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# Bifidogenic and butyrogenic effects of young barely leaf extract in an in vitro human colonic microbiota model

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## Abstract

Young barley leaf extract (YBL) contains beneficial substances such as fructans, minerals, and vitamins. The effects of YBL administration on the human colonic microbiota and its production of metabolites were evaluated using an in vitro model culture system. Fermentations were started by inoculating fecal samples from nine healthy subjects, with or without 1.5% YBL. Bacterial 16S rRNA sequencing results confirmed that YBL administration significantly increased the relative abundances of bacteria related to the genus *Bifidobacterium* ( $p=0.001$ , paired t-test) and those of the genera *Faecalibacterium*, *Roseburia*, Unclassified *Ruminococcaceae*, and *Lachnospira* ( $p=0.013$ ,  $p=0.019$ ,  $p=0.028$ , and  $p=0.034$ , respectively, paired t-test). Increased abundances of the latter genera corresponded to increased butyrate production in human colonic microbiota models following fermentation with 1.5% YBL, when compared to fermentation without 1.5% YBL ( $p=0.006$ , Dunnett's test). In addition, YBL administration significantly increased the production levels of amino acids such as lysine, glutamate, serine, threonine, alanine, isoleucine, leucine, valine, and phenylalanine. Therefore, our results showed the health-promoting bifidogenic and butyrogenic effects of YBL.

**Keywords:** Young barely leaf extract, Intestinal microbiota, In vitro model culture system, *Bifidobacterium*, Butyrate

## Introduction

Barley (*Hordeum vulgare* L.) is a cereal crop that is widely distributed throughout the world, and barley grass is rich in functional ingredients (Zeng et al. 2018). Young barley leaf and its extract are components of a green-colored drink named “Aojiru” in Japan (Yamaura et al. 2012; Ikeguchi et al. 2014; Yamaura et al. 2015). Barley grass (including young green leaves and stems) possesses several pharmacological activities such as anti-cancer activity, anti-oxidant activity, and anti-inflammation activity, and pharmacological interest exists in using barley grass to treat chronic diseases (Lahouar et al. 2015). Young barley leaf extract (YBL) is a natural source of vitamins and minerals, and supplementation with YBL decreased plasma cholesterol levels in hyperlipidemic patients (Yu

et al. 2003). In addition, barley leaves temporarily accumulate fructan, sucrose, and starch as stored carbohydrates, particularly during nitrogen starvation (Wang et al. 2000). Vandeputte et al. (2017) observed that consumption of an inulin-type fructan increased the relative abundances of *Bifidobacterium* species in the fecal microbiota. To date, the impact of YBL interventions on the human colonic microbiota has not yet been investigated in detail.

Recently, we developed an in vitro human colonic microbiota model using a batch fermentation system and human fecal inocula (named as the Kobe University Human Intestinal Microbiota Model [KUHIMM]), which maintained the diversity and overall number of bacterial species in fecal samples (Sasaki et al. 2018). Thus, the KUHIMM served as a convenient model for simulating and evaluating the effect of exogenous functional compounds, such as prebiotics, on the human colonic microbiota. In addition, the KUHIMM could reproduce the production of short-chain fatty acids (SCFAs) in the

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colon. For example, the KUHIMM was used to detect significantly lower butyrogenesis in samples from ulcerative colitis patients (Sasaki et al. 2019). The aim of this study was to assess the impact of YBL administration on the human colonic microbiota composition using the KUHIMM. To this end, we performed high-throughput, next-generation sequencing (NGS) of the bacterial 16S rRNA gene and compared the KUHIMM results obtained with or without YBL supplementation.

## Materials and methods

### YBL acquisition and composition

YBL was supplied by JPD Co., Ltd. (Hyogo, Japan). The manufacturer collected leaves from young barley plants (*Hordeum vulgare* L. var. *nudum* Hook; 20–35 cm in height) in the Oita Prefecture of Japan and extracted juice from the leaves to produce YBL as a spray-dried powder. The YBL contained carbohydrates (46.5%), proteins (29.0%), minerals (17.5%), water (3.4%), fat (0.6%), fructan (9.4 g/100 g), K (4014 mg/100 g), Na (775 mg/100 g), Ca (619 mg/100 g), P (523 mg/100 g), Mg (237 mg/100 g), Fe (15.9 mg/100 g), Mn (4.72 mg/100 g), Zn (2.41 mg/100 g), chlorophyll (938 mg/100 g), and superoxide dismutase (SOD; 7716 U/g).

### Human fecal sample collection from volunteers

Fecal samples were obtained from nine healthy Japanese human volunteers, who had not been treated with antibiotics for more than 2 months prior to the experiment. All participants were recruited according to the inclusion criteria, which comprised an age of the early twenties to the middle forties, being Japanese, a non-smoking status, and good health and physical condition. The exclusion criteria included significant clinical deviation from normal as determined by the investigators; a history or suspicion of having diabetes, liver disease, kidney disease, or a food allergy; or currently taking prebiotics, probiotics, or lipid-lowering medications.

Fecal samples were immediately collected with an anaerobic culture swab (212550 BD BBL Culture Swab; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and used within 24 h.

### Operation of the KUHIMM, with and without YBL

The KUHIMM was operated with or without added YBL, using a multi-channel fermenter (Bio Jr.8; ABLE, Tokyo, Japan), as described in detail previously (Takagi et al. 2016; Sasaki et al. 2018). The cultivations were initiated by inoculating a single fecal suspension (100  $\mu$ L) into each vessel. During cultivation at 37 °C, the culture broth was stirred at 300 rpm with a magnetic stirrer and continuously purged with a filter-sterilized mixture of gas to maintain anaerobic conditions. To evaluate

the effect of YBL, YBL powder was added into one of the vessels at a final concentration of 15 g/L (1.5% per 100 mL medium) prior to cultivation. A control vessel without YBL powder was also prepared. Aliquots (1 mL) of culture broth were sampled from the vessels at 48 h after initiating cultivation. Fecal and culture broth samples were stored at –20 °C until use.

### Extraction of microbial genomic DNA

Microbial genomic DNA was extracted from suspended feces and culture broth from the KUHIMM at 48 h, as described previously (Takagi et al. 2016). Purified DNA was eluted into TE buffer (10 mM Tris HCl containing 1.0 mM ethylenediaminetetraacetic acid) and stored at –20 °C until use.

### Illumina library generation

Bacterial 16S rRNA genes (V3–V4 region) were amplified using genomic DNA as the template and the primers S-D-Bact-0341-b-S-17 (5'-CCTACGGGNGGC WGCAG-3') and S-D-Bact-0785-a-A-21 (5'-GACTAC HVGGGTATCTAATCC-3') (Klindworth et al. 2013), as described previously (Sasaki et al. 2017). Index primers (Nextera XT Index Kit; Illumina Inc., San Diego, CA, USA) overhanging the amplified sequences were added to the gene-specific sequences. Each polymerase chain reaction (PCR) was performed according to the manufacturer's instructions. Amplicons were purified with AMPure XP DNA purification beads (Beckman Coulter, Brea, CA, USA) and eluted in 25  $\mu$ L of 10 mM Tris (pH 8.5). Purified amplicons were quantified using an Agilent Bioanalyzer 2100 with DNA 1000 chips (Agilent Technology, Santa Clara, CA, USA) and a Qubit 2.0 instrument (Thermo Fisher Inc., Waltham, MA, USA), and 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 a MiSeq instrument (Illumina) and the MiSeq Reagent Kit, v3 (600 cycles; Illumina). The PhiX sequences were removed, and paired-end reads with Q scores  $\geq$  20 were joined using the MacQIIME software package, version 1.9.1 (Caporaso et al. 2010). The UCLUST algorithm (Edgar 2010) was used to cluster the filtered sequences into operational taxonomic unit (OTUs) based on a  $\geq$  97% similarity threshold. Chimeric sequences were checked and removed from the library using ChimeraSlayer (Haas et al. 2011). Representative sequences from each OTU were taxonomically classified via the GreenGenes taxonomic database, using the Ribosomal Database Project Classifier (Wang et al. 2007).

### Real-time PCR analysis

Real-time PCR was performed to quantify total bacterial growth during cultivation, using a LightCycler 96 system (Roche, Basel, Switzerland) with a primer set targeting all eubacteria (Matsuki et al. 2004; Rinttila et al. 2004). PCR amplification was performed as described previously (Takagi et al. 2016).

### Measurement of SCFA concentrations

Concentrations of SCFAs such as acetate, propionate, butyrate, lactate, and succinate were measured using a high-performance liquid chromatography (HPLC) instrument (Shimadzu Corporation, Kyoto, Japan) equipped with an Aminex HPX-87H column (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and a RID-10A refractive index detector (Shimadzu Corporation). The HPLC instrument was operated at 65 °C using 5 mM H<sub>2</sub>SO<sub>4</sub> as the mobile phase with a flow rate of 0.6 mL/min.

### Measurement of amino acid concentrations

Free amino acids were extracted from supernatants collected from culture broths after 48 h of fermentation, using a modified cold chloroform–methanol method (Putri et al. 2013). The water phase of the extract (700 µL) was dried under vacuum and stored at –80 °C until further analysis (Bennett et al. 2008).

The dried extract samples were thawed on ice and derivatized at 30 °C for 90 min with 100 µL of 20 mg/mL methoxyamine hydrochloride in pyridine, after which 50 µL *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (GL Sciences, Tokyo, Japan) (Lisec et al. 2006) was added, followed by incubation at 37 °C for 30 min. The derivatized samples (1 µL) were subjected to gas chromatography–quadrupole–mass spectrometry (GC–Q–MS) using a GCMSQP-2010 system (Shimadzu). The details of the GC–Q–MS operating conditions and procedures were described previously (Kato et al. 2012; Sasaki et al. 2014). Free amino acid concentrations were measured in triplicate.

### Bioinformatics and statistical analyses

The  $\alpha$ -diversity value (Shannon–Wiener index) was calculated from OTU numbers using the MacQIIME software package (Caporaso et al. 2010). Principal coordinate analysis (PCoA) was conducted using the OTU information for each sample and calculated based on unweighted UniFrac distances (Lozupone and Knight 2005) using MacQIIME. A nonparametric paired t-test (the Kruskal–Wallis test) and Dunnett's test were performed using Prism 8 (GraphPad Software, Inc., San Diego, CA) and/or JMP 13 software (SAS Institute Inc., Cary, NC, USA).  $p < 0.05$  was considered to reflect a statistically significant difference.

### Data availability

All raw sequence data generated in this study were deposited on the MG-RAST server (Meyer et al. 2008) (<http://metagenomics.anl.gov>) in a file named “Single Batch Fermentation System Simulating Human Colonic Microbiota\_Young Barley Leaf Extract” under Accession Numbers mgm4854581.3–mgm4854607.3. The datasets supporting the conclusions of this article are included within the article (and its additional file).

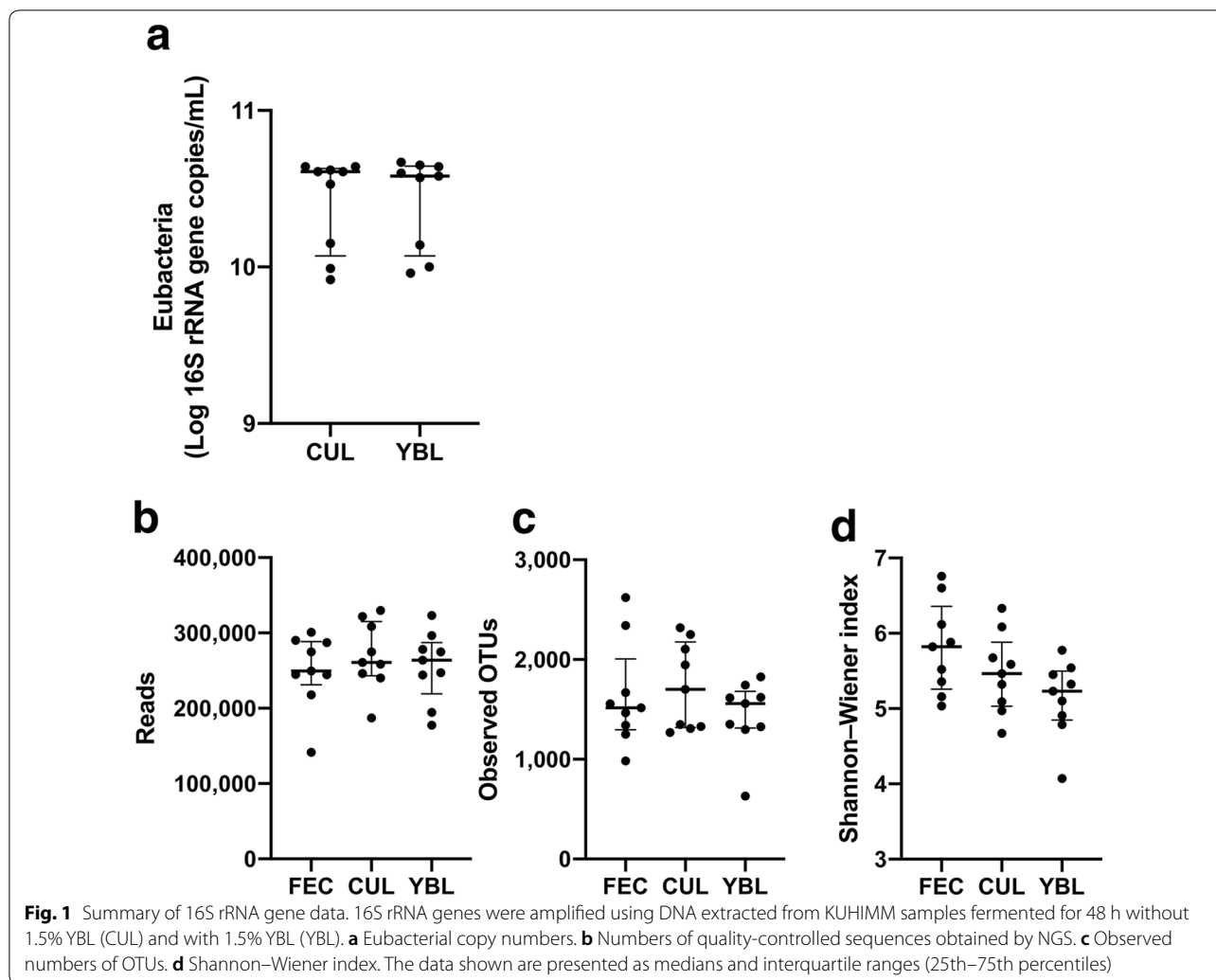
## Results

### The bacterial diversity in feces was maintained after adding 1.5% YBL

One of nine human fecal samples (designated as FEC in Fig. 1) was used as the inoculum, and the *in vitro* human colonic microbiota model, KUHIMM, was constructed with the addition of 1.5% YBL (designated as YBL in Fig. 1). Control cultures without added YBL were also prepared (designated as CUL in Fig. 1). Whole DNA was extracted from the original fecal samples and corresponding culture broths from the KUHIMM, with and without YBL, after 48 h of fermentation. The eubacterial copy numbers reached 0.83–4.73  $\times 10^{10}$  copies/mL using the KUHIMM at 48 h (Fig. 1a), which were comparable to the reported cell densities in human feces (approximately 10<sup>11</sup> copies/g wet feces) (Sender et al. 2016). YBL administration did not affect the eubacterial copy numbers. In addition, bacterial 16S rRNA gene sequences were comprehensively analyzed by NGS. A total of 6977,688 quality-controlled reads were obtained (Fig. 1b). In terms of the OTU numbers (which define the sequence similarities among 16S rRNA gene-sequence clusters) and the Shannon–Wiener indexes of species diversity, no significant differences were observed between the FEC, CUL, and YBL samples ( $p > 0.05$ , Kruskal–Wallis test; Fig. 1b–d). Therefore, the bacterial diversity in the KUHIMM did not change following the addition of 1.5% YBL.

### YBL administration changed the microbiota composition

PCoA of the fecal 16S rRNA gene sequences revealed that most KUHIMMs with 1.5% YBL had microbiota compositions closer to the original fecal samples, when compared to the control KUHIMMs without YBL (Fig. 2 and Additional file 1: Figure S1). These results suggest that YBL administration affected the microbiota composition in the KUHIMM. This possibility was confirmed by performing detailed genus-level compositional analysis of the microbiota for each KUHIMM (Fig. 3). Almost all bacterial genera in the original fecal sample (designated as FEC in Fig. 3) were observed in the KUHIMM without YBL (designated as CUL in Fig. 3). Statistical analyses were carried out to compare the relative abundances of bacterial genera to all

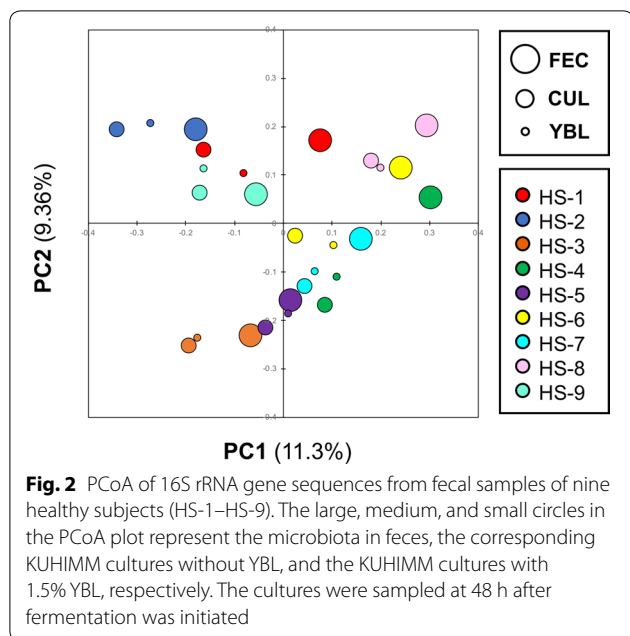


bacteria detected in the CUL and YBL samples. The most remarkable increase was observed in the YBL sample in terms of the relative abundance of commensal bacteria in the genus *Bifidobacterium*, which belongs to the phylum *Actinobacteria* (Fukuda et al. 2012), when compared to the CUL sample (Fig. 4a). In addition, increases in the proportion of other commensal bacteria in the genera *Faecalibacterium*, *Roseburia*, Unclassified *Ruminococcaceae*, and *Lachnospira*, which belong to the phylum *Firmicutes* (Lopetuso et al. 2013), were observed in the YBL sample, when compared to the CUL sample (Fig. 4b). In contrast, significant decreases in the proportions of Unclassified *Peptostreptococcaceae* and the genus *Fusobacterium* (Shang and Liu 2018) were detected in the YBL sample (compared to the CUL sample). Importantly, *Peptoclostridium difficile* (*Clostridium difficile*) and *Peptostreptococcus* spp. (Bourgault et al. 1980; Rupnik et al. 2009) in the family

*Peptostreptococcaceae* and *Fusobacterium* spp. were previously reported as pathogens (Additional file 1: Figure S2).

#### YBL administration enhanced butyrate production

The SCFA concentrations in the KUHIMM after 48 h of fermentation were measured, and the relative ratios were calculated by dividing each concentration measured using the KUHIMM with 1.5% YBL by the corresponding concentration measured with the control KUHIMM without 1.5% YBL (Fig. 5). Although no significant changes in acetate and propionate production were detected between KUHIMMs with or without YBL, butyrate production significantly increased after YBL administration. In addition, the concentrations of 19 free amino acids were measured in the supernatants of KUHIMM cultures, with or without added YBL (Fig. 6). The concentrations of hydrophilic amino acids



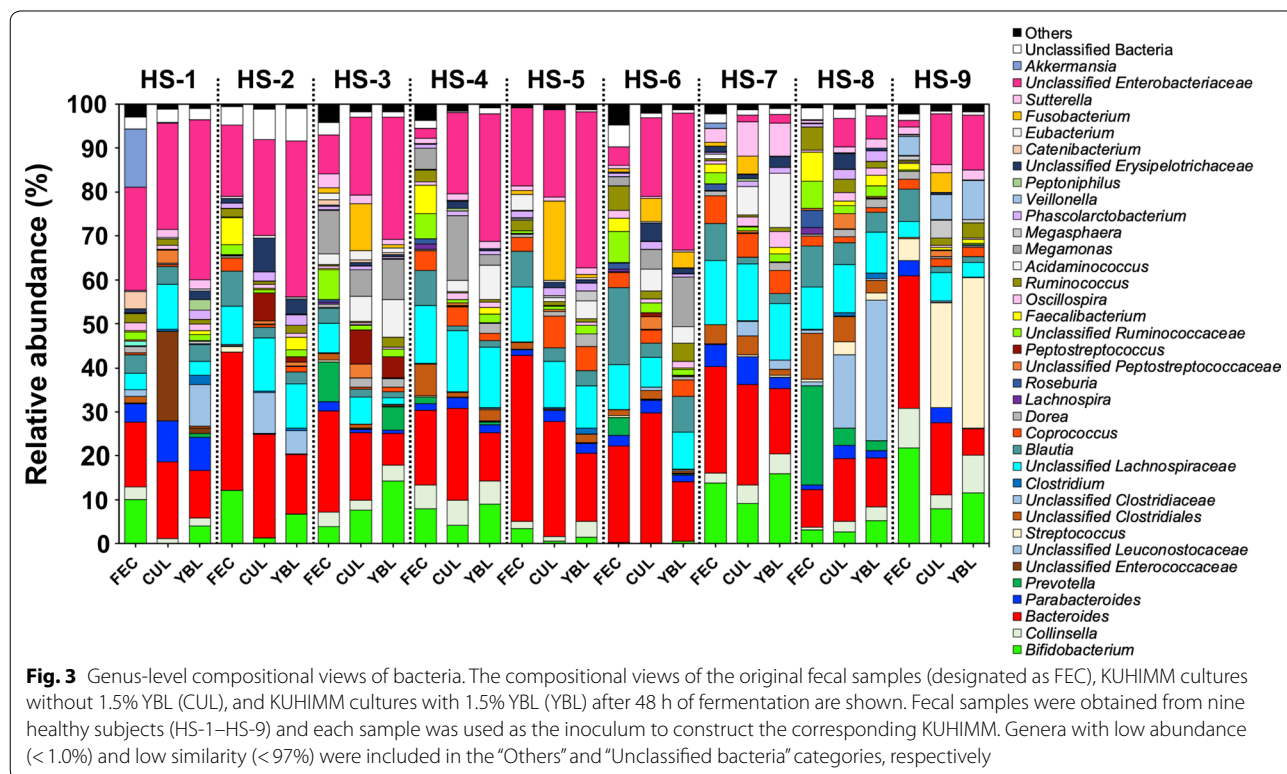
**Fig. 2** PCoA of 16S rRNA gene sequences from fecal samples of nine healthy subjects (HS-1–HS-9). The large, medium, and small circles in the PCoA plot represent the microbiota in feces, the corresponding KUHIMM cultures without YBL, and the KUHIMM cultures with 1.5% YBL, respectively. The cultures were sampled at 48 h after fermentation was initiated

such as lysine, glutamate, serine, and threonine and hydrophobic amino acids such as alanine, isoleucine, leucine, valine, and phenylalanine were significantly higher in the KUHIMMs with 1.5% YBL, compared to those without 1.5% YBL.

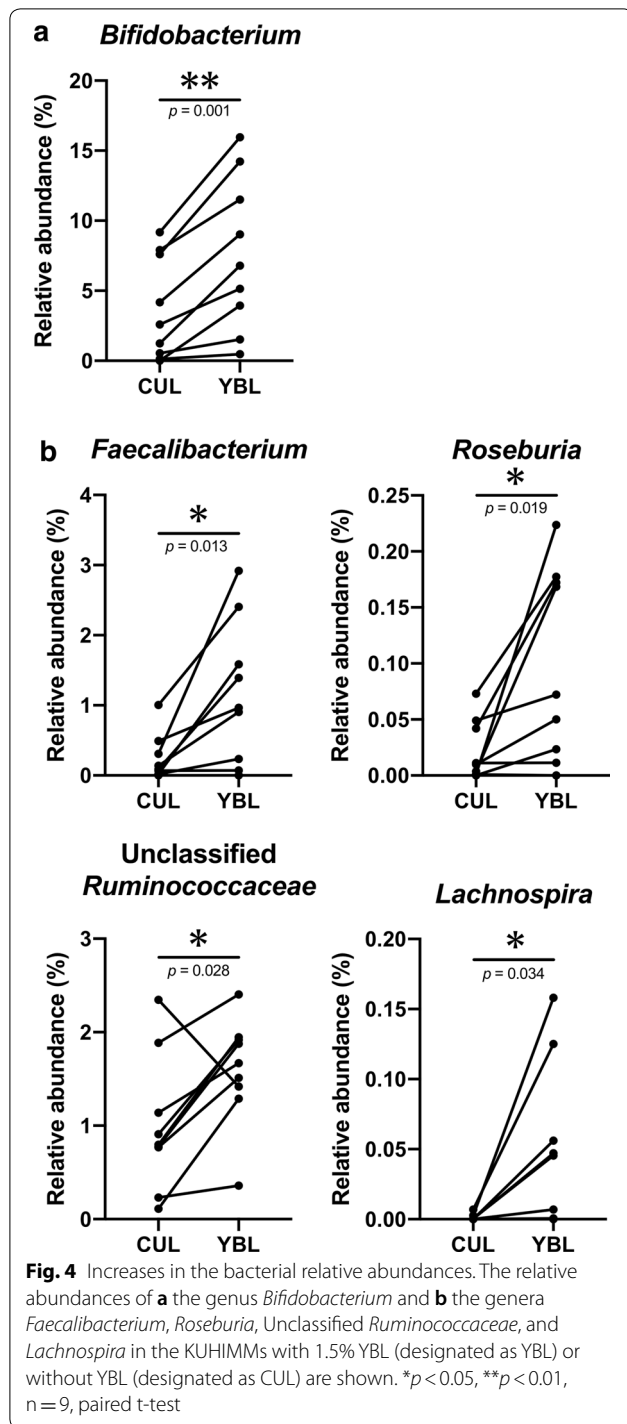
### Discussion

We evaluated the relationships between YBL administration and the responses of human colonic microbiota. YBL exerted a bifidogenic effect by increasing the relative abundances of bacteria related to the genus *Bifidobacterium*. Previous findings showed a bifidogenic effect of barley grain (Martínez et al. 2013), although  $\beta$ -glucan in barley grain impacted the growth of *Bacteroides* species, but not *Bifidobacterium* species (Kristek et al. 2019). Here, we discussed which ingredients in YBL exerted a bifidogenic effect.

The YBL sample contained a substantial amount of fructans (9.4 g/100 g). Fructans consist of fructose-derived oligosaccharides and polysaccharides, and are classified as inulin ( $\beta$  2  $\rightarrow$  1 linkage), levan ( $\beta$  2  $\rightarrow$  6 linkage), and graminan ( $\beta$  2  $\rightarrow$  1 linkage and  $\beta$  2  $\rightarrow$  6 linkages) types based on their structures (Shiomi 2008; Peshev and Van den Ende 2014). Inulin-type fructans selectively stimulate the growth and/or activity of bifidobacteria because bifidobacteria possess  $\beta$ -fructofuranosidase, which can break down and utilize inulin-type fructans, providing a competitive advantage in a mixed culture environment (Kolida and Gibson 2007). In addition, levan-type exopolysaccharides from lactobacilli-enriched *Bifidobacterium* spp. (Bello et al. 2001) and *Bifidobacterium longum* subsp. *infantis* ATCC 15697 by hydrolyzing levan-type fructooligosaccharides (Ávila-Fernández

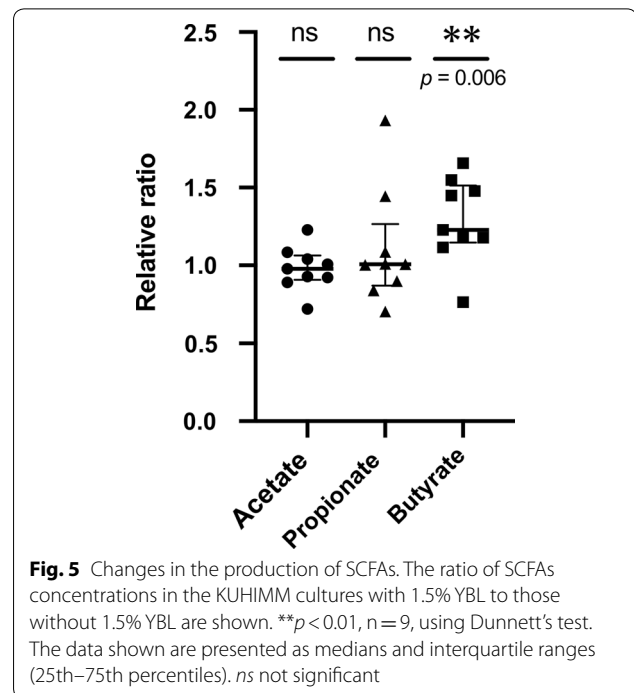


**Fig. 3** Genus-level compositional views of bacteria. The compositional views of the original fecal samples (designated as FEC), KUHIMM cultures without 1.5% YBL (CUL), and KUHIMM cultures with 1.5% YBL (YBL) after 48 h of fermentation are shown. Fecal samples were obtained from nine healthy subjects (HS-1–HS-9) and each sample was used as the inoculum to construct the corresponding KUHIMM. Genera with low abundance (< 1.0%) and low similarity (< 97%) were included in the “Others” and “Unclassified bacteria” categories, respectively

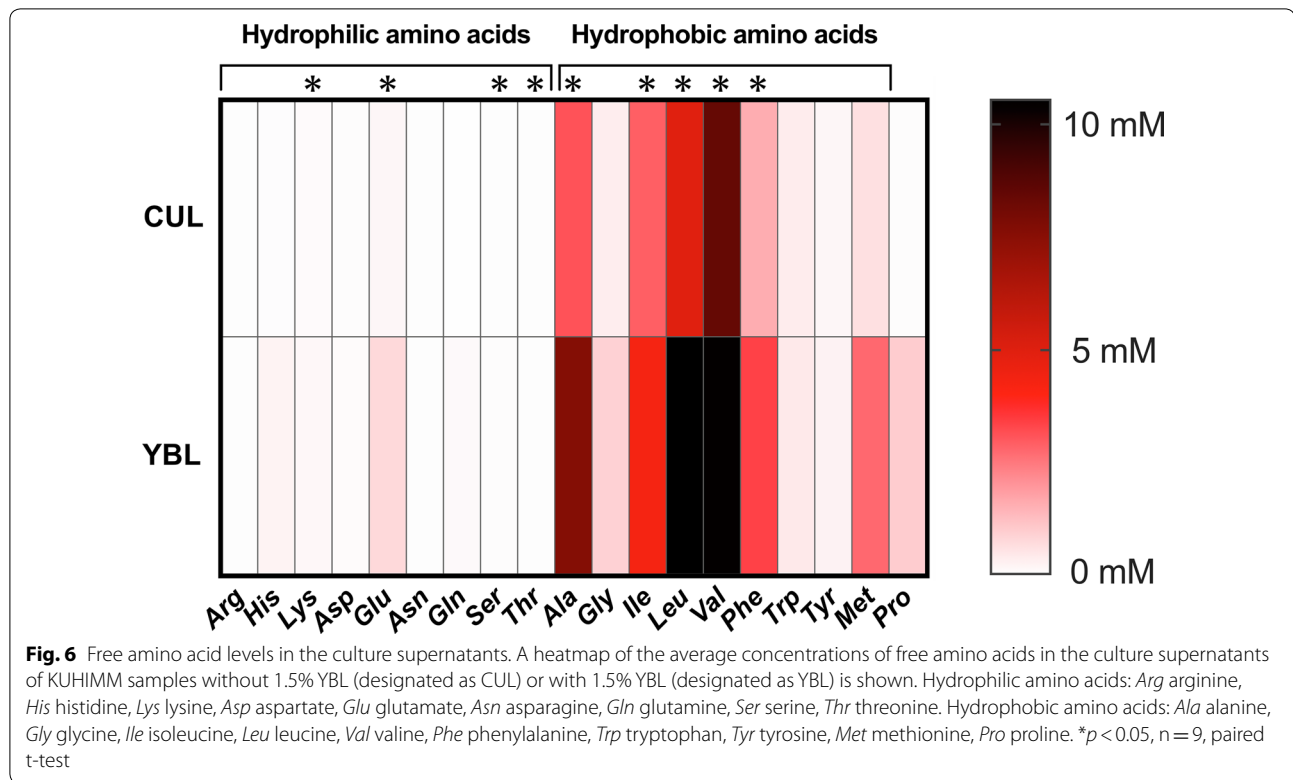


et al. 2016). Thus, the fructans included in YBL would be expected to stimulate bifidobacterial growth.

Administering 1.5% YBL also stimulated the growth of bacteria related to the genera *Faecalibacterium*, *Roseburia*, Unclassified *Ruminococcaceae*, and *Lachnospira*. These microorganisms belong to those that produce



butyrate, i.e., *Clostridium* cluster IV and *Clostridium* cluster XIVa (Duncan 2002, 2006; Ferrario et al. 2014; Vital et al. 2014). The growth stimulation corresponded with increased butyrogenesis in the KUHIMMs treated with 1.5% YBL, compared to those that were not treated with 1.5% YBL (Additional file 1: Figure S3). Recently, it was shown that butyrate producers such as *Faecalibacterium prausnitzii* and *Roseburia* spp. can consume inulin-type fructans (Falony et al. 2009; Moens et al. 2016). The consumption of inulin-type fructans by bifidobacteria provides butyrate-producing bacteria with exogenous acetate for use as a co-substrate to synthesize butyrate when growing on inulin-type fructan (Rivière et al. 2016). Another type of cross-feeding takes place between bifidobacteria that consume inulin-type fructans and produce acetate, and acetate-consuming butyrate-producing bacteria that do not degrade inulin-type fructans (Rivière et al. 2016). The occurrence of such cross-feedings between *Bifidobacterium* spp. and species of butyrate-producing bacteria was supported by our findings that increased acetate production was not detected in KUHIMM cultures with added YBL and that only butyrate production was enhanced. Therefore, our study established the bifidogenic and butyrogenic effects of YBL. Reduced bifidobacterial levels and/or butyrate producers are associated with inflammatory bowel disease and colorectal cancer (Rivière et al. 2016). YBL consumption seems to be a viable approach for counteracting such disorders.



The pH values of the culture broths with or without 1.5% YBL were  $6.38 \pm 0.30$  or  $6.70 \pm 0.11$ , respectively, after 48 h of fermentation. The decrease in pH following YBL administration could explain the growth inhibition of bacteria related to *Peptostreptococcus* and *Fusobacterium*, considering that *Peptostreptococcus* and *Fusobacterium nucleatum* were detected at a relative higher pH (Zilm et al. 2010; Wang et al. 2012). YBL administration also increased the production of certain amino acids. Threonine is necessary for synthesis of the intestinal mucin protein backbone (Ma and Ma 2019). Branched-chain amino acids such as leucine and valine not only are essential for protein biosynthesis, but also are involved in maintaining intestinal barrier function (Ma and Ma 2019). The increased supply of such amino acids from YBL would have beneficial effects in terms of supplying nutrients and regulating the gut immune system.

### Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s13568-019-0911-5>.

**Additional file 1: Figure S1.** A distance matrix was calculated by PCoA of unweighted UniFrac distances. The distances of the PCoA plots between FEC and CUL and between FEC and YBL are referred to as "without YBL" and "with YBL", respectively. Among the nine healthy subjects, seven PCoA plots of the KUHIMMs with 1.5% YBL were closer to those of the original fecal samples than those of the KUHIMMs without 1.5% YBL. **Figure S2.**

Relative decreases in the abundances of bacteria related to Unclassified *Peptostreptococcaceae* and the genus *Fusobacterium* in KUHIMMs, cultured with 1.5% YBL (designated as YBL) or without 1.5% YBL (designated as CUL). \*\* $p < 0.01$ ,  $n = 9$ , paired t-test. ns: not significant. **Figure S3.** Relationship between the sum of the relative abundance (%) of bacteria related to the genera *Faecalibacterium*, *Roseburia*, Unclassified *Ruminococcaceae*, and *Lachnospira* and the butyrate concentration (mM) in KUHIMM cultures with (colored circles) or without (colored triangles) 1.5% YBL, as determined after 48 h of fermentation. The different colors are related to each of the nine healthy subjects (HS-1–HS-9). The solid line and the corresponding line equation indicate the best-fit linear relationship.

### Abbreviations

Ala: alanine; Arg: arginine; Asn: asparagine; Asp: aspartate; CUL: control culture without added YBL; FEC: fecal sample; GC–Q–MS: gas chromatography–quadrupole–mass spectrometry; Gln: glutamine; Glu: glutamate; Gly: glycine; His: histidine; HPLC: high-performance liquid chromatography; Ile: isoleucine; KUHIMM: Kobe University Human Intestinal Microbiota Model; Leu: leucine; Lys: lysine; Met: methionine; NGS: next-generation sequencing; OTU: operational taxonomic unit; PCR: polymerase chain reaction; Phe: phenylalanine; Pro: proline; SCFA: short-chain fatty acid; Ser: serine; SOD: superoxide dismutase; Thr: threonine; Val: valine; Trp: tryptophan; Tyr: tyrosine; YBL: young barley leaf extract.

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### Authors' contributions

DS and KS conceived the study and designed the experiments. DS performed the NGS analyses. KS, DS, YK, and YA wrote the manuscript. AK designed and guided the study. All authors read and approved the final manuscript.

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## Availability of data and materials

All raw sequence data generated in this study were deposited on the MG-RAST server (Meyer et al. 2008) (<http://metagenomics.anl.gov>) in a file named "Single Batch Fermentation System Simulating Human Colonic Microbiota\_Young Barley Leaf Extract" under accession numbers mgm4854581.3–mgm4854607.3.

## Ethics approval and consent to participate

All subjects provided written informed consent prior to specimen collection. 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 Declaration of Helsinki. The authors have no financial or personal relationships that could inappropriately influence this research.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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## References

- Ávila-Fernández Á, Cuevas-Juárez E, Rodríguez-Alegria ME, Olvera C, López-Munguía A (2016) Functional characterization of a novel  $\beta$ -fructofuranosidase from *Bifidobacterium longum* subsp. *infantis* ATCC 15697 on structurally diverse fructans. *J Appl Microbiol* 121:263–276. <https://doi.org/10.1111/jam.13154>
- Bello FD, Walter J, Hertel C, Hammes WP (2001) *In vitro* study of prebiotic properties of levan-type exopolysaccharides from Lactobacilli and non-digestible carbohydrates using denaturing gradient gel electrophoresis. *Syst Appl Microbiol* 24:232–237. <https://doi.org/10.1078/0723-2020-00033>
- Bennett BD, Yuan J, Kimball EH, Rabinowitz JD (2008) Absolute quantitation of intracellular metabolite concentrations by an isotope ratio-based approach. *Nat Protoc* 3:1299–1311. <https://doi.org/10.1038/nprot.2008.107>
- Bourgault AM, Rosenblatt JE, Fitzgerald RH (1980) *Peptococcus magnus*: a significant human pathogen. *Ann Intern Med* 93:244–248. <https://doi.org/10.7326/0003-4819-93-2-244>
- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenkov T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 7:335–336. <https://doi.org/10.1038/nmeth.f.303>
- Duncan SH (2002) Growth requirements and fermentation products of *Fusobacterium prausnitzii*, and a proposal to reclassify it as *Faecalibacterium prausnitzii* gen. nov., comb. nov. *Int J Syst Evol Microbiol* 52:2141–2146. <https://doi.org/10.1099/ijs.0.02241-0>
- Duncan SH (2006) Proposal of *Roseburia faecis* sp. nov., *Roseburia hominis* sp. nov. and *Roseburia inulinivorans* sp. nov., based on isolates from human faeces. *Int J Syst Evol Microbiol* 56:2437–2441. <https://doi.org/10.1099/ijs.0.64098-0>
- Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26:2460–2461. <https://doi.org/10.1093/bioinformatics/btq461>
- Falony G, Verschaeren A, De Bruycker F, De Preter V, Verbeke K, Leroy F, De Vuyst L (2009) *In vitro* kinetics of prebiotic inulin-type fructan fermentation by butyrate-producing colon bacteria: implementation of online gas chromatography for quantitative analysis of carbon dioxide and hydrogen gas production. *Appl Environ Microbiol* 75:5884–5892. <https://doi.org/10.1128/AEM.00876-09>
- Ferrario C, Taverniti V, Milani C, Fiore W, Laureati M, De Noni I, Stuknyte M, Chouaia B, Riso P, Guglielmetti S (2014) Modulation of fecal *Clostridiales* bacteria and butyrate by probiotic intervention with *Lactobacillus paracasei* DG varies among healthy adults. *J Nutr* 144:1787–1796. <https://doi.org/10.3945/jn.114.197723>
- Fukuda S, Toh H, Taylor TD, Ohno H, Hattori M (2012) Acetate-producing bifidobacteria protect the host from enteropathogenic infection via carbohydrate transporters. *Gut Microbes* 3:449–454. <https://doi.org/10.4161/gmic.21214>
- Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, Giannoukos G, Ciulla D, Tabbaa D, Highlander SK, Sodergren E, Methé B, DeSantis TZ, Petrosino JF, Knight R, Birren BW (2011) Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Res* 21:494–504. <https://doi.org/10.1101/gr.112730.110>
- Ikeguchi M, Tsubata M, Takano A, Kamiya T, Takagaki K, Ito H, Sugawa-Katayama Y, Tsuji H (2014) Effects of young barley leaf powder on gastrointestinal functions in rats and its efficacy-related physicochemical properties. *Evid Based Complement Alternat Med*. <https://doi.org/10.1155/2014/974840>
- Kato H, Izumi Y, Hasunuma T, Matsuda F, Kondo A (2012) Widely targeted metabolic profiling analysis of yeast central metabolites. *J Biosci Bioeng* 113:665–673. <https://doi.org/10.1016/j.jbiosc.2011.12.013>
- Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 41:e1. <https://doi.org/10.1093/nar/gks808>
- Kolida S, Gibson GR (2007) Prebiotic capacity of inulin-type fructans. *J Nutr* 137:2503S–2506S. <https://doi.org/10.1093/jn/137.11.2503S>
- Kristek A, Wiese M, Heuer P, Kosik O, Schär MY, Soykan G, Alsharif S, Kuhnle GGC, Walton G, Spencer JPE (2019) Oat bran, but not its isolated bioactive  $\beta$ -glucans or polyphenols, have a bifidogenic effect in an *in vitro* fermentation model of the gut microbiota. *Br J Nutr* 121:549–559. <https://doi.org/10.1017/S0007114518003501>
- Lahour L, El-Bok S, Achour L (2015) Therapeutic potential of young green barley leaves in prevention and treatment of chronic diseases: an overview. *Am J Chin Med* 43:1311–1329. <https://doi.org/10.1142/S0192415X15500743>
- Lisee J, Schauer N, Kopka J, Willmitzer L, Fernie AR (2006) Gas chromatography mass spectrometry-based metabolite profiling in plants. *Nat Protoc* 1:387–396. <https://doi.org/10.1038/nprot.2006.59>
- Lopetuso LR, Scaldaferrri F, Petito V, Gasbarrini A (2013) Commensal *Clostridia*: leading players in the maintenance of gut homeostasis. *Gut Pathog* 5:23. <https://doi.org/10.1186/1757-4749-5-23>
- Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol* 71:8228–8235. <https://doi.org/10.1128/AEM.71.12.8228-8235.2005>
- Ma N, Ma X (2019) Dietary amino acids and the gut-microbiome-immune axis: physiological metabolism and therapeutic prospects. *Compr Rev Food Sci Food Saf* 18:221–242. <https://doi.org/10.1111/1541-4337.12401>
- Martínez I, Lattimer JM, Hubach KL, Case JA, Yang J, Weber CG, Louk JA, Rose DJ, Kyureghian G, Peterson DA, Haub MD, Walter J (2013) Gut microbiome composition is linked to whole grain-induced immunological improvements. *ISME J* 7:269–280. <https://doi.org/10.1038/ismej.2012.104>
- Matsuki T, Watanabe K, Fujimoto J, Kado Y, Takada T, Matsumoto K, Tanaka R (2004) Quantitative PCR with 16S rRNA-gene-targeted species-specific primers for analysis of human intestinal bifidobacteria. *Appl Environ Microbiol* 70:167–173. <https://doi.org/10.1128/aem.70.1.167-173.2004>
- Meyer F, Paarmann D, D'Souza M, Olson R, Glass E, Kubal M, Paczian T, Rodriguez A, Stevens R, Wilke A, Wilkening J, Edwards R, Venter J, Remington K, Heidelberg J, Halpern A, Rusch D, Eisen J, Wu D, Paulsen I, Nelson K,



- Nelson W, Tyson G, Chapman J, Hugenholtz P, Allen E, Ram R, Richardson P, Solovoyev V, Rubin E, Rokhsar D, Banfield J, Huse S, Huber J, Morrison H, Sogin M, Welch D, Overbeek R, Begley T, Butler R, Choudhuri J, Diaz N, Chuang HY, Cohoon M, de Crécy-Lagard V, Disz T, Edwards R, McNeil L, Reich C, Aziz R, Bartels D, Cohoon M, Disz T, Edwards R, Gerdes S, Hwang K, Kubal M, Margulies M, Egholm M, Altman W, Attiya S, Bader J, Bembler L, Berka J, Braverman M, Chen Y, Chen Z, Aziz R, Bartels D, Best A, DeJongh M, Disz T, Edwards R, Formisano K, Gerdes S, Glass E, Kubal M, Field D, Morrison N, Selengut J, Sterk P, Sf Altschul, Madden T, Schaffer A, Zhang J, Zhang Z, Miller W, Lipman D, Jarvie T, DeSantis T, Hugenholtz P, Larsen N, Rojas M, Brodie E, Keller K, Huber T, Dalevil D, Hu P, Andersen G, Cole J, Chai B, Farris R, Wang Q, Wuyts J, de Peer YV, Winkelmans T, de Wachter R, Leplae R, Hebrant A, Wodak S, Toussaint A, Meyer F, Overbeek R, Rodriguez A, Tringe S, von Mering C, Kobayashi A, Salamov A, Chen K, Chang H, Podar M, Short J, Mathur E, Dettler J, Rodriguez-Brito B, Rohwer F, Edwards R, McHardy A, Martin H, Tsirigos A, Hugenholtz P, Rigoutsos I, Edwards R, Rodriguez-Brito B, Wegley L, Haynes M, Breitbart M, Peterson D, Saar M, Alexander S, Alexander E, Rohwer F, Fierer N, Breitbart M, Nulton J, Salamon P, Lozupone C, Jones R, Robeson M, Edwards R, Felts B, Rayhawk S, Wegley L, Edwards R, Rodriguez-Brito B, Liu H, Rohwer F, Mou X, Edwards R, Hodson R, Moran M, Dinsdale E, Edwards R, Hall D, Angly F, Breitbart M, Brulc J, Furlan M, Desnues C, Haynes M, Li L, Krause L, Diaz N, Bartels D, Edwards R, Puhler A, Rohwer F, Meyer F, Stoye J, Liang F, Holt I, Pertege G, Karamycheva S, Salzberg S, Quackenbush J, Rohwer F (2008) The metagenomics RAST server—a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinform* 9:386. <https://doi.org/10.1186/1471-2105-9-386>
- Moens F, Weckx S, De Vuyst L (2016) Bifidobacterial inulin-type fructan degradation capacity determines cross-feeding interactions between bifidobacteria and *Faecalibacterium prausnitzii*. *Int J Food Microbiol* 231:76–85. <https://doi.org/10.1016/j.jfoodmicro.2016.05.015>
- Peshev D, Van den Ende W (2014) Fructans: prebiotics and immunomodulators. *J Funct Foods* 8:348–357. <https://doi.org/10.1016/j.jfff.2014.04.005>
- Putri SP, Nakayama Y, Matsuda F, Uchikata T, Kobayashi S, Matsubara A, Fukusaki E (2013) Current metabolomics: practical applications. *J Biosci Bioeng* 115:579–589. <https://doi.org/10.1016/j.jbiosc.2012.12.007>
- Rinttilä T, Kassinen A, Malinen E, Krogius L, Palva A (2004) Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *J Appl Microbiol* 97:1166–1177. <https://doi.org/10.1111/j.1365-2672.2004.02409.x>
- Rivière A, Selak M, Lantin D, Leroy F, De Vuyst L (2016) Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. *Front Microbiol* 7:979. <https://doi.org/10.3389/fmicb.2016.00979>
- Rupnik M, Wilcox MH, Gerding DN (2009) *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. *Nat Rev Microbiol* 7:526–536. <https://doi.org/10.1038/nrmicro2164>
- Sasaki D, Sasaki K, Tsuge Y, Morita M, Kondo A (2014) Comparison of metabolomic profiles of microbial communities between stable and deteriorated methanogenic processes. *Bioresour Technol* 172:83–90. <https://doi.org/10.1016/j.biortech.2014.08.054>
- Sasaki K, Sasaki D, Okai N, Tanaka K, Nomoto R, Fukuda I, Yoshida K, Kondo A, Osawa R (2017) Taurine does not affect the composition, diversity, or metabolism of human colonic microbiota simulated in a single-batch fermentation system. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0180991>
- Sasaki D, Sasaki K, Ikuta N, Yasuda T, Fukuda I, Kondo A, Osawa R (2018) Low amounts of dietary fibre increase in vitro production of short-chain fatty acids without changing human colonic microbiota structure. *Sci Rep* 8:435. <https://doi.org/10.1038/s41598-017-18877-8>
- Sasaki K, Inoue J, Sasaki D, Hoshi N, Shirai T, Fukuda I, Azuma T, Kondo A, Osawa R (2019) Construction of a model culture system of human colonic microbiota to detect decreased *Lachnospiraceae* abundance and butyrogenesis in the feces of ulcerative colitis patients. *Biotechnol J*. <https://doi.org/10.1002/biot.201800555>
- Sender R, Fuchs S, Milo R (2016) Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol*. <https://doi.org/10.1371/journal.pbio.1002533>
- Shang F-M, Liu H-L (2018) *Fusobacterium nucleatum* and colorectal cancer: a review. *World J Gastrointest Oncol* 10:71–81. <https://doi.org/10.4251/wjgo.v10.i3.71>
- Shiomi N (2008) Food biochemical study on fructans and related synthesis enzymes. *J Appl Glycosci* 55:25–33. <https://doi.org/10.5458/jag.55.25>
- Takagi R, Sasaki K, Sasaki D, Fukuda I, Tanaka K, Yoshida K, Kondo A, Osawa R (2016) A single-batch fermentation system to simulate human colonic microbiota for high-throughput evaluation of prebiotics. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0160533>
- Vandeputte D, Falony G, Vieira-Silva S, Wang J, Sailer M, Theis S, Verbeke K, Raes J (2017) Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. *Gut* 66:1968–1974. <https://doi.org/10.1136/gutjnl-2016-313271>
- Vital M, Howe AC, Tiedje JM (2014) Revealing the bacterial butyrate synthesis pathways by analyzing (meta)genomic data. *MBio*. <https://doi.org/10.1128/mBio.00889-14>
- Wang C, Van den Ende W, Tillberg JE (2000) Fructan accumulation induced by nitrogen deficiency in barley leaves correlates with the level of sucrose: fructan 6-fructosyltransferase mRNA. *Planta* 211:701–707. <https://doi.org/10.1007/s004250000335>
- Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 73:5261–5267. <https://doi.org/10.1128/AEM.00062-07>
- Wang T, Cai G, Qiu Y, Fei N, Zhang M, Pang X, Jia W, Cai S, Zhao L (2012) Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. *ISME J* 6:320–329. <https://doi.org/10.1038/ismej.2011.109>
- Yamaura K, Shimada M, Fukata H, Nakayama N, Bi Y, Ueno K (2012) Antidepressant-like effects of young green barley leaf (*Hordeum vulgare* L.) in the mouse forced swimming test. *Pharmacognosy Res* 4:22. <https://doi.org/10.4103/0974-8490.91030>
- Yamaura K, Tanaka R, Bi Y, Fukata H, Oishi N, Sato H, Mori C, Ueno K (2015) Protective effect of young green barley leaf (*Hordeum vulgare* L.) on restraint stress-induced decrease in hippocampal brain-derived neurotrophic factor in mice. *Pharmacogn Mag* 11:86. <https://doi.org/10.4103/0973-1296.157702>
- Yu YM, Chang WC, Liu CS, Tsai CE (2003) Effect of young barley leaf extract and adlay on plasma lipids and LDL oxidation in hyperlipidemic smokers. *Plant Foods Hum Nutr* 58:1–8. <https://doi.org/10.1023/B:QUAL.0000041159.81153.84>
- Zeng Y, Pu X, Yang J, Du J, Yang X, Li X, Li L, Zhou Y, Yang T (2018) Preventive and therapeutic role of functional ingredients of barley grass for chronic diseases in human beings. *Oxid Med Cell Longev*. <https://doi.org/10.1155/2018/3232080>
- Zilm PS, Mira A, Bagley CJ, Rogers AH (2010) Effect of alkaline growth pH on the expression of cell envelope proteins in *Fusobacterium nucleatum*. *Microbiology* 156:1783–1794. <https://doi.org/10.1099/mic.0.035881-0>

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