SANK 70258 increased bacteria related to the family *Lachnospiraceae* ($P = 0.031$), thereby enhancing butyrate production ($P = 0.031$) in the microbiota models of healthy subjects. However, these changes were not observed in the microbiota models of ulcerative colitis patients, likely owing to the low abundance of *Lachnospiraceae* species. This study demonstrates the potential of *B. coagulans* SANK 70258 to exhibit antimicrobial activity against harmful organisms in patients with ulcerative colitis; while improving the intestinal microenvironment by increasing butyrogenesis in healthy persons.

Keywords

Bacillus coagulans, probiotics, microbiota, ulcerative colitis

Key Points

- *B. coagulans* SANK 70258 treatment reduced colonic *Enterobacteriaceae* species.
- *B. coagulans* SANK 70258 treatment enhanced butyrogenesis in healthy individuals.

- 63 • *B. coagulans* SANK 70258 treatment increased *Lachnospiraceae* in healthy persons.
- 64 • *B. coagulans* SANK 70258 improves the colonic microenvironment in ulcerative
- 65 colitis.

Introduction

Bacillus species are aerobic or facultatively anaerobic gram-positive spore formers and have been extensively used as probiotics in human dietary supplements and animal feeds to prevent gastrointestinal infections, via immunomodulation and synthesis of antimicrobials (Hong et al. 2005; Sanders et al. 2003). Spore-forming bacteria can survive in the extreme acidity of the stomach, bile salts, and the hostile conditions of the gastrointestinal tract (Cutting 2011). Thus, *Bacillus* spores can be stored without refrigeration and ingested species will reach the small intestine (Elshaghabe et al. 2017).

Several strains of a spore-forming lactic acid bacterium, *Bacillus coagulans*, have received Generally Recognized As Safe (GRAS) status from the Food and Drug Administration in the US and probiotic supplements containing *B. coagulans* are available in global markets (Elshaghabe et al. 2017; Cutting 2011). Intake of *B. coagulans* SANK 70258 increased stool frequency and alleviated constipation or diarrhea in humans (Watanabe et al. 2018; Ara et al. 2002). Supplementation with *B. coagulans* GBI-30, 6086 inhibited the pathogenic strain, *Clostridium difficile*, both in the *in vitro* culture system (Honda et al. 2011) and in mice with colitis induced by *C. difficile* infection (Fritzpatrick et al. 2011 and 2012), and daily consumption of *B. coagulans* GBI-30, 6086 by adults aged 65–80 years could increase populations of the commensal bacterium, *Faecalibacterium prausnitzii* in the human gut (Nyangale et al. 2015). In addition, a dose of simethicone and *B. coagulans* GBI-30, 6086 relieved abdominal pain and bloating for patients with irritable bowel syndrome (Ugresi et al. 2014). This was supported by the results of Majeed et al. (2018) which showed that a dose of *B. coagulans* MTCC 5856 reduced serum myeloperoxidase, an inflammatory biomarker, in irritable bowel syndrome patients.

We previously exploited the *in vitro* human colonic microbiota model [referred to as Kobe University Human Intestinal Microbiota Model (KUHIMM)] in a culture

system (Sasaki et al. 2018). The corresponding KUHIMM culture maintained most of bacterial species present in the original human feces, for the first time in an *in vitro* microbiota model. The administration of prebiotic oligosaccharides reproduced the bifidogenic property that increased the number of bacteria of genus *Bifidobacterium* and acetate production in the KUHIMM (Takagi et al. 2016). Thus, KUHIMM served as a tool for evaluating the functionality of food components. By combining next-generation sequencing technology with KUHIMM, a detailed examination of gut microbiota was possible (Sasaki et al. 2018). In addition, there is growing evidence that dysbiosis of the gut microbiota is associated with pathogenesis of intestinal disorders (Carding et al. 2015). KUHIMM reproduced disordered microbiota composition in ulcerative colitis patients, which decreased the proportion of butyrate-producing bacteria related to the family *Lachnospiraceae* and butyrogenesis, and increased the proportion of family *Enterobacteriaceae* that includes pathogens (Sasaki et al. 2019). Administration of *Clostridium butyricum* MIYAIRI restored butyrogenesis in the KUHIMM of ulcerative colitis patients. Hence, KUHIMM was useful to simulate the effect of probiotics in a deregulated intestinal environment.

Here, we utilized *B. coagulans* SANK 70258 that was isolated from green malt by Mashita et al. (1964) and Nakayama et al. (1967a, b). The aim of our study was to clarify in detail the effect of *B. coagulans* SANK 70258 administration on the colonic microbiota of healthy human subjects using KUHIMM. In addition, we investigated the effect of this strain on deregulated colonic microbiota of ulcerative colitis patients.

Materials and methods

Probiotics preparation

B. coagulans SANK 70258 (Lacris[®]-S powdered form; Mitsubishi-Chemical Foods Corporation, Tokyo, Japan) was propagated aerobically (0.01 g/L) in Gifu anaerobic

medium (GAM) broth (code 05422; Nissui Pharmaceuticals Co., Ltd, Tokyo, Japan), also containing KH_2PO_4 (2.67 g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1.33 g/L), NaCl (0.27 g/L), and trace elements ($\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$: 0.10 g/L; $\text{CaCl}_2 \cdot 5\text{H}_2\text{O}$: 0.10 g/L), for 17 h at 37 °C and rotated at 160 r/min. After propagation, *B. coagulans* SANK 70258 was added to the KUHIMM at an initial concentration of 4×10^7 - total cells/mL prior to fermentation.

Fecal collection from human volunteers

Fecal samples were obtained from seven healthy subjects and eight ulcerative colitis patients who had not been treated with antibiotics for more than 2 months prior to the sampling, as described previously (Sasaki et al. 2019). Written informed consent was obtained from all participants. Demographic data and ulcerative colitis characteristics (disease activity, location, and medical treatment) are presented in Additional File 1: Table S2. Disease activity was determined using the partial Mayo score at the time of fecal sampling. The study was performed in accordance with the principles of the Declaration of Helsinki and the guidelines of our institution and was approved by the intestinal ethics review board at Kobe University (research code 1902, approval date 10 May, 2016). All methods used in this study were performed in accordance with guidelines approved by the Medical Ethics Committee at Kobe University. After collection, fecal samples were immediately stored in an anaerobic culture swab (212559 BD BBL culture swab; BD Biosciences, Franklin Lakes, NJ, USA).

Operation of the model culture system with and without *B. coagulans*

The model culture system was operated using a multi-channel fermenter (Bio Jr.8; ABLE, Tokyo, Japan) to construct KUHIMM, as described previously (Sasaki et al. 2018; Takagi et al. 2016). Briefly, each vessel of the system contained autoclaved GAM (100 mL; Nissui Pharmaceutical Co.), with the initial pH adjusted to 6.5. Anoxic conditions were

constructed by purging vessels with a filter-sterilized mixture of N₂ and CO₂ (80:20) gas (15 mL/min) at 37 °C prior to fermentation. To prepare the inoculum, each fecal sample was suspended in 2 mL of 0.1 M phosphate buffer (pH 6.5, containing of a 2:2 mixture of 0.1 M NaH₂PO₄ and 0.1 M Na₂HPO₄), supplemented with 1% L-ascorbic acid (Wako Pure Chemical Industries, Osaka, Japan). Fermentation was initiated by inoculation of each medium-containing vessel with 100 µL of the above-mentioned fecal suspension. To test the effect of lactate, 30 mM of sodium L-lactate (Sigma) was added to a separate vessel containing medium. During fermentation, the medium was continuously purged with a filter-sterilized gas mixture. Fecal samples and aliquots of fermentation cultures were stored at –20 °C until use.

Extraction of microbial DNA

Microbial DNA was extracted from fecal samples and fermentation cultures of the KUHIMM using 0.1 mm glass beads, TE (10 mM Tris-HCl and 1 mM ethylenediaminetetraacetic acid [EDTA])-saturated phenol, and sodium dodecyl sulfate, as described previously (Takagi et al. 2016). Purified DNA was stored at –20°C until use.

16S rRNA gene sequencing

The bacterial 16S rRNA gene V3-V4 region was amplified from genomic DNA using the primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 (Klindworth et al. 2013). Illumina adapter overhang nucleotide sequences (Illumina, San Diego, CA, USA) were added to the gene-specific sequences. PCR was performed according to the manufacturer's instructions. Confirmed amplicons were purified with AMPure XP DNA purification beads (Beckman Coulter, Brea, CA, USA) and eluted in 25 µL of 10 mM Tris (pH 8.5). Amplicons were quantified on Agilent Bioanalyzer 2100 DNA 1000 chips (Agilent Technologies, Santa Clara, CA, USA) and pooled in equimolar concentrations

(5 nM). The pooled 16S rRNA gene products, along with an internal control (PhiX control V3; Illumina), were subjected to paired-end sequencing using a MiSeq sequencer (Illumina) with a 600-cycle MiSeq reagent kit (Illumina). PhiX sequences were removed, and paired-end reads with Q scores ≥ 20 were joined using QIIME version 1.9.1 software (Caporaso et al. 2010). The UCLUST algorithm was used to cluster filtered sequences into OTUs based on a 97% similarity threshold (Edgar et al. 2010). Chimeric sequences were identified and excluded from the library using USEARCH. Paired-end reads were taxonomically classified via the Greengenes taxonomic database using the Ribosomal Database Project Classifier (Wang et al. 2007). OTUs were used to calculate Chao 1 richness estimator and the Shannon and Simpson diversity indices.

Real-time PCR analysis

Real-Time PCR was performed using a TP700 Thermal Cycler Dice Real Time System Lite (Takara Bio, Shiga, Japan). Amplification with a primer set targeting all eubacteria was carried out as described previously (Takagi et al. 2016).

Short chain fatty acid identification

The concentrations of lactate, acetate, propionate, and butyrate were measured using high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan), equipped with an Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA, USA) and a RID-10A refractive index detector (Shimadzu), as described previously (Sasaki et al. 2018).

Bioinformatics and statistical analysis

The Chao 1, Shannon index, and Simpson index were calculated using the QIIME software package (Caporaso et al. 2010). Data were compared between the groups using the Mann–Whitney *U*-test, Wilcoxon signed-rank test, or Student’s *t* test in the JMP version 12. *P*-values below 0.05 were considered to be statistically significant.

Data availability

All raw sequence data generated in this study have been deposited in MG-RAST as “Model Culture System of Human Colonic Microbiota_*Bacillus coagulans* SANK 70258” under the accession numbers mgm4856204.3–mgm4856248.3.

Results

Evaluation of *B. coagulans* in KUHIMMs harboring similar bacterial richness to feces

The effect of *B. coagulans* SANK 70258 on colonic microbiota of healthy subjects was investigated using the *in vitro* human colonic microbiota model, KUHIMM. To initiate the fermentation, a fecal sample from each of seven healthy subjects was inoculated into the model culture system. The KUHIMM including *B. coagulans* SANK 70258 (initial concentration: 4×10^7 cells/mL) was compared to that without *B. coagulans* SANK 70258.

Eubacterial copy numbers were initially $(1.08 \pm 0.47) \times 10^7$ copies/mL in fecal samples of healthy subjects and reached $(6.42 \pm 1.33) \times 10^{10}$ copies/mL in the KUHIMM after 30 h of fermentation. Using Illumina MiSeq sequencing, more than 2,800,000 quality reads were yielded for bacteria from original feces and the KUHIMMs in corresponding fermentation cultures with and without *B. coagulans* SANK 70258 (Table 1). The KUHIMMs with and without *B. coagulans* SANK 70258 harbored similar numbers of operational taxonomic unit (OTU) to those in original feces (Mann–Whitney *U*-test, *P* = 0.16 and 0.52, respectively). The Chao1 richness estimator indicated no

significant differences between original feces and KUHIMMs without *B. coagulans* SANK 70258 or KUHIMMs with and without *B. coagulans* SANK 70258 (Mann–Whitney *U*-test, $P = 0.13$ or 0.31 , respectively). Bacterial community diversity indices (Shannon and Simpson) were lower in the KUHIMMs without *B. coagulans* SANK 70258, compared to those in original feces (Mann–Whitney *U*-test, $P = 0.04$ and 0.02 , respectively); however, no significant changes in the Shannon and Simpson indices were observed between KUHIMMs with *B. coagulans* SANK 70258 and KUHIMMs without *B. coagulans* SANK 70258 (Mann–Whitney *U*-test, $P = 0.80$ and 0.61 , respectively).

Effect of *B. coagulans* on taxonomic composition

Figure 1 shows the mean bacterial genus level-distribution in original feces, and KUHIMMs with and without *B. coagulans* SANK 70258 for seven healthy human subjects. Most of bacterial genera found in the feces belonged to the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Fusobacteria*, *Proteobacteria*, and *Verrucomicrobia* and almost all of these phyla were also found in the KUHIMMs (Fig. 1a). The mean relative abundance of genus *Bacillus* was 2.65 % in the KUHIMMs with *B. coagulans* SANK 70258 after 30 h of fermentation. Interestingly, the relative proportion of bacteria related to unclassified *Enterobacteriaceae* was significantly lower in the KUHIMMs with *B. coagulans* SANK 70258 than in KUHIMMs without *B. coagulans* SANK 70258 (Wilcoxon signed-rank test, $P = 0.016$, Fig. 1b). In addition, the relative proportion of bacteria related to unclassified *Lachnospiraceae* was significantly higher in the KUHIMMs with *B. coagulans* SANK 70258 than in KUHIMMs without *B. coagulans* SANK 70258 (Wilcoxon signed-rank test, $P = 0.031$, Fig. 1c).

The impact of *B. coagulans* SANK 70258 supplementation on the production of short chain fatty acids was examined after 30 h of fermentation. The concentration of butyrate was significantly higher in the cultures of KUHIMM with *B. coagulans* SANK

70258, compared to that in the cultures of KUHIMM without *B. coagulans* SANK 70258 (Wilcoxon signed-rank test, $P = 0.031$, Fig. 2a). In contrast, propionate production was unchanged (Wilcoxon signed-rank test, $P = 0.16$, Fig. 2b) and acetate production was lower (Wilcoxon signed-rank test, $P = 0.016$, Fig. 2c) with *B. coagulans* SANK 70258 supplementation. Lactate was reduced to 0 mM in the all cultures after 30 h of fermentation. We hypothesized that the increase in butyrate production resulted from lactate produced by *B. coagulans* SANK 70258, as lactate accumulation was observed in cultures of KUHIMM with *B. coagulans* SANK 70258 (25.1 ± 3.1 mM), compared to KUHIMM cultures without *B. coagulans* SANK 70258 (6.5 ± 6.3 mM), at the early stage of fermentation. Productions of butyrate, propionate, and acetate were compared in the cultures of KUHIMMs with and without supplementation of 30 mM lactate for three healthy subjects. Only butyrate production was increased by lactate supplementation (Student's t test, $P = 0.016$), while productions of propionate and acetate were unchanged (Student's t test, $P = 0.97$ and 0.93 , respectively) after 30 h of fermentation (Additional File 1: Table S1).

Effect of *B. coagulans* on microbiota in ulcerative colitis patients

Reportedly, a relative increase in the abundance of *Enterobacteriaceae* has been associated with irritable bowel disease (Lane et al. 2017), and a decrease in predominant butyrate producers, resulting in reduced butyrate level, contributed to the etiology of ulcerative colitis (Kumari et al. 2013). From these previous reports, we were interested in the effect of *B. coagulans* SANK 70258 on the colonic microbiota of ulcerative colitis patients. We administered *B. coagulans* SANK 70258 (initial concentration: 4×10^7 cells/mL) to each *in vitro* human colonic microbiota model (KUHIMM) of eight ulcerative colitis patients. Eubacterial copy numbers were initially $(0.99 \pm 0.51) \times 10^7$ copies/mL in the fecal samples of ulcerative colitis patients and reached $(8.40 \pm 4.44) \times$

10¹⁰ copies/mL in the KUHIMM after 30 h of fermentation. Illumina MiSeq sequencing yielded more than 4,500,000 quality reads for bacteria in original feces and the KUHIMMs in corresponding fermentation cultures with and without *B. coagulans* SANK 70258 after 30 h of fermentation (Table 1). The KUHIMMs with and without *B. coagulans* SANK 70258 harbored similar numbers of OTUs to those in original feces (Mann–Whitney *U*-test, *P* = 0.56 and 0.16, respectively). In addition, values of the alpha diversity metrics (Chao1, Shannon, and Simpson) for the KUHIMMs with and without *B. coagulans* SANK 70258 were similar to those for original feces.

Figure 3 shows the mean bacterial genus level-distribution in original feces, and KUHIMMs with and without *B. coagulans* SANK 70258 for eight ulcerative colitis patients. The mean relative abundance of the *Bacillus* genus was 0.77 % in the KUHIMMs after 30 h of fermentation with *B. coagulans* SANK 70258. As expected, the relative proportion of bacteria related to unclassified *Enterobacteriaceae* was significantly increased (Mann–Whitney *U*-test, *P* = 0.018) and that of bacteria related to unclassified *Lachnospiraceae* was significantly decreased in the KUHIMMs of ulcerative colitis patients compared to those in the KUHIMMs of healthy subjects (Mann–Whitney *U*-test, *P* = 0.0014). The relative proportion of bacteria related to unclassified *Enterobacteriaceae* was significantly lower in the KUHIMMs with *B. coagulans* SANK 70258 than in KUHIMMs without *B. coagulans* SANK 70258 (Wilcoxon signed-rank test, *P* = 0.023, Fig. 3b). However, the relative proportion of bacteria related to unclassified *Lachnospiraceae* was not significantly different in the KUHIMMs with and without *B. coagulans* SANK 70258 (Mann–Whitney *U*-test, *P* = 0.38, Fig. 3c). As expected, butyrate concentrations in KUHIMM cultures of ulcerative colitis patients were lower than those in KUHIMM cultures of healthy subjects (Wilcoxon signed-rank test, *P* = 0.032). Concentrations of butyrate, propionate, and acetate were not significantly different in the KUHIMM cultures with and without *B. coagulans* SANK 70258

(Wilcoxon signed-rank test, $P = 0.055$, 0.641 , and 0.109 , respectively, Fig. 4).

Discussion

Here, we clarified the effect of the spore-forming and lactic acid bacterium, *B. coagulans* SANK 70258 on human colonic microbiota, using the *in vitro* microbiota model, KUHIMM. The KUHIMM uses a rich fermentation medium without the adverse conditions of stomach acid and bile; therefore, sporulation of *B. coagulans* is not induced. At first, *B. coagulans* SANK 70258 had antimicrobial activity against members of the *Enterobacteriaceae*, both in healthy subjects and ulcerative colitis patients. Gram-positive soil inhabiting bacteria, *Bacillus* spp. can produce several antimicrobial compounds, which are safe to use (Joseph et al. 2013). The *B. coagulans* I₄ strain, isolated from cattle feces, is reported to produce coagulin (Hyronimus et al. 1998) and lactosporin with antimicrobial activity against pathogenic microorganisms (Riazi et al. 2009). Thus, we speculate that *B. coagulans* SANK 70258 produced the antimicrobial compound that suppressed bacteria related to *Enterobacteriaceae*. An increased relative abundance of proinflammatory bacterial species, including *Enterobacteriaceae*, in the irritable bowel syndrome microbiota has been reported (Rodiño-Janeiro et al. 2018). Our finding of an inhibitory effect of *B. coagulans* SANK 70258 on the *Enterobacteriaceae* corresponded with the previous research showing that *B. coagulans* administration improved abdominal pain and diarrhea in irritable bowel syndrome patients (Rogha et al. 2014).

The second effect of *B. coagulans* SANK 70258 administration was the increased relative abundance of bacteria related to the *Lachnospiraceae*, with a simultaneous increase in the production of butyrate. However, this effect was only observed in the microbiota of healthy subjects, and not in ulcerative colitis patients. This effect on the butyrate-producing *Lachnospiraceae* would result from the ability of *B. coagulans* to produce lactic acid (Wang et al. 2014). Certain members of the

Lachnospiraceae can consume lactate and produce butyrate (Louis et al. 2009). In this study, bacteria related to unclassified *Lachnospiraceae* formed a major part of the microbiota of healthy subjects. In fact, lactate administration enhanced butyrate production in the KUHIMM of healthy subjects. Therefore, it was speculated that lactate produced by *B. coagulans* SANK 70258 was metabolized by some members of *Lachnospiraceae* family to produce butyrate. This bacterial cross-feeding resulted in increased numbers of unclassified *Lachnospiraceae* and an increased butyrate level. In contrast, the microbiota of ulcerative colitis patients was comparatively depleted in unclassified *Lachnospiraceae*. Thus, in the microbiota of ulcerative colitis patients, the effect of bacterial cross-feeding would be less clear than in healthy subjects. Butyrate exerts multiple benefits in the gastrointestinal tract, including anticarcinogenesis, antiinflammation and antioxidant effects, and the improvement of the colonic defense barrier function (Hamer et al. 2008). Future research should investigate additional options to increase the relative abundance of indigenous *Lachnospiraceae* and stimulate butyrate formation in ulcerative colitis patients. For now, however, at least, *B. coagulans* SANK 70258 has been shown to be effective for preventing expansion in *Enterobacteriaceae*. Clinical trials are necessary to determine if *B. coagulans* SANK 70258 has efficacy as a probiotics and can join the ranks of known probiotics, such as *Escherichia coli* Nissle 1917 (Chibbar and Dieleman 2015).

Declarations

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Conflict of Interest

The authors have no competing interests to declare.

Ethical approval

All subjects provided written informed consent prior to specimen collection. The study was performed in accordance with the guideline of Kobe University Hospital and was approved by the institutional ethics review board of Kobe University. All methods used in this study were in accordance with the Declaration of Helsinki.

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Table 1 Summary of bacterial 16S rRNA gene sequencing data and α -diversity values (Chao1 estimator, Shannon index, and Simpson index).

	Healthy subjects			Ulcerative colitis patients		
	Feces (n = 7)	Culture (n = 7)	Culture+ <i>B. coagulans</i> (n = 7)	Feces (n = 8)	Culture (n = 8)	Culture+ <i>B. coagulans</i> (n = 8)
Read counts	124,132 $\pm$ 28,613	169,591 $\pm$ 39,797	120,397 $\pm$ 52,254	189,881 $\pm$ 12,862	194,928 $\pm$ 20,875	194,928 $\pm$ 20,875
Observed OTUs	1640 $\pm$ 460	1890 $\pm$ 316	1588 $\pm$ 434	1277 $\pm$ 522	924 $\pm$ 283	896 $\pm$ 316
Chao 1	2362 $\pm$ 571	2774 $\pm$ 445	2382 $\pm$ 606	2348 $\pm$ 850	2002 $\pm$ 559	2011 $\pm$ 648
Shannon index	6.06 $\pm$ 0.55	5.45 $\pm$ 0.35*	5.54 $\pm$ 0.35	5.12 $\pm$ 0.92	4.59 $\pm$ 0.57	4.64 $\pm$ 0.74
Simpson index	0.960 $\pm$ 0.017	0.935 $\pm$ 0.022*	0.939 $\pm$ 0.027	0.910 $\pm$ 0.068	0.898 $\pm$ 0.040	0.900 $\pm$ 0.060

Note: The values show the mean $\pm$ standard deviation. Statistical differences between samples were evaluated with respect to observed operational taxonomic units (OTUs), Chao 1 estimator, Shannon index, and Simpson index. The asterisks (*) indicate a significant difference (* $P < 0.05$ by Mann–Whitney’s U test) between microbiota in original feces and microbiota models in corresponding cultures. No significant differences were observed between microbiota models in cultures with and without *Bacillus coagulans* SANK 70258.

Figure legends

Fig. 1 Effect of *Bacillus coagulans* SANK 70258 on bacterial taxonomic composition.

(a) Genus level classification of bacteria in original fecal samples (Feces) and *in vitro* human colonic microbiota model (KUHIMM) without (-) and with *B. coagulans* SANK 70258 (+ *B. coagulans*) after 30 h of fermentation. Means are shown for seven healthy human subjects. Relative abundances of bacteria related to (b) Unclassified *Enterobacteriaceae* and (c) Unclassified *Lachnospiraceae*. * $P < 0.05$.

Fig. 2 Concentrations of (a) butyrate, (b) propionate, and (c) acetate. The *in vitro* human colonic microbiota model (KUHIMM) cultures without (-) and with *Bacillus coagulans* SANK 70258 (+ *B. coagulans*) were examined after 30 h of fermentation. A fecal sample from each of seven healthy subjects was used as the inoculum. * $P < 0.05$.

Fig. 3 Effect of *Bacillus coagulans* SANK 70258 on bacterial taxonomic composition.

(a) Genus level classification of bacteria in original fecal samples (Feces) and *in vitro* human colonic microbiota model (KUHIMM) without (-) and with *B. coagulans* SANK 70258 (+ *B. coagulans*) after 30 h of fermentation. Means are shown for eight ulcerative colitis patients. Relative abundances of bacteria related to (b) Unclassified *Enterobacteriaceae* and (c) Unclassified *Lachnospiraceae*. * $P < 0.05$.

Fig. 4 Concentrations of (a) butyrate, (b) propionate, and (c) acetate. The *in vitro* human colonic microbiota model (KUHIMM) cultures without (-) and with *Bacillus coagulans* SANK 70258 (+ *B. coagulans*) were examined after 30 h of fermentation. A fecal sample from each of eight ulcerative colitis patients was used as the inoculum.

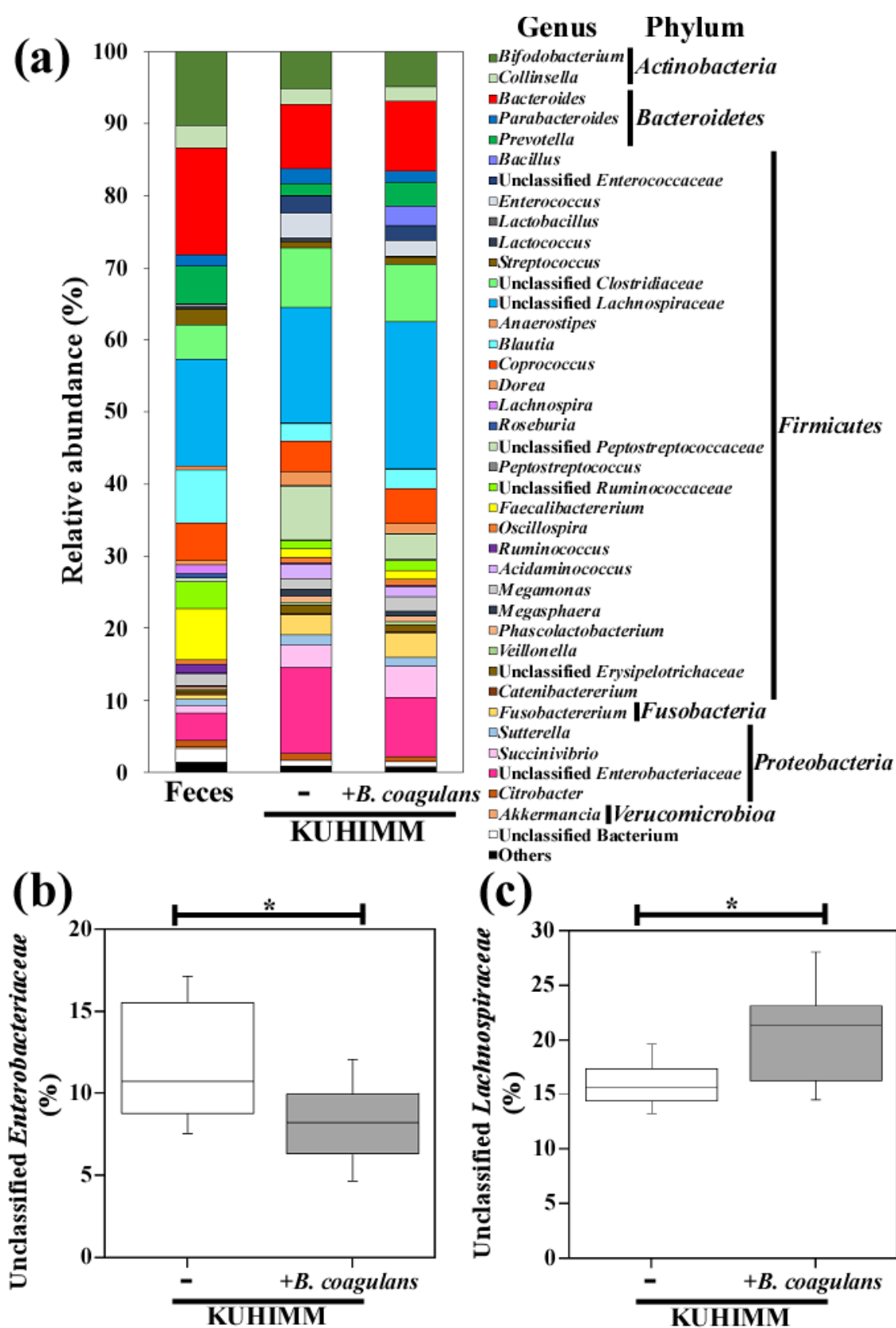


Fig. 1

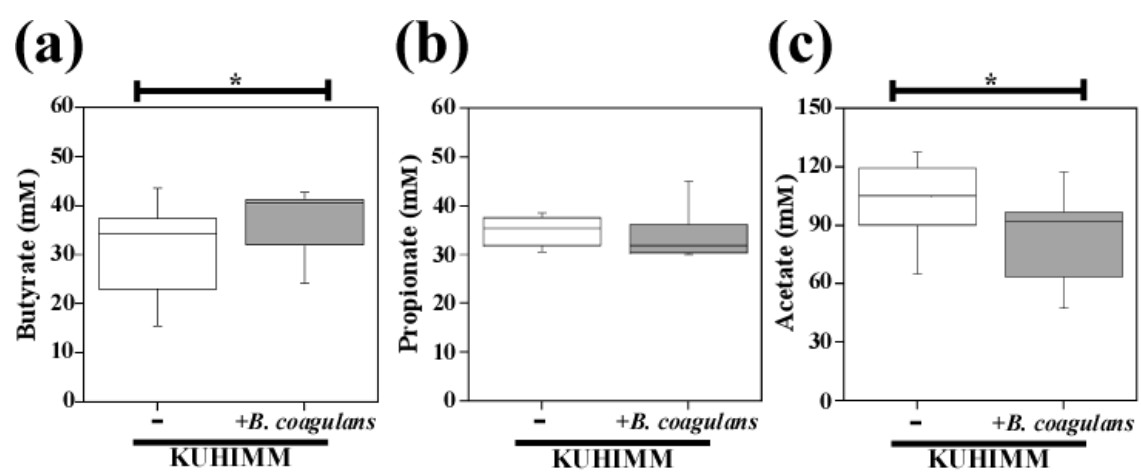


Fig. 2

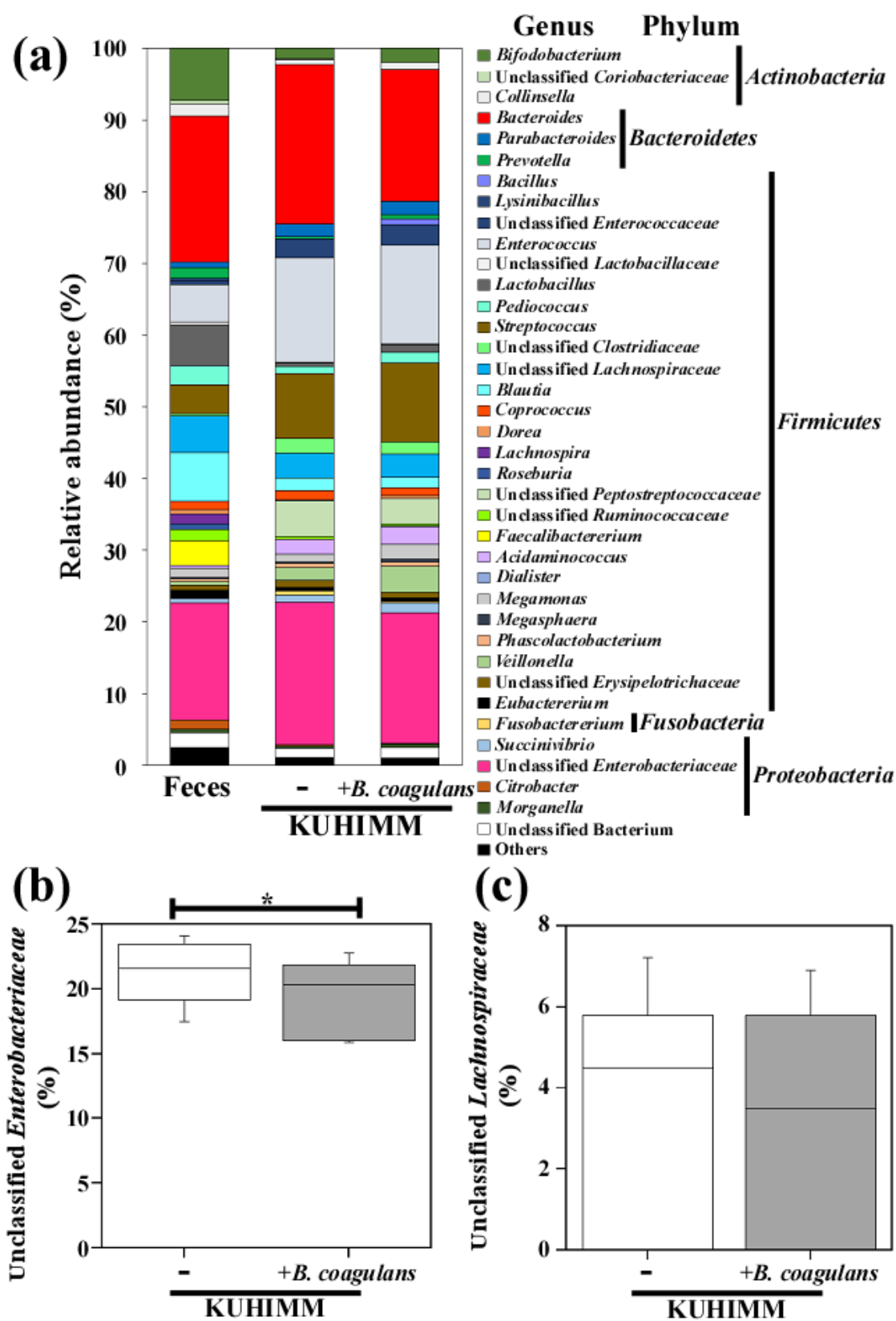


Fig. 3

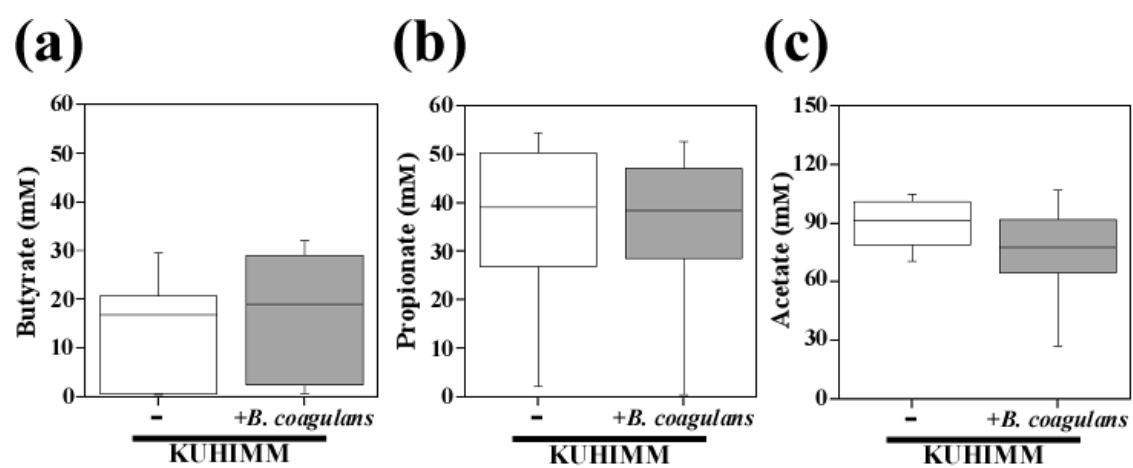


Fig. 4

Submitted to *Applied Microbiology and Biotechnology*

Title: *Bacillus coagulans* SANK 70258 suppresses *Enterobacteriaceae* in the microbiota of ulcerative colitis *in vitro* and enhances butyrogenesis in healthy microbiota

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Table S1 Concentrations of butyrate, propionate, and acetate. The *in vitro* human colonic microbiota model (KUHIMM) cultures without (-) and with 30 mM lactate (+ Lactate) were examined after 30 h of fermentation. The asterisk (*) indicates a significant difference between KUHIMM cultures with and without lactate (* $P < 0.05$ by Student's t test).

	Butyrate	Propionate	Acetate
	(mM)	(mM)	(mM)
–	38.5 ± 4.2	29.2 ± 6.2	108.9 ± 12.2
+ Lactate	52.4 ± 4.2*	28.2 ± 16.8	108.5 ± 8.3

Table S2 Characteristics of study volunteers comprising both healthy subjects and ulcerative colitis patients.

	Healthy subjects (n = 7)	Ulcerative colitis patients (n = 8)
Age in years (mean $\pm$ SD)	46.0 $\pm$ 14.2	48.3 $\pm$ 17.8
Sex (F/M)	2/5	4/4
Race		
Asian	100 %	100 %
Mean disease duration, years (mean $\pm$ SD)	NA	11.4 $\pm$ 8.2
Disease activity		
Partial Mayo score	NA	1.1 $\pm$ 0.9 (n = 8)
Disease location		
E1/E2/E3	NA	0/3/5
Medical treatment ^a		
5-ASAs	0	8
Oral corticosteroids	0	0
Purine analogues	0	5
TNF inhibitors	0	4
Other immunosuppressants	0	0

NA: Not applicable; 5-ASA: 5-aminosalicylic acid; TNF: Tumor necrosis factor.

^aMedical treatment at the time of sample collection.