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Full Paper

Soy sauce-like seasoning enhances the growth of *Agathobacter rectalis* and the production of butyrate, propionate, and lactate

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The short-chain fatty acids responsible for gut homeostasis are volatile fatty acids produced by commensal bacteria in the gut as fermentation products from undigested food components. Among the short-chain fatty acids, butyrate is important for maintaining intestinal tract anaerobic conditions, promoting epithelial barrier functions, and inducing regulatory T cells that suppress inflammatory bowel disease and allergic diarrhea. However, the type of food-derived molecular components and mechanisms by which they regulate the growth and butyrate production of butyrate-producing bacteria are not clearly understood. *Agathobacter rectalis* is a butyrate-producing bacterium highly colonized in the gut of the Japanese population. In this study, we investigated the effects on *A. rectalis* of a soy sauce-like seasoning made by brewing with a low salt concentration. The soy sauce-like seasoning promoted the growth of *A. rectalis* 2.6-fold. An ethanol precipitate prepared from the soy sauce-like seasoning was critical for promoting the growth of *A. rectalis* and the production of butyrate, propionate, and lactate. Fourier transform infrared spectroscopy (FT-IR) analysis suggested that polysaccharides were active ingredients in the ethanol precipitate of the soy sauce-like seasoning. Inulin, a representative prebiotic with effects against butyrate-producing bacteria, had a limited effect on the growth of *A. rectalis* compared with the soy sauce-like seasoning. Our results indicate that polysaccharides in a soy sauce-like seasoning contributed to the growth of *A. rectalis* and enhanced production of butyrate, propionate, and lactate.

Key words: soy sauce-like seasoning, butyrate, polysaccharides, *Agathobacter rectalis*

INTRODUCTION

Approximately 1,000 species of bacteria reside in the human gastrointestinal tract, and the number of colonic bacteria is as high as 10^{11} per gram of luminal contents in the colon [1–3]. These bacterial species form a complicated ecosystem in the gut, which is called the gut microbiota. Accumulating evidence has revealed that bacterial populations in the gut microbiota are affected by various environmental factors, including diet as well as host age and genetic background [2]. The gut microbiota contributes to host health by modulating the local and systemic immune systems, digesting resistant starch and dietary fiber, and inhibiting pathogen colonization [4]. Disruption of the homeostasis of the gut microbiota, called dysbiosis, is involved in the development of various diseases, including type II diabetes, obesity, colon cancer, inflammatory bowel disease (IBD), and infection [4–6].

Dietary fiber is converted by colonic microbial fermentation to short-chain fatty acids (SCFAs), acetate (C=2), propionate (C=3), and butyrate (C=4), which account for 90–95% of SCFAs in the colon [7, 8]. These SCFAs have pleiotropic effects on the host, contributing to strengthening the integrity of the intestinal barrier system, maintaining glucose homeostasis, lipid metabolism, appetite control, immune system regulation, and anti-inflammatory responses [9]. Of these SCFAs, butyrate is used as a major energy

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source by intestinal epithelial cells (IECs), which consume oxygen and convert it to carbon dioxide during metabolism to maintain anaerobic conditions in the intestinal tract [10]. Butyrate also promotes intestinal barrier function by strengthening tight junctions and producing mucin and anti-microbial peptides [11]. In the context of immune system regulation, butyrate is essential for the differentiation of CD4-positive regulatory T cells (Tregs), which suppress immune responses, and are expected to ameliorate IBD and allergic diarrhea [12, 13]. The importance of butyrate-producing bacteria and butyrate in regulating the pathogenesis of IBD has been suggested, as both are significantly decreased in patients with IBD [14].

It was reported that *Faecalibacterium prausnitzii* of *Clostridium* cluster IV, *Eubacterium rectale* (*Agathobacter rectalis*) of cluster XIVa, and *Clostridium butyricum* are representative butyrate-producing human intestinal bacteria [15, 16]. *A. rectalis*, first isolated from the feces of healthy Japanese-Hawaiian males, is a Gram-positive, anaerobic, rod-shaped bacterium resident in the human intestine [17, 18]. Because *A. rectalis* preferentially colonizes the mucin layer and produces butyrate, IECs can easily access and utilize it [19]. Therefore, *A. rectalis* has the potential to exert physiological and beneficial effects on the host. Indeed, *A. rectalis* has been associated with various diseases, including IBD, intestinal lymphoma, brain tumors, COVID-19, and coronary heart disease [20–24]. Notably, metagenomic analysis of a Japanese cohort revealed that *A. rectalis* was a dominant bacterial species in the colon [25, 26]. In contrast, *F. prausnitzii* was more predominant than *A. rectalis* in a European cohort [27]. Although there are interracial differences between the colonization of each butyrate-producing bacterial species, the factors contributing to these differences remain unclear.

The genetic background of the host immune system and various environmental factors, especially the daily diet of the host, are involved in the colonization of commensal bacteria [28, 29]. Therefore, it is possible that *A. rectalis* is dominant in the colon of Japanese people because of unique components of the Japanese diet.

In Japan, soy sauce, miso, mirin, Japanese sake, and various other seasonings produced by traditional koji-fermentation techniques are routinely used [30]. Some koji-fermented foods have been reported to help improve the intestinal environment [31, 32]. However, the effects of most koji-fermented foods or seasonings, such as soy sauce-like seasoning, on intestinal bacteria and their functions have yet to be investigated.

In this study, we examined the effects of a soy sauce-like seasoning on the growth of *A. rectalis* and production of acetate, propionate, butyrate, and lactate by *A. rectalis*. The soy sauce-like seasoning has unique features, partly due to its fermentation process occurring at a lower salt concentration than regular soy sauce. We hypothesized that this deviation from the normal fermentation process might lead to the production of unique dietary substances with a distinct impact on the growth of *A. rectalis* and a consequential increase in the production of SCFAs, primarily butyrate.

MATERIALS AND METHODS

Bacteria strains

Freeze-dried *A. rectalis* JCM 17463^T was provided by the Japan Collection of Microorganisms (JCM), RIKEN BioResource Research Center, which is participating in the National BioResource Project of the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Preparation of bacterial culture medium

A vinyl anaerobic chamber (Coy Laboratory Products, Grass Lake, MI, USA) containing 82.5–83.8% nitrogen, 6.2–7.5% hydrogen, and 10% carbon dioxide was used for the culture of *A. rectalis*, a strict anaerobic bacterium. Modified yeast casitone fatty acid (YCFA) was prepared by improving YCFA medium (Table 1). First, 2% (v/v) pre-cultured *A. rectalis* adjusted to an optical density $(OD)_{600}$ of 0.15–0.25 was inoculated into YCFA medium and cultured at 37°C for 16 hr. After centrifugation, the culture supernatant was sterilized twice using a filter system (0.20 µm pore size; Sartorius, Göttingen, Germany) for *in vitro* culture assay.

Soy sauce-like seasoning

Soy sauce koji was obtained by adding the spores of *Aspergillus sojae* to a solid medium containing equal amounts of heat-denatured soybeans and wheat and then incubated at 25–40°C for 42 hr. Next, heat-denatured soybeans and soy sauce koji were mixed at a ratio of 1:2 or more, brine was mixed in so the salt concentration was less than 4% (w/v), and the mixture, called moromi (or moromi mash), was digested at 50°C for 24 hr. After cooling to room temperature, lactate was added, and the pH was adjusted to 4.0–5.0. Then, the moromi was sterilized at 121°C for 5 min, and the enzymes in the digested moromi were inactivated at the same time. Soy sauce yeast (*Zygosaccharomyces rouxii*) was added to the moromi at a concentration of 1×10^7 cells/g moromi, and fermentation was carried out for 5 days at a temperature of 25°C with aeration.

After adding salt at a concentration of 10% to the moromi, it was pressed and filtered, and the resulting unrefined soy sauce was heated to 80°C for 30 min. It was then placed in a clarifying tank for 3 days (omission pulling) to obtain a soy sauce-like seasoning.

Ethanol precipitation of the soy sauce-like seasoning

First, 99.5% ethanol was added to the soy sauce-like seasoning at a final concentration of 75% (v/v). After centrifugation, the pellet was dissolved in Milli-Q waterand adjusted to the same volume as the original soy sauce-like seasoning sample. The solution was dialyzed against Milli-Q water using a dialysis tube (100–500 Da; Repligen, Waltham, MA, USA) with a cut-off molecular weight of 100–500 Da at 4°C for 24 hr. The sample was lyophilized using an SP VirTis AdVantage Pro Freeze Dryer (SP Industries, Warminster, PA, USA) for 48 hr.

In vitro culture assay

First, 5% (v/v) pre-cultured *A. rectalis* with an adjusted OD_{600} of 0.15–0.25 was inoculated into modified YCFA medium including each sample and cultured at 37°C for 15 hr. Then, the OD_{600} was measured.

Measurement of SCFA concentrations in culture supernatants

SCFA concentrations in culture supernatants were measured by a reverse-phase high-performance liquid chromatography system. Sterilized culture supernatant was added to an ultrafiltration tube with a cut-off molecular weight of 10,000 Da, and 10 µL of flow-

Table 1. Preparation method for the yeast casitone fatty acid (YCFA) medium

The composition of YCFA medium

Reagent	Weight or Volume
Tryptone	5 g
Yeast extract	1.25 g
$NaHCO_3$	2 g
Glucose	1 g
Maltose	1 g
Cellobiose	1 g
L-Cysteine • HCl • H_2O	0.5 g
Resazurin	0.5 mg
Mineral solution I (see below)	75 mL
Mineral solution II (see below)	75 mL
VFA mix (see below)	3.1 mL
Hemin solution (see below)	5 mL
Vitamin solution I (see below)	0.5 mL
Vitamin solution II (see below)	0.5 mL
Distilled water	330 mL

The composition of Mineral solution I

Reagent	Weight or Volume
K_2HPO_4	0.225 g
Distilled water	75 mL

The composition of Mineral solution II

Reagent	Weight or Volume
KH_2PO_4	0.225 g
$(NH_4)_2SO_4$	0.45 g
NaCl	0.45 g
$MgSO_4 • 7H_2O$	0.045 g
$CaCl_2 • 2H_2O$	0.045 g
Distilled water	75 mL

The composition of VFA (volatile fatty acid) mix

Reagent	Weight or Volume
Acetic acid	17.0 mL
Propionic acid	6.0 mL
n-Valeric acid	1.0 mL
iso-Valeric acid	1.0 mL
iso-Butyric acid	1.0 mL
10N NaOH	26.0 mL

The composition of Hemin solution

Reagent	Weight or Volume
Potassium hydroxide	0.14 g
Hemin	0.05 g
Ethanol	12.5 mL
Distilled water	37.5 mL

The composition of Vitamin solution I

Reagent	Weight or Volume
Biotin	0.5 mg
Vitamin B_{12}	0.5 mg
p-Aminobenzoic acid	1.5 mg
Folic acid	2.5 mg
Pyridoxine • HCl	7.5 mg
Distilled water	50 mL

The composition of Vitamin solution II

Reagent	Weight or Volume
Thiamine • HCl	2.5 mg
Riboflavin	2.5 mg
Distilled water	50 mL

The pH of the YCFA medium was adjusted to 7.45 using 10 N sodium hydroxide. After 20 min of autoclaving at 121°C, 0.5 mL filter-sterilized vitamin solution II was added.

through solution was chromatographed on a Nexera HPLC system (Shimadzu, Kyoto, Japan) equipped with Shim-pack SCR-102H columns run at 0.8 mL min^{-1} in 2.5 mmol/L p-toluenesulfonic acid. The eluate from the column was mixed with 2.5 mmol/L p-toluenesulfonic acid, 10 mmol/L Bis-Tris, and 0.05 mmol/L EDTA-4H buffer and detected using an electrical conductivity meter (polarity: +).

Fourier transform infrared spectroscopy (FTIR) analysis

Infrared spectra were measured using a Fourier transform infrared spectrophotometer (IRAffinity-1S, Shimadzu) equipped with an L-alanine-doped deuterated triglycine sulfate (DLaTGS) detector. Measurements were performed using the transmission method, with a measurement wavenumber range of 4000–400 cm^{-1}, a resolution of 4 cm^{-1}, and 45 integrations.

Statistical analysis

All data are presented as the mean ± standard deviation (SD). Comparisons between controls and 5% (v/v) soy sauce-like seasoning and between controls and each concentration of ethanol precipitate from soy sauce-like seasoning sample were analyzed by Student's t-test or Dunnett's test. All statistical analyses were performed using GraphPad Prism ver. 10.0.2 (GraphPad Software Inc, San Diego, CA, USA).

RESULTS

Soy sauce-like seasoning promoted the growth of A. rectalis

To investigate the diet-derived materials that control the physiology of *A. rectalis*, we evaluated the effect of soy sauce-like seasoning on the growth of *A. rectalis*. *A. rectalis* was cultured anaerobically in modified YCFA medium with or without the soy sauce-like seasoning added at 5% (v/v). The soy sauce-like seasoning promoted the growth of *A. rectalis* 2.6-fold compared with modified YCFA medium without seasoning (Fig. 1), suggesting a positive impact of the soy sauce-like seasoning on the growth of *A. rectalis*.

Ethanol precipitate of the soy sauce-like seasoning promoted the growth of A. rectalis

Next, we identified which soy sauce-like seasoning-derived components facilitated the growth of *A. rectalis*. The soy sauce-

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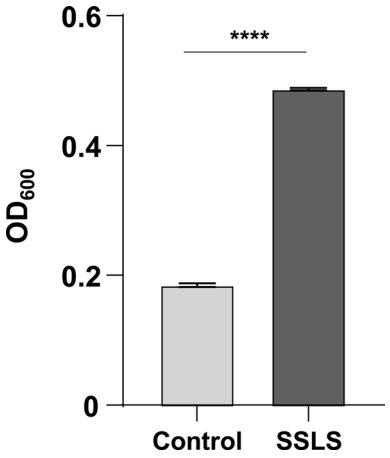

Fig. 1. The soy sauce-like seasoning (SSLS) promotes the growth of *A. rectalis.*

A. rectalis was cultured without or with 5% (v/v) SSLS for 15 hr, and the OD_{600} was measured. Values are given as means ± standard deviation (n=3 per group). ****p<0.0001 vs. control (Student's t-test).

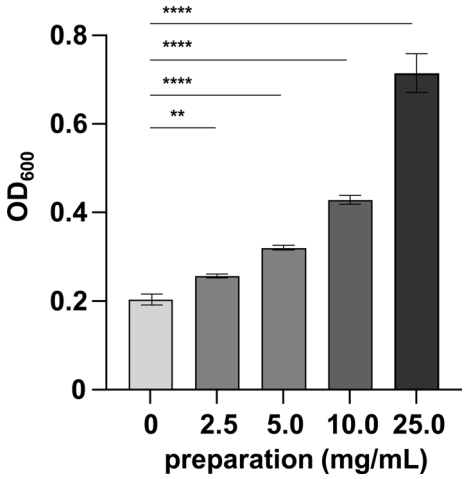

Fig. 2. Ethanol precipitate from soy sauce-like seasoning (SSLS) promotes the growth of *A. rectalis.*

A. rectalis was cultured with or without 2.5, 5.0, 10, or 25 mg/mL SSLS precipitate for 15 hr, and the OD_{600} was measured. Values are given as means ± standard deviation (n=3 per each group). **p<0.01 vs. control (Dunnett's test), ****p<0.0001 vs. control (Dunnett's test).

like seasoning was rich in carbohydrates, which were expected to be metabolized as nutrients for luminal commensal bacteria in the gut. To extract the carbohydrate content, we prepared an ethanol precipitate from the soy sauce-like seasoning and assessed whether the ethanol precipitate affected the growth of *A. rectalis*. The ethanol precipitate from the soy sauce-like seasoning promoted the growth of *A. rectalis* in a concentration-dependent manner, with 3.5-fold enhanced growth at a concentration of 25% (w/v) compared with the control (Fig. 2). These data indicate that carbohydrates in the soy sauce-like seasoning have the potential to promote the growth of *A. rectalis*.

Ethanol precipitate of the soy sauce-like seasoning enhanced butyrate, lactate, and propionate production by A. rectalis

A. rectalis is reported to be a butyrate-producing commensal bacterium. To evaluate the effect of the soy sauce-like seasoning and its ethanol precipitate on the production of metabolites by *A. rectalis*, we quantified the amounts of typical short-chain fatty acids (acetate, propionate, and butyrate) as well as the organic acid lactate in the culture supernatant of *A. rectalis*. When *A. rectalis* was cultured in modified YCFA medium supplemented with the soy sauce-like seasoning at 5% (v/v), the amounts of n-butyrate and lactate in the culture supernatant increased 3.3- and 1.5-fold, respectively (Fig. 3A, 3B). We next examined whether the ethanol precipitate of the soy sauce-like seasoning affected butyrate, acetate, propionate, and lactate production by *A. rectalis* in a concentration-dependent manner. At a 25 mg/mL concentration, n-butyrate, lactate, and propionate in the supernatant of *A. rectalis* increased 5-, 4.1-, and 2.7-fold, respectively, compared with the control (Fig. 3C–3E). By contrast, acetate production did not change at any ethanol precipitate concentration (Fig. 3F). We found no acetate in the control modified YCFA medium or in the modified YCFA medium supplemented with the ethanol precipitate of soy sauce-like seasoning, indicating that acetate from the sample was not being consumed to increase butyrate production. These results indicate that the soy sauce-like seasoning and ethanol precipitate enhance butyrate, lactate, and propionate production by *A. rectalis*.

Composition of the ethanol precipitate of the soy sauce-like seasoning was inferred to contain polysaccharides

Evidence that the ethanol precipitate of the soy sauce-like seasoning had a positive impact on the growth of *A. rectalis* and the robust butyrate production by *A. rectalis* led us to investigate the constituent elements of the ethanol precipitate using Fourier transform infrared spectroscopy. Peak wavenumbers of 3,279 cm^{-1}, corresponding to hydroxyl groups; 1,584 cm^{-1}, corresponding to carbonyl groups; 1,402 cm^{-1}, corresponding to a carbon-hydrogen bond; and 1,042 cm^{-1}, corresponding to ether groups, were detected (Fig. 4). As these functional groups constitute polysaccharides and components with a molecular weight less than 500 Da were excluded during dialysis, the composition of this ethanol precipitate was inferred to contain polysaccharides with molecular weights greater than oligosaccharides.

Inulin had a limited effect on the growth of A. rectalis *compared with the soy sauce-like seasoning*

Inulin, a polymerized fructose polysaccharide, is a soluble fiber that is obtained from chicory roots, Jerusalem artichokes, dahlia tubers, yacon, asparagus, leeks, onions, bananas, wheat, and garlic [33] and has been reported to promote the growth of *A. rectalis* [34]. We evaluated its effect on the growth of *A. rectalis* and compared it with the effect of the soy sauce-like seasoning. Although inulin promoted the growth of *A. rectalis* in a concentration-dependent manner, the effect was limited compared with the soy sauce-like seasoning, suggesting the significance of the ethanol precipitate in the soy sauce-like seasoning as a growth stimulation agent of *A. rectalis* (Figs. 2, 5).

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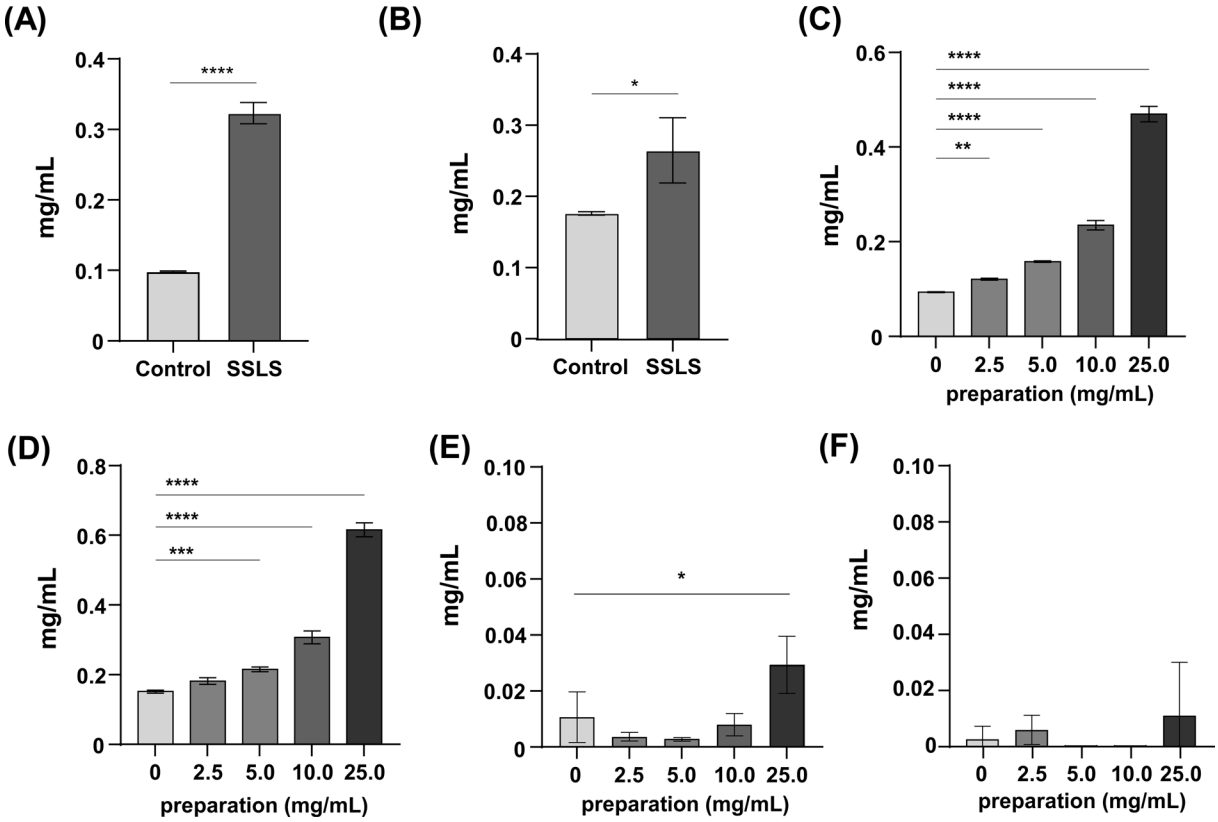

Fig. 3. Soy sauce-like seasoning (SSLS) and its precipitate enhance butyrate, lactate, and propionate production by *A. rectalis.*

(A) Butyrate and (B) lactate production levels when cultured without or with 5% SSLS. (C) butyrate, (D) lactate, (E) propionate, and (F) acetate production levels when cultured with or without 2.5, 5.0, 10, or 25 mg/mL SSLS precipitate. Measurement of butyrate, lactate, propionate, and acetate production levels was performed using HPLC. Values are given as means ± standard deviations (n=3 per group). *p<0.05 vs. control, **p<0.01 vs. control, ***p<0.001 vs. control, ****p<0.0001 vs. control (Student's t-test, Fig. 3A, B; Dunnett's test, Fig. 3C–3F).

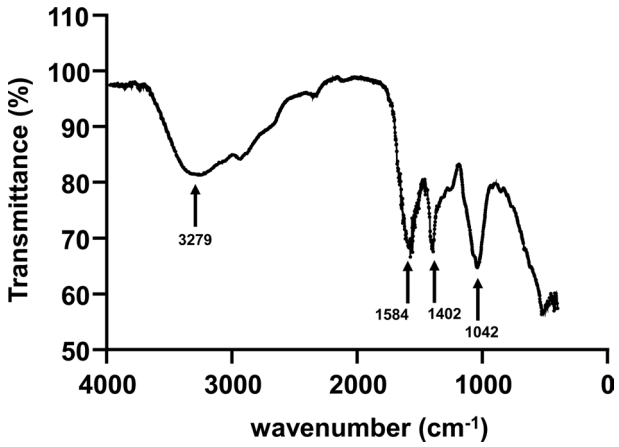

Fig. 4. Fourier transform infrared spectroscopy (FTIR) analysis of the soy sauce-like seasoning (SSLS) precipitate.

FTIR analysis was performed for the SSLS precipitate.

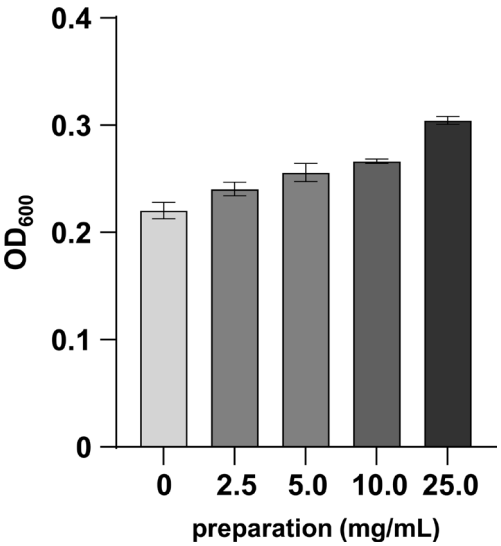

Fig. 5. The effects of inulin on the growth of *A. rectalis* are limited compared with soy sauce-like seasoning (SSLS).

A. rectalis was cultured with or without 2.5, 5.0, 10, or 25 mg/mL inulin for 15 hr, and the OD_{600} was measured. Values are given as the mean ± standard deviation (n=2 per each group).

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DISCUSSION

In this study, we evaluated the effects of a soy sauce-like seasoning on the growth and butyrate production of *A. rectalis*, a ubiquitous butyrate-producing bacteria that colonizes the colons of Japanese people. We found that the soy sauce-like seasoning promoted the growth of *A. rectalis*. An ethanol-insoluble compound prepared from the soy sauce-like seasoning enhanced the growth of *A. rectalis* as well as its production of butyrate and lactate. The *A. rectalis* used in this paper (synonymous with CIP105953^T) is also known to produce lactate as well as butyrate [34]. Lactate is the substrate for butyrate production by certain butyrate-producing bacteria: *Anaerostipes caccae*, *Anaerostipes butyraticus*, *Anaerostipes hadrus*, and *Eubacterium hallii* [11]. Therefore, increased lactate production by *A. rectalis* is expected to induce butyrate production by other resident butyrate-producing bacteria. The composition of the ethanol insoluble fraction was presumed to contain polysaccharides with hydroxyl, carbonyl, carbon-hydrogen bonds, and ether groups. The effect of the ethanol precipitate of the soy sauce-like seasoning on the growth of *A. rectalis* was greater than that of inulin. These results suggest that polysaccharides in the soy sauce-like seasoning are utilized by *A. rectalis* as an active nutritional agent to maintain gut homeostasis. *In vivo*, the effects of the soy sauce-like seasoning are presumably affected by the genetic background of the host, digestive enzymes produced by the digestive organs, and various other environmental factors. In the future, it will be necessary to investigate the effects of the soy sauce-like seasoning on digestive enzymes and other environmental factors, as well as the methods for administration and intestinal delivery of the soy sauce-like seasoning to *A. rectalis*.

The unique traits of polysaccharides in the soy sauce-like seasoning may be derived from their molecular structures. Because of the lyophilization process used during preparation of the ethanol precipitate sample from the soy sauce-like seasoning, we speculate that the hydroxyl groups are derived from the composition of the soy sauce-like seasoning and not from water. In addition, monosaccharides and oligosaccharides consisting of two or three monosaccharides were not included in the sample because they were below the 500 Da cutoff during the preparation of the sample. Therefore, oligosaccharides or polysaccharides with molecular weights of 500 Da or more are the active ingredients that promote the growth of *A. rectalis* and their production of butyrate and lactate.

A. rectalis JCM 17463^T produces a large number of glycohydrolases. Its genome encodes 52 glycoside hydrolases, including β-fructofuranosidases, α-arabinofuranosidases, β-xylosidases, exo-oligoxylanases, α-amylases, α- and β-glucosidases, α- and β-galactosidases, and cellulases [11]. These enzymes work together to break down the polysaccharides in the soy sauce-like seasoning and contribute to the increased butyrate and lactate production of *A. rectalis*. In addition to *A. rectalis*, the *Bacteroides* group was reported to contain various glycohydrolase gene sets [35]. Because the polysaccharides in the soy sauce-like seasoning have pleiotropic effects and unique features, they might have a positive impact on resident microbes as well as *A. rectalis*.

Inulin, fructooligosaccharides, galactooligosaccharides, xylooligosaccharides, and starch are dietary fibers that promote the growth of *A. rectalis* [36]. In this study, the polysaccharides in the soy sauce-like seasoning had a higher growth-promoting effect on *A. rectalis* than that previously reported for inulin, suggesting that the polysaccharides in the soy sauce-like seasoning might be a more effective target for *A. rectalis* compared with the previously reported dietary fiber (Figs. 2, 5). Another report found that the *E. rectale* A1-86 strain effectively utilized inulin, especially HP inulin, resulting in a significant increase in bacterial growth [17]. This discrepancy between the previous report and our data may be explained by the different characteristics of the strains used, namely *E. rectale* A1-86 and *A. rectalis* JCM 17463^T (synonymous with *E. rectale* VPI0990 and CIP105953^T, respectively) [17]. It has been reported that the distribution of *E. rectale* subspecies varies by region and that the families and number of carbohydrate-active enzyme (CAZy) genes also differ [37]. In addition, another report suggested that the growth of *E. rectale* is influenced by environmental conditions such as the growth medium and culture chamber [37]. Therefore, the differences in culture conditions between the previous study and our study may have led to the discrepancies in the results. In the future, it is important to investigate the cause of the differences in saccharide and polysaccharide metabolism among *A. rectalis* strains, as well as the effects of culture conditions.

The detailed structures and features of polysaccharides in the soy sauce-like seasoning are still unknown. When comparing the ethanol precipitate from the soy sauce-like seasoning and inulin, the former had a greater effect on *A. rectalis* growth and butyrate and lactate production, suggesting that the constituents and structure of the polysaccharides are more readily available to *A. rectalis*. The soy sauce-like seasoning was brewed at low salt concentrations; thus, the activity profiles of the polysaccharide-degrading enzymes derived from *Aspergillus sojae* may have been altered during moromi fermentation. Additionally, the brewing temperature was also higher than normal, and this may have facilitated glycosylation reactions between low-molecular-weight polysaccharides that had been degraded, producing polysaccharides that stimulate *A. rectalis*.

Among the butyrate-producing bacteria, there are several glycoside hydrolases only possessed by *A. rectalis*, including enzymes belonging to the GH13, GH36, and GH133 families. In addition, the GH2 family, which contains many active glycoside hydrolases, is highly expressed in *A. rectalis* [38]. It is possible that these enzymes work together to degrade polysaccharides derived from the soy sauce-like seasoning. The detailed structures of the polysaccharides in the soy sauce-like seasoning and the mechanism by which the polysaccharides positively regulate *A. rectalis* growth will be investigated in future studies.

In conclusion, we found that polysaccharides extracted from a soy sauce-like seasoning significantly increased the growth of a butyrate-producing bacteria, *A. rectalis,* and their production of butyrate, propionate, and lactate. Our study findings suggest that polysaccharides contained in the traditional Japanese diet, which uses fermented seasonings, may have an important role in regulating gut immune homeostasis by promoting butyrate-producing bacteria. Examining the detailed structures of the polysaccharides and investigating their effects on butyrate production by *A. rectalis*, including the butyrate production pathway, might provide a therapeutic target for the development of novel anti-inflammatory agents.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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REFERENCES

1. Weinstock GM. 2012. Genomic approaches to studying the human microbiota. Nature 489: 250–256. [Medline] [CrossRef]
2. Hippe B, Zwielehner J, Liszt K, Lassl C, Unger F, Haslberger AG. 2011. Quantification of butyryl CoA: acetate CoA-transferase genes reveals different butyrate production capacity in individuals according to diet and age. FEMS Microbiol Lett 316: 130–135. [Medline] [CrossRef]
3. Sender R, Fuchs S, Milo R. 2016. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol 14: e1002533. [Medline] [CrossRef]
4. Koropatkin NM, Cameron EA, Martens EC. 2012. How glycan metabolism shapes the human gut microbiota. Nat Rev Microbiol 10: 323–335. [Medline] [CrossRef]
5. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, *et al.* 2012. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 490: 55–60. [Medline] [CrossRef]
6. Stanislawski MA, Dabelea D, Lange LA, Wagner BD, Lozupone CA. 2019. Gut microbiota phenotypes of obesity. NPJ Biofilms Microbiomes 5: 18. [Medline] [CrossRef]
7. Daskova N, Heczkova M, Modos I, Videnska P, Splichalova P, Pelantova H, Kuzma M, Gojda J, Cahova M. 2021. Determination of butyrate synthesis capacity in gut microbiota: quantification of *but* gene abundance by qPCR in fecal samples. Biomolecules 11: 1303. [Medline] [CrossRef]
8. Ríos-Covián D, Ruas-Madiedo P, Margolles A, Gueimonde M, de Los Reyes-Gavilán CG, Salazar N. 2016. Intestinal short chain fatty acids and their link with diet and human health. Front Microbiol 7: 185. [Medline] [CrossRef]
9. Blanco-Morales V, Garcia-Llatas G, Yebra MJ, Sentandreu V, Lagarda MJ, Alegría A. 2020. Impact of a plant sterol- and galactooligosaccharide-enriched beverage on colonic metabolism and gut microbiota composition using an *in vitro* dynamic model. J Agric Food Chem 68: 1884–1895. [Medline] [CrossRef]
10. Litvak Y, Byndloss MX, Bäumler AJ. 2018. Colonocyte metabolism shapes the gut microbiota. Science 362: eaat9076. [Medline] [CrossRef]
11. 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. [Medline] [CrossRef]
12. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, *et al.* 2013. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature 504: 446–450. [Medline] [CrossRef]
13. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, *et al.* 2013. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 500: 232–236. [Medline] [CrossRef]
14. Gasaly N, Hermoso MA, Gotteland M. 2021. Butyrate and the fine-tuning of colonic homeostasis: implication for inflammatory bowel diseases. Int J Mol Sci 22: 3061. [Medline] [CrossRef]
15. Soto-Martin EC, Warnke I, Farquharson FM, Christodoulou M, Horgan G, Derrien M, Faurie JM, Flint HJ, Duncan SH, Louis P. 2020. Vitamin biosynthesis by human gut butyrate-producing bacteria and cross-feeding in synthetic microbial communities. MBio 11: e00886–e20. [Medline] [CrossRef]
16. Stoeva MK, Garcia-So J, Justice N, Myers J, Tyagi S, Nemchek M, McMurdie PJ, Kolterman O, Eid J. 2021. Butyrate-producing human gut symbiont, *Clostridium butyricum*, and its role in health and disease. Gut Microbes 13: 1–28. [Medline] [CrossRef]
17. Duncan SH, Flint HJ. 2008. Proposal of a neotype strain (A1-86) for *Eubacterium rectale*. Request for an opinion. Int J Syst Evol Microbiol 58: 1735–1736. [Medline] [CrossRef]
18. Mukherjee A, Lordan C, Ross RP, Cotter PD. 2020. Gut microbes from the phylogenetically diverse genus *Eubacterium* and their various contributions to gut health. Gut Microbes 12: 1802866. [Medline] [CrossRef]
19. Van den Abbeele P, Belzer C, Goossens M, Kleerebezem M, De Vos WM, Thas O, De Weirdt R, Kerckhof FM, Van de Wiele T. 2013. Butyrate-producing *Clostridium* cluster XIVa species specifically colonize mucins in an in vitro gut model. ISME J 7: 949–961. [Medline] [CrossRef]
20. Heinken A, Ravcheev DA, Baldini F, Heirendt L, Fleming RMT, Thiele I. 2019. Systematic assessment of secondary bile acid metabolism in gut microbes reveals distinct metabolic capabilities in inflammatory bowel disease. Microbiome 7: 75. [Medline] [CrossRef]
21. Lu H, Xu X, Fu D, Gu Y, Fan R, Yi H, He X, Wang C, Ouyang B, Zhao P, *et al.* 2022. Butyrate-producing *Eubacterium rectale* suppresses lymphomagenesis by alleviating the TNF-induced TLR4/MyD88/NF-κB axis. Cell Host Microbe 30: 1139–1150.e7. [Medline] [CrossRef]
22. Yang J, Moon HE, Park HW, McDowell A, Shin TS, Jee YK, Kym S, Paek SH, Kim YK. 2020. Brain tumor diagnostic model and dietary effect based on extracellular vesicle microbiome data in serum. Exp Mol Med 52: 1602–1613. [Medline] [CrossRef]
23. Liu Y, Chan MTV, Chan FKL, Wu WKK, Ng SC, Zhang L. 2023. Lower gut abundance of *Eubacterium rectale* is linked to COVID-19 mortality. Front Cell Infect Microbiol 13: 1249069. [Medline] [CrossRef]
24. Li W, Li H, Wang S, Han K, Liu Y, An Z, Wu H, Li J, Song J, Wu W. 2022. Regional pattern and signatures of gut microbiota in rural residents with coronary heart disease: a metagenomic analysis. Front Cell Infect Microbiol 12: 1007161. [Medline] [CrossRef]
25. Nishijima S, Suda W, Oshima K, Kim SW, Hirose Y, Morita H, Hattori M. 2016. The gut microbiome of healthy Japanese and its microbial and functional uniqueness. DNA Res 23: 125–133. [Medline] [CrossRef]
26. Nagata N, Nishijima S, Miyoshi-Akiyama T, Kojima Y, Kimura M, Aoki R, Ohsugi M, Ueki K, Miki K, Iwata E, *et al.* 2022. Population-level metagenomics uncovers distinct effects of multiple medications on the human gut microbiome. Gastroenterology 163: 1038–1052. [Medline] [CrossRef]
27. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, *et al.* MetaHIT Consortium 2010. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464: 59–65. [Medline] [CrossRef]
28. Brown K, DeCoffe D, Molcan E, Gibson DL. 2012. Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. Nutrients 4: 1095–1119. [Medline] [CrossRef]
29. Bibbò S, Ianiro G, Giorgio V, Scaldaferri F, Masucci L, Gasbarrini A, Cammarota G. 2016. The role of diet on gut microbiota composition. Eur Rev Med Pharmacol Sci 20: 4742–4749. [Medline]
30. Yamashita H. 2021. Koji starter and koji world in Japan. J Fungi (Basel) 7: 569. [Medline] [CrossRef]
31. Nakano H, Setoguchi S, Kawano K, Miyagawa H, Sakao K, Hou DX. 2023. Effects of amazake produced with different *Aspergillus* on gut barrier and microbiota. Foods 12: 2568. [Medline] [CrossRef]
32. Kosakai T, Kato H, Sho C, Kawano K, Iwai KI, Takase Y, Ogawa K, Nishiyama K, Yamasaki M. 2019. Dietary fermented products using *koji* mold and sweet potato-*shochu* distillery by-product promotes hepatic and serum cholesterol levels and modulates gut microbiota in mice fed a high-cholesterol diet. PeerJ 7: e7671. [Medline] [CrossRef]
33. Shoaib M, Shehzad A, Omar M, Rakha A, Raza H, Sharif HR, Shakeel A, Ansari A, Niazi S. 2016. Inulin: properties, health benefits and food applications. Carbohydr Polym 147: 444–454. [Medline] [CrossRef]
34. Moens F, De Vuyst L. 2017. Inulin-type fructan degradation capacity of *Clostridium* cluster IV and XIVa butyrate-producing colon bacteria and their associated metabolic outcomes. Benef Microbes 8: 473–490. [Medline] [CrossRef]
35. Hooper LV. 2009. Do symbiotic bacteria subvert host immunity? Nat Rev Microbiol 7: 367–374. [Medline] [CrossRef]
36. Scott KP, Martin JC, Duncan SH, Flint HJ. 2014. Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, *in vitro*. FEMS Microbiol Ecol 87: 30–40. [Medline] [CrossRef]
37. Karcher N, Pasolli E, Asnicar F, Huang KD, Tett A, Manara S, Armanini F, Bain D, Duncan SH, Louis P, *et al.* 2020. Analysis of 1321 *Eubacterium rectale* genomes from metagenomes uncovers complex phylogeographic population structure and subspecies functional adaptations. Genome Biol 21: 138. [Medline] [CrossRef]
38. Nilsen M, Madelen Saunders C, Leena Angell I, Arntzen MØ, Lødrup Carlsen KC, Carlsen KH, Haugen G, Hagen LH, Carlsen MH, Hedlin G, *et al.* 2020. Butyrate levels in the transition from an infant- to an adult-like gut microbiota correlate with bacterial networks associated with *Eubacterium Rectale* and *Ruminococcus gnavus*. Genes (Basel) 11: 1245. [Medline] [CrossRef]

