# W27 IgA suppresses growth of Escherichia in an in vitro model of the human intestinal microbiota

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# Abstract

W27 monoclonal immunoglobulin A (IgA) suppresses pathogenic *Escherichia coli* cell growth; however its effect on the human intestine remains unclear. We thus aimed to determine how W27 IgA affects the human colonic microbiota using the *in vitro* microbiota model. This model was established using fecal samples collected from 12 healthy volunteers; after anaerobic cultivation, each model was found to retain the genera found in the original human fecal samples. After pre-incubating W27 IgA with the respective fecal sample in aerobic condition, the mixture of W27 IgA (final concentration, 0.5 μg/mL) and each fecal sample was added to the *in vitro* microbiota model and cultured under anaerobic condition,. Next-generation sequencing of the bacterial 16S rRNA gene revealed that W27 IgA addition significantly decreased the relative abundance of bacteria related to the genus *Escherichia* in the model. Additionally, at a final concentration of 5 μg/mL, W27 IgA delayed growth in pure culture of *Escherichia coli* isolated from human fecal samples. Our study thus revealed the suppressive effect of W27 IgA on the genus *Escherichia* at relatively low-concentrations and the usefulness of an *in vitro* microbiota model to evaluate the effect of IgA as a gut microbiota regulator.


# Introduction

The human gastrointestinal tract harbors a complex community of over trillions of microbial cells, which play a central role in human health [1]. The gut microbiota promotes food digestion and xenobiotic metabolism and regulates innate and adaptive immunological processes [2]. Many studies have shown that dysbiosis, defined as a persistent imbalance of gut microbiota, is associated with diseases such as inflammatory bowel disease, irritable bowel syndrome, diabetes, obesity, cancer, cardiovascular, and central nervous system disorders [2, 3]. Manipulating the intestinal microbiota using prebiotics, probiotics, and fecal microbiota transplants is an important strategy for disease prevention and treatment [4].

Secretory immunoglobulin A (IgA) is the most abundant class of antibody isotypes found in the intestinal lumen [5]. Secretory IgA interacts with various intestinal antigens including self-antigens, food components, and intestinal microbiota [6]. Predominant IgA involves polyreactive specificities, mostly to coat commensal bacteria during homeostasis [7]. Other typical non-polyreactive IgA are predominantly triggered by pathogens and exhibit classical features of T cell-dependent, extensive somatic hypermutation and affinity maturation (generating high-affinity) [8]. Recently, Okai et al. demonstrated that a unique mouse monoclonal IgA antibody (clone W27) had high affinity for a variety of bacteria (showing a polyreactive nature) and suppressed the *in vitro* growth of *Escherichia coli* cells [9, 10]. Oral administration of W27 IgA prevented the development of dextran sodium sulfate-induced colitis in mice, by modulating the intestinal microbiota [9, 11]. These results have aroused much interest in the effect of W27 IgA on the human gut microbiota.

We previously reported an *in vitro* human colonic microbiota model (named as Kobe University human intestinal microbiota model, KUHIMM), which can capture most of the microbial species in a fecal sample [12]. Although *in vitro* models cannot provide the complete host factors, they are useful tools for studying

and uncovering the microbiota response to different compounds [13]. Here, we examined the effect of W27 IgA on the human colonic microbiota using KUHIMM.

# Results

### W27 IgA suppresses genus *Escherichia* at low concentration

To evaluate the effective W27 dose in human microbial culture, the optimal W27 IgA concentration was assessed using an *in vitro* human colonic microbiota model, KUHIMM. First, we preincubated one fecal sample with W27 IgA at 200, 1000, and 5000 μg/mL under aerobic conditions at 37°C for 3 h. This mixture was then transferred to the model culture system to establish KUHIMM, in which the final concentration of W27 IgA was 0, 0.5, 2.5, or 12.5 μg/mL. Total bacterial DNA was extracted from the original fecal inoculum and the corresponding KUHIMM cultures after 48 h of fermentation. In all 4 KUHIMM samples, eubacterial copy numbers, evaluated by quantitative real-time PCR, reached $(9.69 \pm 1.46) \times 10^{11}$ copies/mL from the fecal inoculum ($8.47 \times 10^{8}$ copies/mL). Next-generation sequencing on the Illumina MiSeq system was performed for analyzing the V3-V4 region of bacterial 16S rRNA gene sequences in the fecal inoculum and the corresponding KUHIMM cultures (Fig. 1). The relative abundance of bacteria related to the genus *Escherichia* was found to decreased with decreasing W27 IgA concentrations. A lower concentration of W27 IgA was found more effective at suppressing the genus *Escherichia* in KUHIMM. Thus, W27 IgA was applied to subsequent KUHIMM experiments at final concentration of 0.5 μg/mL.

### W27 IgA at final concentration of 0.5 μg/mL, suppresses genus *Escherichia* in KUHIMMs

KUHIMM was established using the 12 human fecal samples as inoculums. Each fecal sample was preincubated with 200 μg/mL of W27 IgA or mouse monoclonal IgA in aerobic conditions at 37°C for 3 h. Each fecal sample was also prepared and incubated for 3 h without W27 IgA. Then, to examine the effect of IgA on the microbiota, particularly on bacteria related to the genus *Escherichia*, we added each of these fecal samples to the model culture system. The final concentration of W27 IgA or mouse monoclonal IgA in KUHIMM was 0.5 μg/mL. DNA was extracted from the fecal inoculums and corresponding KUHIMM cultures without IgA (control) and with W27 IgA (+ W27 IgA) or mouse monoclonal IgA (+ Mouse IgA) after 48 h of fermentation. In all KUHIMM samples, the eubacterial copy numbers reached $1.44–5.53 \times 10^{11}$ copies/mL.

Next-generation sequencing obtained a total of 1,213,033 quality reads from the 12 fecal samples and the corresponding KUHIMMs with and without IgA (Table 1). The number of operational taxonomic units (OTUs) was lower in the KUHIMMs compared to the original fecal samples (Wilcoxon signed-rank test, $p = 0.0005$). However, there was no significant difference in the number of OTUs between KUHIMMs without IgA and with IgA (Wilcoxon signed-rank test, $p > 0.05$). The other alpha diversity measures (Shannon index, and Faith's phylogenetic diversity) were also lower in the KUHIMMs than in the original fecal samples (Wilcoxon signed-rank test, $p < 0.05$); however, there was no significant difference between

KUHIMMs without IgA and with IgA (Wilcoxon signed-rank test, $p > 0.05$). Thus, the microbial richness and diversity in the KUHIMMs did not change with the addition of W27 IgA or mouse monoclonal IgA at 0.5 μg/mL.

Table 1

**Summary of 16S rRNA gene sequencing data and α-diversity values [Shannon index and Faith's phylogenetic diversity (PD)].** In addition to twelve human fecal samples (Feces), the corresponding cultures without IgA (Control), the corresponding cultures with W27 IgA (+ W27 IgA), and the corresponding cultures with mouse monoclonal IgA (+ Mouse IgA) were sampled after 48 h of fermentation. The values are the mean ± standard deviation. Asterisks (*) represent significant differences (*$p < 0.05$) (n = 12) between the microbiota in the original feces and in corresponding cultures without IgA using Wilcoxon signed rank test. Significant differences were not detected between KUHIMMs without and with IgA.

| | Feces | KUHIMM | | |
|---|---|---|---|---|
| | | Control | + W27 IgA | + Mouse IgA |
| Read counts | 22,340 ± 3,797 | 26,063 ± 9,806 | 27,090 ± 5,774 | 25,593 ± 6,594 |
| Observed OTUs | 380.2 ± 113.7 | 215.2 ± 74.7* | 210.0 ± 56.8 | 213.0 ± 46.0 |
| Shannon index | 7.72 ± 0.54 | 6.28 ± 0.46* | 6.32 ± 0.46 | 6.35 ± 0.45 |
| Faith's PD | 17.5 ± 5.2 | 10.6 ± 2.8* | 11.7 ± 3.0 | 10.7 ± 1.4 |

Bacterial genus-level compositional analyses of the microbiota in the feces, control, + W27 IgA, and + mouse IgA are shown in Fig. 2. Almost all bacterial genera in the original feces were also detected in the corresponding KUHIMMs. The most significant decrease in the relative abundance of the genus *Escherichia* was observed in KUHIMMs with W27 IgA (+ W27 IgA), compared to those without IgA (Control) and those with mouse monoclonal IgA (Wilcoxon signed-rank test, $p = 0.002$ and 0.02, respectively); however, no significant difference was observed between + mouse IgA and control (Wilcoxon signed-rank test, $p = 0.90$) (Fig. 3). For almost all other genera, no major difference was observed between + mouse IgA and control. Thus, addition of 0.5 μg/mL W27 IgA selectively decreased the bacteria related to genus *Escherichia*.

### W27 IgA suppressesEscherichia coliisolated from human feces

We investigated the effect of W27 IgA on *E. coli*, isolated from a human fecal sample, using nutrient broth with 0.5% NaCl under aerobic conditions. Isolated *E. coli* (800 cells/mL) was preincubated with or without W27 IgA at 200 or 20 μg/mL at 37°C for 1 h under aerobic condition. We then added isolated *E. coli* or the mixture with W27 IgA to Gifu Anaerobic Medium under anaerobic conditions and incubated at 37°C for 24 h. The final concentration of W27 IgA was 5.0 or 0.5 μg/mL. As indicated by the optical density at 600 nm ($OD_{600}$), W27 IgA delayed the growth of isolated *E. coli* at a final concentration of 5.0 μg/mL (Fig. 4). However, the growth of isolated *E. coli* was similar in the absence of W27 IgA and with W27 IgA at a final concentration of 0.5 μg/mL. However, microbial concentrations were similar between isolated *E. coli* with and without W27 IgA after 24h of incubation,.

# Discussion

W27 IgA at a final concentration of 0.5 μg/mL specifically suppressed bacteria related to the genus *Escherichia* in the *in vitro* human colonic microbiota model, KUHIMM, which harbor complex microbiota at more than $10^{11}$ copies/mL. This microbial number was comparable to the reported cell densities in the human colon (approximately $10^{11}$ cells/mL) [14]. Our results suggested that W27 IgA showed high specificity to the genus *Escherichia*. This result corresponded with previous data showing that W27 IgA specifically recognized the metabolic enzyme, serine hydroxymethyltransferase, from *E. coli*, rather than that from *Bifidobacterium bifidum*, *Blautia coccoides*, and *Lactobacillus casei* [9]. To our knowledge, this is the first report on the effect of W27 IgA on the human colonic microbiota model. In addition, W27 IgA at a final concentration of 5 μg/mL suppressed isolated *E. coli* in an *in vitro* model harboring *E. coli* alone. The final concentration of W27 IgA in the *E. coli* model was ten times higher than that in the KUHIMMs. The relative abundance of the Enterobacteriaceae family, including the *Escherichia* genus, was found to be approximately 2% in the colon of 13 healthy human subjects [15]. Thus, 0.5 μg/mL of W27 IgA as a daily dose would be sufficient to suppress the growth of the *Escherichia* genus in healthy human subjects. This amount (0.5 μg/mL) corresponds to 200 μg in the human colon, as the human colon content has been estimated at approximately 400 mL [14]. On the contrary, the relative abundance of the Enterobacteriaceae family was found to be increased to an average of approximately 24% (12 times higher than that in healthy subjects) in the colon of 13 patients with ulcerative colitis [15]. Several studies have demonstrated that proliferation of *E. coli* may influence the inflammatory process in the gastrointestinal tract [16]. Suppression of *E. coli* is thus important to prevent the progression of inflammation. Thus, patients with ulcerative colitis might require 6 (= 0.5 × 12) μg/mL of W27 IgA, corresponding to 2.4 mg/day, for suppressing *E. coli*.

To the best of our knowledge, this is the first study to investigate the role of IgA using an *in vitro* model mimicking the human colonic microbiota. We utilized an *in vitro* batch fermentation system that is fast, easy to operate, and reproducible [17]. The limitation of KUHIMM is the simple batch fermentation for 48 h; therefore, the long-term-effective dose of IgA was not evaluated. Thus, an increase in the relative abundance of Lachnospiraceae was not observed in the KUHIMM by addition of W27 IgA, whereas Lachnospiraceae abundance was increased in mice supplemented with W27 IgA for four weeks [9]. In addition, differences were observed in the microbiota composition between KUHIMM and fecal samples, such as an increase in unclassified Peptostreptococcaceae, *Streptococcus*, and *Enterococcus* in the KUHIMM culture. Therefore, further improvement of KUHIMM to address these limitations is currently underway.

In conclusion, these results demonstrate that KUHIMM is useful for simulating the effect of therapeutic IgA as a gut microbial regulator in human patients. Further, we confirmed that W27 IgA can suppress *Escherichia* growth *in vitro* in KUHIMM harboring complex human microbial species at relatively low concentration (0.5 μg/mL), indicating its potential for treating intestinal diseases with a disturbed balance of *Escherichia* species.

# Methods

### Fecal specimen collection.

Fecal samples were obtained from 12 healthy Japanese volunteers, who had not been treated with antibiotics for more than six months prior to the experiment. All participants were recruited according to the following inclusion criteria: age 20 to 60 years, Japanese, non-smoking, and with good health and physical condition. All subjects provided written informed consent prior to specimen collection. Immediately after collection, each fecal sample was stored in an anaerobic culture swab (212550 BD BBL Culture Swab; Becton, Dickinson and Company, New Jersey, USA) and used within 24 h. The study was performed in accordance with the guidelines 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 principles of the Declaration of Helsinki.

### Operation of the model culture system with and without IgA.

We used a small-scale multi-channel fermenter (Bio Jr. 8; ABLE, Tokyo, Japan) comprising eight parallel and independent anaerobic culturing vessels, as described by Sasaki et al. [12]. Each vessel contained autoclaved Gifu anaerobic medium (GAM [Code 05422]; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), with the initial pH adjusted to 6.5. The anaerobic conditions in the vessel were achieved by purging with a mixture of $N_2$ and $CO_2$ (80:20, 15 mL/min), which was filter-sterilized through a 0.2-μm polytetrafluoroethylene membrane filter (Pall Corporation, Port Washigton, Ny, USA) at 37°C for 1 h prior to cultivation. To prepare the fecal suspension, the fecal sample in the swab was suspended in phosphate buffer (0.1 M, 2.0 mL, pH 6.5, comprising of mixture of $NaH_2PO_4$ and $Na_2HPO_4$ at 61.65:28.35) supplemented with L-ascorbic acid (1.0% w/v; Wako Pure Chemical Industries, Osaka, Japan) in aerobic conditions.

W27 IgA was prepared as described previously (Okai et al. 2016). Mouse IgA-LE/AF was purchased from Southern Biotechnologies (0106-14). The fecal suspension was preincubated with IgA or without IgA at 37°C under aerobic condition for 3 h. Cultivation in the fermantation jar was initiated by inoculating one fecal suspension or mixture with IgA (approximately 250 μL) into each vessel. During fermentation at 37°C, the culture broth was stirred at 300 rpm with a magnetic stirrer and continuously purged with a filter-sterilized gas mixture. Aliquots of the culture broth were collected from the vessel 48 h after initiating the culture. The fecal suspensions and culture broth samples were then stored at −20°C until use.

### Profiling of bacterial 16S rRNA

Microbial genomic DNA was extracted from the fecal suspension and culture broth obtained from KUHIMM, as described previously [12]. Purified DNA was eluted into TE buffer (10 mM Tris–HCl, 1.0 mM EDTA) and stored at −20°C until use. Bacterial 16S rRNA genes (V3—V4 region) were amplified using genomic DNA as the template with the primers S-D-Bact-0341-b-S-17 (5'-CCTACGGGNGGCWGCAG-3') and

S-D-Bact-0785-a-A-21 (5'-GACTACHVGGGTATCTAATCC-3') [18]. PCR and amplicon pool preparation were performed according to the manufacturer's instructions (Illumina, San Diego, CA, USA). PCR amplicons were purified using AMPure XP DNA purification beads (Beckman Coulter, Brea, CA, USA) and were eluted in 25 μL of 10 mM Tris (pH 8.5). Purified amplicons were quantified using the Agilent Bioanalyzer 2100 with DNA 1000 chips (Agilent Technology, Santa Clara, CA, USA) and the Qubit 2.0 instrument (Thermo Fisher Inc., Waltham, MA, USA), and were pooled at equimolar concentrations (5 nM). The 16S rRNA genes and an internal control (PhiX control v3; Illumina) were subjected to paired-end sequencing using the MiSeq instrument (Illumina) and the MiSeq Reagent Kit v3 (600 cycles; Illumina). The PhiX sequences were removed, and paired-end reads with Q scores ≥ 20 were joined using the Automated CASAVA 1.8 paired-end demultiplexed fastq, which was performed with the FASTQ Generation program on the Illumina Basespace Sequence Hub (https://bases pace.illum ina.com/). Sequence quality control and feature table construction of the sequence data were performed and corrected using QIIME 2 version 2020.8 (https://qiime 2.org) (Bolyen et al. 2019) and the DADA2 pipeline (Callahan et al. 2016). The taxonomic compositions of the OTUs were classified using the naive Bayes classifier trained on the Greengenes 13_8 99% OTU full-length sequence database (https://data.qiime2.org/2020.8/common/gg-13-8-99-nb-classifier.qza). The OTU data were then used for α-diversity estimation of Faith's phylogenetic diversity (Faith 1992) and Shannon's indices (Shannon 1948, Shannon 1948).

### Real-time PCR analysis

Real-time PCR was performed to quantify total bacteria, using the LightCycler 96 system (Roche, Basal, Switzerland) with a universal primer set (5'-ACTCCTACGGGAGGCAGCAGT-3' and 5'-GTATTACCGCGGCTGCTGGCAC-3') targeting eubacteria (Nordeste et al. 2017). PCR amplification was performed as described previously (Takagi et al. 2016).

### Isolation of *E. coli* from human feces

Fresh fecal samples derived from one human volunteer were prepared and cultured in Gifu anaerobic medium as described above. The human fecal fermentation culture was plated on the surface of autoclaved nutrient broth agar with 0.5% NaCl (composition per liter was 15 g agar, 5 g Bacto peptone, 3 g beef extract, and 5 g NaCl). The agar plate was incubated at 37°C under aerobic conditions for 1 d. A single colony was picked, subcultured in nutrient broth medium with 0.5% NaCl, and then stored as the stock culture at −80°C after adding glycerol (20% [vol/vol]). Genomic DNA was extracted from the 24-h culture in each culture medium as described previously.

### Growth assayof isolated *E. coli*

Isolated *E. coli* were pre-cultured overnight in nutrient broth medium with 0.5% NaCl at 37°C under aerobic conditions. The culture was then diluted to 800 cells/mL in phosphate-buffered saline and preincubated with or without 200 μg/mL of W27 IgA at 37°C under aerobic conditions for 1 h. Then, *E. coli* with or without W27 IgA (final concentration: 5 μg/mL) was cultured in Gifu anaerobic medium at 37°C under

anaerobic conditions ($N_2$: 80%, $H_2$: 10%, $CO_2$: 10%) for 24 h. Finally, the $OD_{600}$ was measured using a spectrophotometer (UVmini-1240; Shimadzu, Japan).

### Statistical analysis

Data were compared between groups using the Wilcoxon signed-rank test in JMP version 12. Statistical significance was set at $p < 0.05$.

### Data Availability Statement

All sequences from the original fecal samples and corresponding KUHIMMs were deposited in MG-RAST as “Model Culture System of Human Colonic Microbiota IgA” under accession number mgm4922092.3-mgm4922148.3.

## Declarations


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### Author Contributions

KS, TM, NH, and RS conceived the major study design. TM and RS prepared the IgA. KS, NH, and JI operated and analyzed the model culture system. DS performed the NGS analyses. KS and AK conducted the cultures. KS, TM, NH, RS, and AK drafted and revised the manuscript. All authors read and approved the final manuscript.

### Additional Information

**Competing financial interests:** The authors declare no competing financial interests.

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# Figures

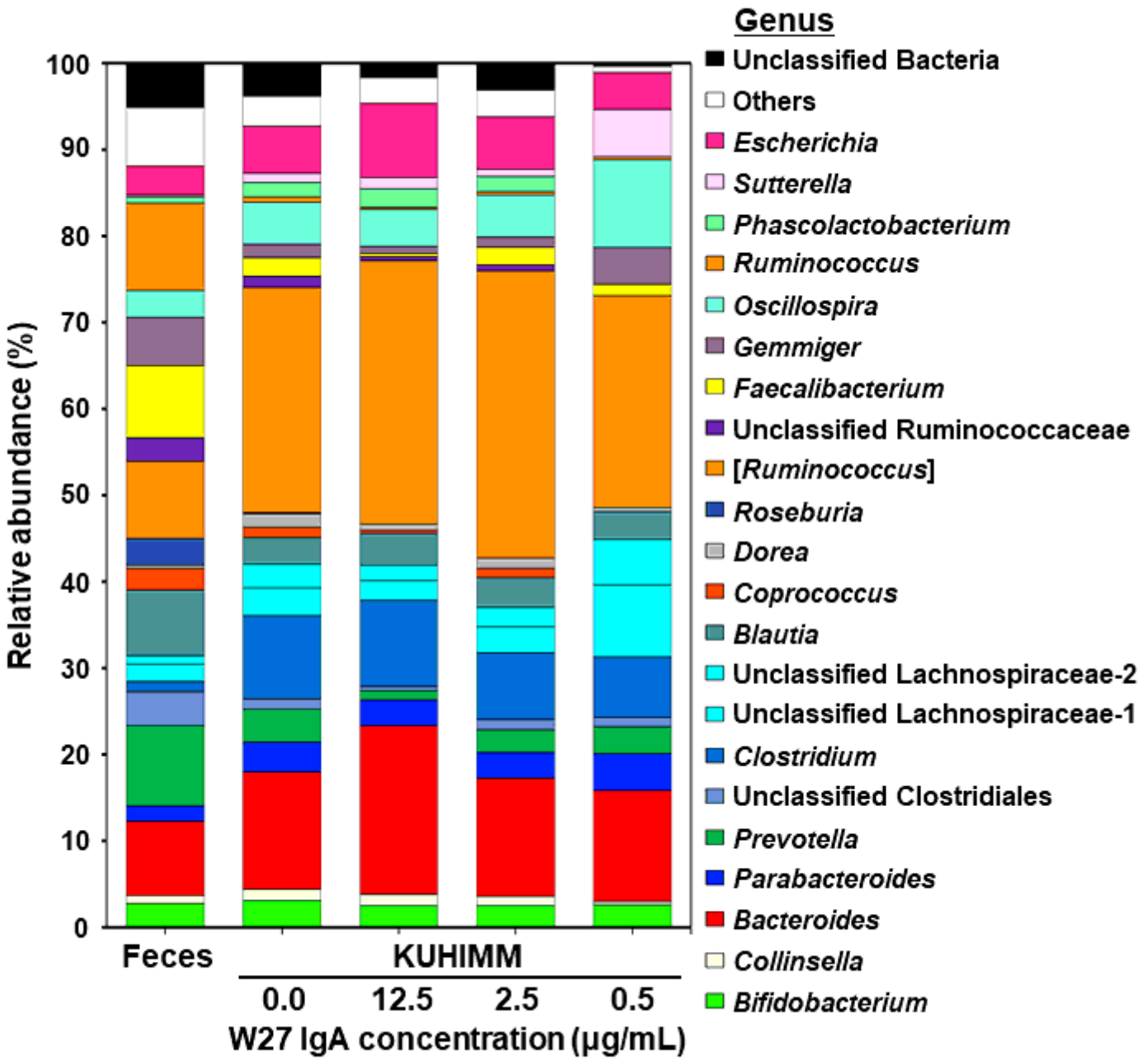
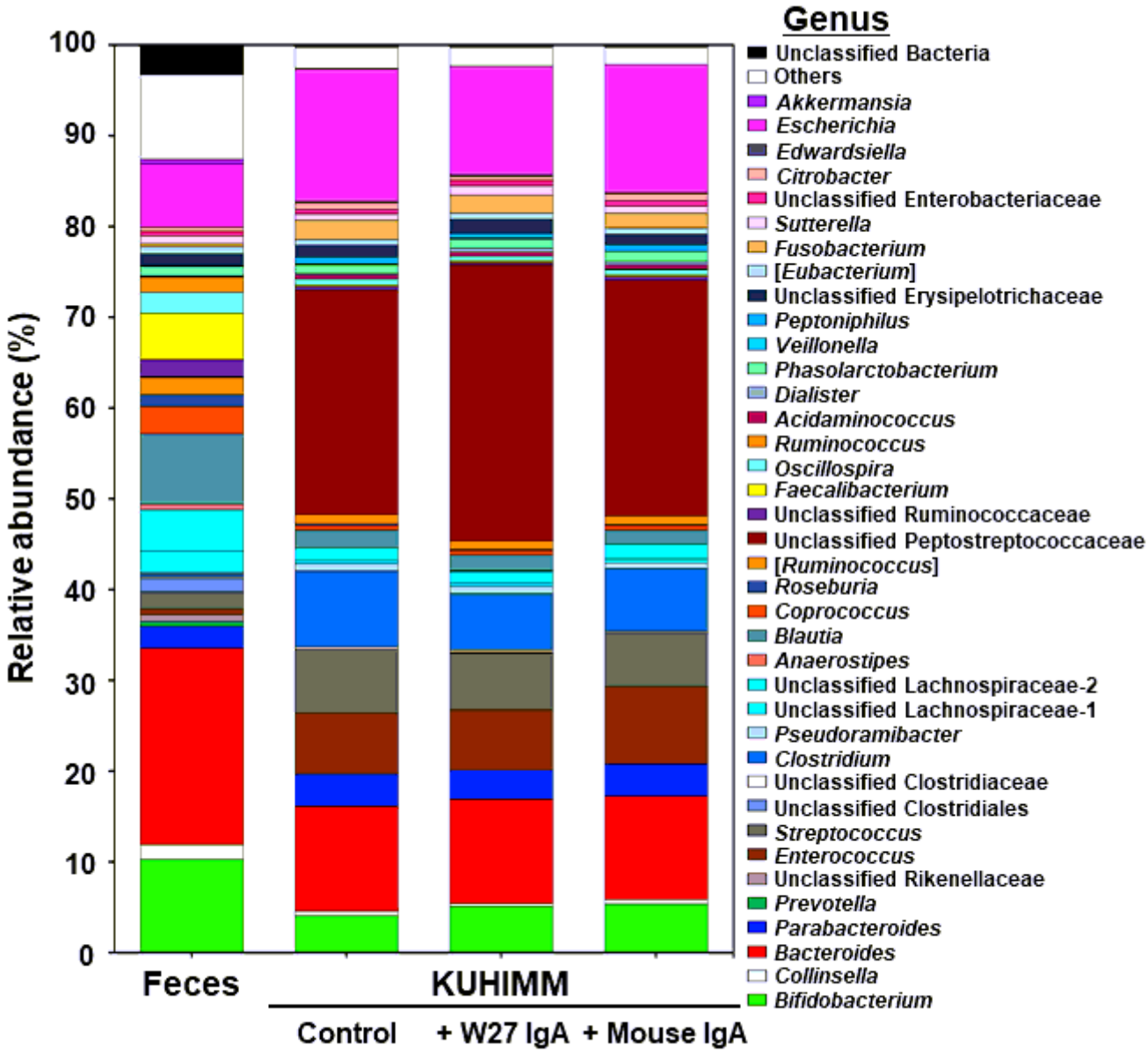



**Figure 1**

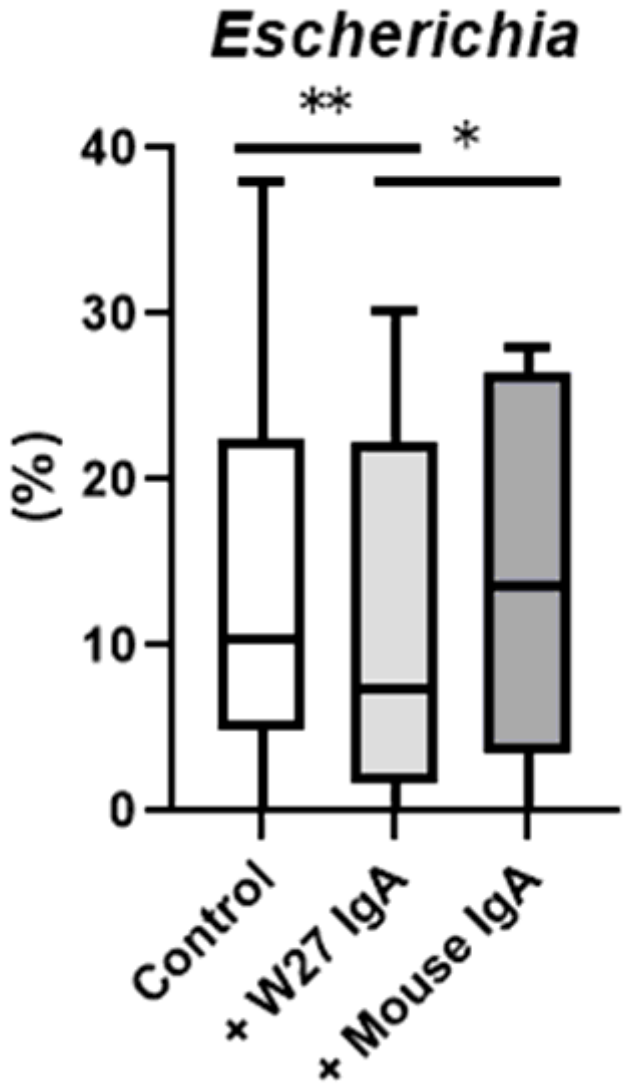
Effect of W27 IgA on bacterial taxonomic composition. Genus-level classification of bacteria in the original fecal inoculum (n = 1) (Feces) and in vitro human colonic microbiota model (KUHIMM) without (0.0 µg/mL) and with W27 IgA (12.5, 2.5, and 0.5 µg/mL) after 48 h of fermentation. Genera with lower

abundance (<1.0%) and lower similarity (< 97%) were included as ‘Others’ and ‘Unclassified Bacteria’, respectively.




## Figure 2

Genus-level compositional views of bacteria in twelve human samples. The means are shown. Fecal samples (Feces) (n = 12), the corresponding KUHIMM cultures without IgA (Control), corresponding cultures with W27 IgA (+ W27 IgA), and corresponding cultures with mouse monoclonal IgA (+ Mouse IgA) were sampled after 48 h of fermentation. Genera with lower abundance (< 1.0%) and lower similarity (< 97%) were included as ‘Others’ and ‘Unclassified Bacteria’, respectively.




## Figure 3

Box-and-whisker plot of the relative abundance distributions of genus Escherichia in KUHIMM (n = 12). The distribution for each group is shown (Control, +W27 IgA, and + Mouse IgA). The box-and-whisker plots show high, low, and median values with the lower and upper edges of each box denoting the first and third quartiles, respectively. **, $p < 0.01$, and * $p < 0.05$, Wilcoxon signed-rank test.

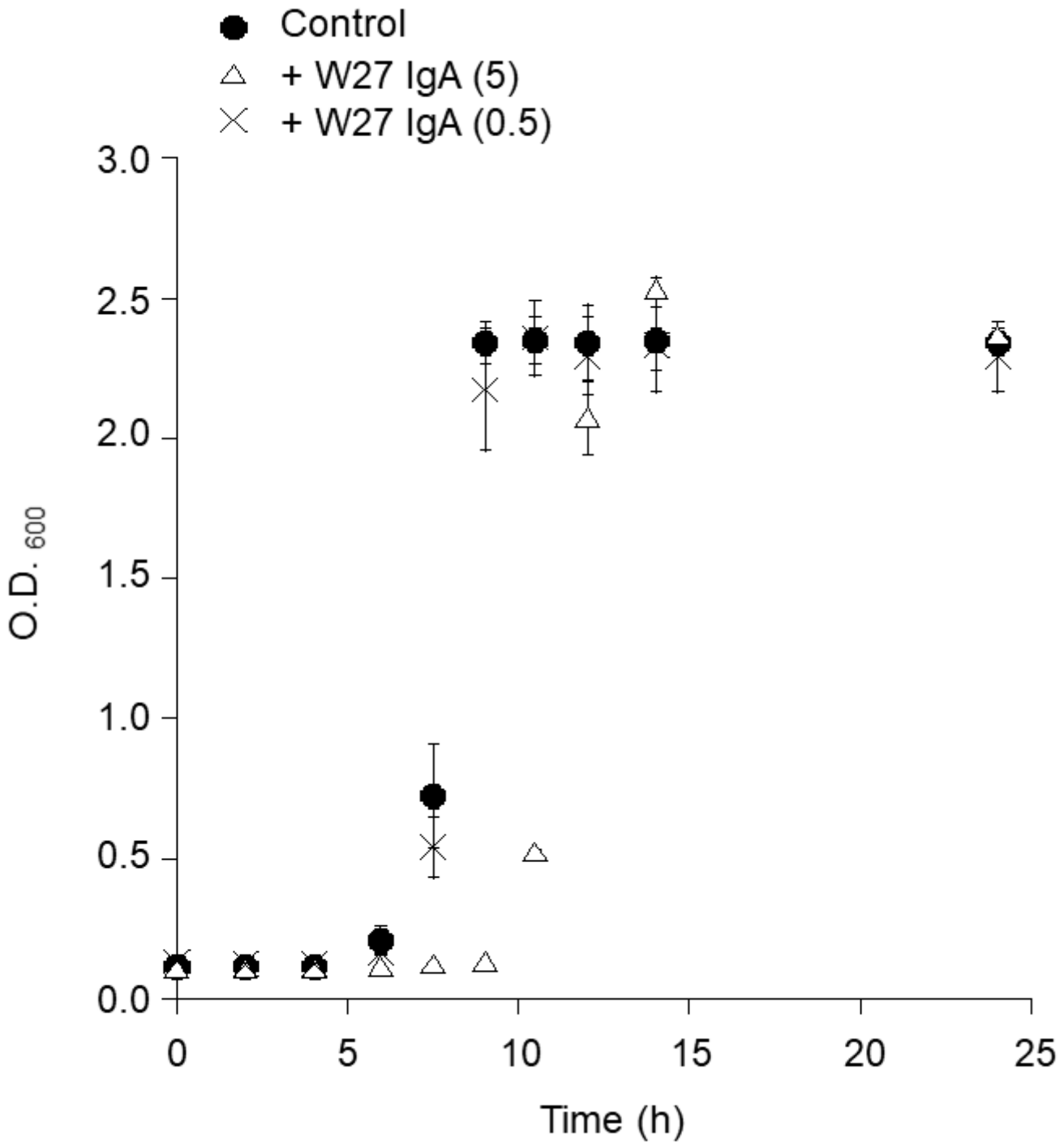



**Figure 4**

Growth of isolated Escherichia coli subcultured in Gifu Anaerobic Medium with 5.0 or 0.5 µg/mL W27 IgA [+W27 IgA (5) or +W27 IgA (0.5)] or without IgA (Control). Growth is represented as the OD600. Data are shown as the means ± standard deviation.