Administration of *Aspergillus oryzae* suppresses DSS-induced colitis

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ARTICLE INFO

**Keywords:**
Aspergillus oryzae
Gut microbiome
Bifidobacterium pseudolongum
DSS-induced colitis
Prebiotics

**ABSTRACT**

*Aspergillus oryzae*, a filamentous fungus, has long been used for the production of traditional Japanese foods. Here, we analyzed how *A. oryzae* administration affects the intestinal environment in mice. The results of 16S rRNA gene sequencing of the gut microbiota indicated that after the administration of heat-killed *A. oryzae* spores, the relative abundance of an anti-inflammatory *Bifidobacterium pseudolongum* strain became 2.0-fold greater than that of the control. Next, we examined the effect of *A. oryzae* spore administration on the development of colitis induced by dextran sodium sulfate in mice; we found that colitis was alleviated by not only heat-killed *A. oryzae* spores, but also the cell wall extracted from the spores. Our findings suggest that *A. oryzae* holds considerable potential for commercial application in the production of both traditional Japanese fermented foods and new foods with prebiotic functions.

1. Introduction

Prebiotics are non-digestible food ingredients that enhance host health by stimulating the growth and/or activity of certain groups of beneficial bacteria in the gut (Gibson et al., 2004). For example, polysaccharides present in dietary fibers, such as pectin, β-(1,3)-glucan, β-(1,3)-/β-(1,6)-glucan, and chitin, act as prebiotics (Jayachandran et al., 2018; Sanders et al., 2019), and *Bacteroides*, *Bifidobacterium*, and *Lactobacillus* species exhibit a strong ability to decompose these polysaccharides by producing various polysaccharide-degrading enzymes. The bacteria convert polysaccharides into oligosaccharides and monosaccharides, which are then metabolized to various metabolites, such as short-chain fatty acids (Ndeh and Gilbert, 2018; Ndeh et al., 2017). Notably, the short-chain fatty acids produced by *Bacteroides*, *Bifidobacterium*, and *Lactobacillus* species are associated with certain benefits, such as improvement of the intestinal barrier function, induction of anti-obesity effects, and enhancement of the immune system (Nicholson et al., 2012).

The human gut microbiome profoundly influences host health by affecting various intestinal functions (Bäumler and Sperandio, 2016; Sánchez et al., 2017). Recently, the diversity of the gut microbiome among different human populations was suggested to be influenced by geographical, cultural, and traditional differences (Lawley et al., 2019; Rubel et al., 2020). For example, among the gut microbes in Japanese people, the *Bifidobacterium* and *Blautia* populations were found to be large, whereas the *Clostridium* population was found to be small (Nakayama et al., 2015; Nishijima et al., 2016), and the *Bifidobacterium* population, in particular, was found to be larger in the gut microbiome of Japanese people than in that of people from other countries (Nakayama et al., 2015; Nishijima et al., 2016). Therefore, the high abundance of *Bifidobacterium* could potentially result from the consumption of various traditional and unique Japanese foods. However, the nutrients in traditional Japanese foods that contribute to the abundance of *Bifidobacterium* species remain to be elucidated.

Recently, UNESCO registered Washoku, a traditional Japanese cuisine, as an intangible cultural heritage. In Japan, over the past 1000 years, the filamentous fungus *Aspergillus oryzae* has been used for the production of sake, miso, soy sauce, amazake, and other traditional...
The consumption of *A. oryzae* is generally regarded to be safe by the US Food and Drug Administration, and the fungus has been used for producing fermented foods, organic acids, beneficial secondary metabolites, and various enzymes. Thus, Japanese fermented foods contain various organic acids, beneficial secondary metabolites, proteins, and enzymes produced by the fungus, and several polysaccharides that are components of the fungal cell wall, such as \( \beta-(1,3) \)-glucan, \( \beta-(1,3) \)/\( \beta-(1,6) \)-glucan, chitin, and galactosaminogalactan (Beauvais et al., 2014; Gow et al., 2017; Yoshimi et al., 2016). Intriguingly, fungal cell-wall polysaccharides, such as \( \beta-(1,3) \)-glucan, \( \beta-(1,3) \)/\( \beta-(1,6) \)-glucan, and galactosaminogalactan, have recently been reported to alleviate dextran sodium sulfate (DSS)-induced colitis in mice (Briard et al., 2020; Sun et al., 2019). These findings suggest that *A. oryzae* metabolites, proteins, and/or cell wall components present in foods can favorably affect host health. Here, we analyzed the effects of *A. oryzae* administration on the intestinal environment in mice.

2. Material and methods

2.1. Fungal strain, culture, and medium

The *A. oryzae* strain RIB40 was obtained from NBRC (http://www.nbrc.nite.go.jp) and cultured on Czapek-Dox agar (pH 6.0) at 30 °C (Hata et al., 1992). After cultivation for 3 days, spores were collected in saline.

2.2. Cell wall preparation from *A. oryzae*

*A. oryzae* RIB40 was cultured in Czapek-Dox liquid medium for 3 days, as described in Section 2.1. After cultivation, *A. oryzae* spores were collected in 50 mL tubes and centrifuged at 3500 g for 10 min at 4 °C, following which the supernatant was discarded, and the pellet was resuspended in 30 mL of 0.1 mol/L sodium phosphate buffer (pH 6.0) and incubated at 120 °C for 60 min. The sample was cooled and then centrifuged at 3500 g for 10 min at 4 °C, the supernatant was discarded,
and the pellet was resuspended in a 0.5 mol/L NaOH solution. After incubation at 40 °C for 12 h, the sample was again centrifuged at 3500g for 10 min at 4 °C, the supernatant was discarded, and the pellet was resuspended in a 2 mol/L NaOH solution. Lastly, after incubation at 4 °C for 12 h, the sample was centrifuged at 3500g for 10 min at 4 °C, the supernatant was discarded, and the pellet was resuspended in saline, dialyzed, and freeze-dried.

2.3. Animals

Six-week-old male ICR mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). The mice were housed individually with free access to food (AIN-93G) and drinking water and maintained on a 12 h/12 h light/dark cycle (lights on at 07:00 and off at 19:00) at 25 °C and 50% humidity. At the end of the experiment, the mice were sacrificed by over-anesthetization with isoflurane (FUJIFILM Wako, Osaka, Japan). This study was approved by the Institutional Animal Care and Use Committee of Meijo University (No. 2021A006) and carried out in accordance with the guidelines of Meijo University Animal experiments.

2.4. Administration of A. oryzae spores to mice

Six-week-old male ICR mice were acclimated for 7 days and then separated into two groups: (1) control mice (n = 7), and (2) mice administered heat-killed (70 °C, 30 min) A. oryzae spores (0.13 mg; 5.0 × 10^6 cells/100 μL) (n = 6). Heat-killed A. oryzae spores were administered once daily for 21 days (Fig. 1A). To analyze the gut bacterial composition using a metagenomic approach, feces were collected at 7 days after spore administration and immediately stored at −80 °C until use for the metagenomic analysis. The body weight and weight of the food consumed by mice (food intake) were assessed daily.

2.5. Metagenomic analysis of gut microbiota

Amplification preparation for 16S rRNA analysis and sequencing using a MiSeq platform (Illumina, San Diego, CA, USA) were conducted by Bioengineering Lab. Co., Ltd. (Kanagawa, Japan). Briefly, total genomic DNA was extracted from 70 mg of feces using the MPure Bacterial DNA Extraction Kit (MP Biomedicals, Tokyo, Japan) per the manufacturer’s instructions, following which the DNA concentration was measured using a Synergy H1 reader and the QuantiFluor dsDNA System, and the quality of the prepared libraries was confirmed using a Fragment Analyzer System with a dsDNA 915 Reagent Kit (Advanced Analytical Technologies, Inc., Ankeny, IA, USA). The libraries were purified using the MPure Bacterial DNA Library Prep Kit (Advanced Analytical Technologies, Inc., Ankeny, IA, USA). The libraries were sequenced using an Illumina MiSeq platform (300 bp, paired-end reads) at Bioengineering Lab. Co., Ltd. The obtained raw FASTQ files were processed using the microbiome package QIIME 2 (version 2021.4) (https://qiime2.org). Reads were filtered using a quality score of 20 and dereplicated using q2-dada2. The representative sequence sets obtained using the quality-control procedure were subjected to diversity and taxonomy analyses. In the taxonomy analysis, the representative sequences were classified into taxonomic groups using the Greengenes 16S rRNA gene database.

2.6. Administration of A. oryzae spores to mice with DSS-induced colitis

As described in Section 2.4, 6-week-old male ICR mice were acclimated for 7 days and then administered 2.5% DSS (36–50 kDa; MP Biomedicals, Illkirch, France) in drinking water for 7 days to induce acute colitis. The mice used in this experiment were separated into four groups: (1) mice without DSS treatment (n = 16), (2) mice with DSS treatment (n = 16), (3) DSS-treated mice administered heat-killed A. oryzae spores (0.13 mg; 5.0 × 10^6 cells/100 μL) (n = 15), and (4) DSS-treated mice administered the cell wall of A. oryzae spores (0.10 mg/100 μL) (n = 15). The administration of fungal spores or cell wall was continued for 14 days (from Day 8 to Day 26), and the body weights of the mice were measured daily. The mice were sacrificed on the 26th day, and colon and spleen tissues were collected. To evaluate the damage caused by DSS-induced colitis, the colon lengths were measured and colon tissues were stained with hematoxylin and eosin (H&E) (Feldman and Wolfe, 2014). Colon tissues fixed in 4% paraformaldehyde solution were embedded in paraffin, sectioned (thickness = 4 μm), and stained using H&E according to the manufacturer’s instructions.

2.7. Quantification of inflammatory cytokines

The blood samples of the mice were collected and centrifuged at 1000g for 15 min at 4 °C. The supernatant was separated and stored at −80 °C for measurement. The plasma concentrations of inflammatory cytokines (IL-6 and IL-1β) were determined using enzyme-linked immunosorbent assay kits (Cosmo Bio Co., Ltd., Tokyo, Japan) according to the manufacturer’s instructions.

2.8. Quantitative PCR

The total DNA of the microbial population present in the feces samples was extracted using a QIAamp Fast DNA Stool Mini Kit (QIA-GEN, Hilden, Germany). The 16S rRNA gene of bifidobacteria was amplified using the following universal primers: Bifidobacteria F, 5′-AGGGTGTTGATTTGCTACGAG-3′, and Bifidobacteria R, 5′-GATCTCAGGTACTTGTTAGG-3′ (Kosmidis et al., 2007). The following amplification reaction mixture (25 μL) contained 12.5 μL of 2 × TB Green Premix Ex Taq II (TaKaRa Bio Inc., Shiga, Japan), 0.08 μL of forward and reverse primers (10 pmol/μL), 2 μL of bacterial DNA (10 ng), and 8.5 μL of ddH₂O. The following PCR protocol was used: initial denaturation at 95 °C for 10 min, followed by 40 cycles at 95 °C for 10 s, 62 °C for 30 s, and 72 °C for 30 s, and a final extension step at 72 °C for 5 min. The relative abundance of the 16S rRNA gene of bifidobacteria was normalized against that of total bacteria.

2.9. Quantification of A. oryzae glycosylceramide in fermented foods

To determine the A. oryzae contents in commercial fermented foods, such as komekoji, amazake, and shiokoji, we quantified glycosylceramide extracted from A. oryzae (Ferdouse et al., 2019). Spores (10–100 mg) from cultures grown on Czapek-Dox agar plates (pH 6.0) were collected and lyophilized, and the fermented foods (3.0 g) were also lyophilized. The spores and the fermented foods were aseptically disrupted using liquid nitrogen in a mortar and pestle, and the fine powder obtained was dissolved in 2 mL of chloroform:methanol (1:1, v/v) and vortexed for 5 min. After sonication for 30 min, 2 mL of chloroform and 9 mL of distilled water were added, and the mixture was vortexed until saponification. After centrifugation at 800g for 10 min at 25 °C, the lower phase was recovered, evaporated under vacuum, and dissolved in...
200 μL of chloroform:methanol (2:1, v/v). An aliquot of the resultant solution was spotted onto a thin-layer chromatography (TLC) plate (Silica gel 60 plate, Merck Millipore Inc., Darmstadt, Germany) and, subsequently, the dried plate was developed using a saturated chloroform:methanol:acetic acid:water solution (20:3.5:2.3:0.7, v/v). As a standard, 10 μL of 0.1–10.0 mg/mL cerebroside (Matreya Inc., Pleasant Gap, PA, USA) was spotted onto the TLC plates. To detect glycosyleramide, the TLC plates were sprayed with 2 mg/mL orcinol (MP Biomedicals) in 15% sulfuric acid and heated at 100 °C for 5 min. The intensities and areas of the glycosyleramide spots on the TLC plates were determined and quantified using ImageJ software. Glycosyleramide from A. oryzae was analyzed using MALDI-TOF-MS, as previously described (Hirata et al., 2012).

Fig. 2. Effect of Aspergillus oryzae administration on gut microbiota. (A) Taxonomy bar plot at species level. Biological replicates (6 or 7 replicates in each group) are displayed in separate bars. (B) Relative abundance of major taxa detected among the intestinal microbiota of mice. Error bars: standard error (n = 6 or 7). Differences were assessed using Welch’s t-test (*0.01 < p < 0.05, **0.001 < p < 0.01, ***p < 0.001).
3. Results

3.1. The administration of A. oryzae spores affects gut microbiota in mice

We investigated how the administration of heat-killed A. oryzae spores affects the body weight and food intake (weight of diet consumed) of mice. The body weight and food intake of mice were not altered in response to the administration of A. oryzae spores (Fig. 1B and C).

Next, we determined the effects of A. oryzae spore administration on the structure and constitution of the gut microbial community (Fig. 2 and Supplementary Table 1). Feces were collected from control mice and mice treated with A. oryzae spores, and the V3-V4 region of the 16S rRNA gene isolated from microbes present in the feces was sequenced using the Illumina MiSeq platform; following the removal of unqualified sequences, we obtained 332,902 reads in total and an average of 25,608 reads (standard deviation = 5412) per sample. After the 7-day administration of the spores, the bacterial compositions of the gut microbiota in the two groups were compared (Fig. 2 and Supplementary Table 1). At the phylum level, the population of Actinobacteria in the gut microbiota of mice treated with heat-killed A. oryzae spores was larger than that of the control, whereas the relative abundance of Firmicutes was lower (Supplementary Fig. 1). To further investigate the differences among the groups, the bacterial taxa were identified (Fig. 2A and B). After the administration of heat-killed A. oryzae spores, Bifidobacterium pseudolongum, from the phylum Actinobacteria, was found to be relatively more abundant, with a population 2.0-fold larger than that in the control and accounting for 40% of the total bacterial population (Fig. 2A and B). Conversely, the relative abundances of family Erysipelotrichaceae and family Erysipelotrichaceae genus Allobaculum were significantly lower than those in the control, although genus Lactococcus was more abundant after spore administration (Fig. 2A and B). These results indicate that mice treated with heat-killed A. oryzae spores exhibited a distinct bacterial composition compared with mice that were not treated with A. oryzae spores.

3.2. The administration of A. oryzae spores alleviates DSS-induced colitis in mice

We examined the effects of A. oryzae spore administration on the development of DSS-induced colitis in mice. Mice were continuously administered A. oryzae spores for 7 days before DSS treatment, following which colitis was induced by including 2.5% DSS in drinking water for 7 days (Fig. 3A); the A. oryzae spores were administered daily until the end of the experiment (Fig. 3A). The body weight and colon length of DSS-treated mice decreased by 19.2% and 31.0%, respectively, as compared with the corresponding measurements in non-treated mice (Fig. 3B, C, and D). Next, H&E staining was used to evaluate tissue damage in the mouse colon. In control mice, the colon exhibited normal morphology, characterized by an intact mucosa, abundance of goblet cells, and thin muscularis and submucosa layers; in contrast, in DSS-treated mice, the colon exhibited severe damage, including major damage to the epithelial surface, colonocyte loss, goblet cell exhaustion, crypt distortion, myometrial edema and thickening, mucosal inflammation, and extensive inflammatory cell infiltration (Supplementary Fig. 2). Notably, the administration of A. oryzae spores attenuated the pathological changes induced by DSS, which helped maintain an intact colon tissue morphology (Fig. 3B, C, and D and Supplementary Fig. 2). These results suggest that the administration of A. oryzae spores reduced DSS-induced damage in colon tissues.

DSS treatment is known to increase spleen weight and size, which is generally correlated with the extent of inflammation and anemia (van Tilburg Bernardes et al., 2020; Yan et al., 2018). Here, DSS treatment increased the spleen weight and size, whereas the administration of A. oryzae spores suppressed DSS-induced spleen enlargement (Fig. 3E and F).

The role of pro-inflammatory cytokines in inflammatory bowel disease (IBD) pathogenesis has been studied extensively, with their dysregulation considered a major sign of colon damage during IBD development (Chi et al., 2018; Neurath, 2014). In addition, pro-inflammatory cytokines, including IL-6 and IL-1β, have been shown to enhance paracellular permeability (Chang et al., 2021). Accordingly, we measured the levels of these cytokines in the plasma of colitis-induced mice to evaluate the anti-inflammatory effects of A. oryzae spore administration. As shown in Supplementary Fig. 3, the plasma IL-6 and IL-1β levels increased significantly in DSS-treated mice (to levels approximately 4–5-fold higher than those in controls). This indicated that inflammatory responses mounted after DSS treatment. Conversely, mice that were administered A. oryzae spores showed significantly lower pro-inflammatory cytokine levels. These results suggest that cytokine regulation in response to the administration of A. oryzae spores is conducive to the alleviation of inflammatory responses, which helps preserve the intestinal barrier integrity.

Various polysaccharides have been reported to function as prebiotics and increase the population of Bifidobacterium species in the gut microbiota (Mu et al., 2021; Zheng et al., 2018). Intriguingly, the polysaccharides β-(1,3)-glucan, β-(1,3)−/β-(1,6)-glucan, and chitin are known to possess anti-inflammatory properties (Jayachandran et al., 2018; Sun et al., 2019; Wagener et al., 2014), and the fungal cell wall has been shown to contain these polysaccharides (Beauvais et al., 2014; Gow et al., 2017; Yoshimi et al., 2016). Thus, we examined how the administration of the cell wall of A. oryzae spores affects the development of DSS-induced colitis in mice. We found that treatment with spore cell wall suppressed colitis development (Fig. 3B and C). Moreover, in agreement with findings from a previous study (Jing et al., 2017), spleen enlargement was observed in DSS-treated mice, but not in mock-treated mice or DSS-treated mice that were administered the spore cell wall (Fig. 3E and F). Mice administered the spore cell wall preparation exhibited a significantly low IL-1β level, and the IL-6 level also showed a decreasing trend (Supplementary Fig. 3). These results indicate that polysaccharides present in the cell wall of A. oryzae partially alleviate DSS-induced colitis.

We next used quantitative PCR to ascertain how the B. pseudolongum population in the gut microbiota is affected after spore cell wall treatment (Fig. 4). As observed after the 7- and 14-day administration of A. oryzae spores, B. pseudolongum was relatively more abundant after the administration of the spore cell wall, as the population was 2.0-fold greater than that in the control (Fig. 4). These results indicate that the polysaccharides in the fungal cell wall partially function as prebiotics.

3.3. A. oryzae contents in Japanese fermented foods

Lastly, the A. oryzae content in commercially available fermented foods was determined based on the glycosylceramide concentrations (Supplementary Fig. 4). Glycosylceramide extracted from A. oryzae spores and the Japanese fermented food shiokoji was analyzed on TLC plates and identified using MALDI-TOF-MS (Supplementary Fig. 4). The main peak was consistent with the mass of glycosylceramide (m/z776.6) (Supplementary Fig. 4D). A similar spectrum of glycosylceramide has been reported previously (Hirata et al., 2012). The glycosylceramide content was 4.6 µg/10 mg of spores (Supplementary Fig. 4A and 4B). Next, the glycosylceramide contents of the Japanese fermented foods shiokoji, amazake, and pulverized komeko A/B/C were measured (Supplementary Fig. 4C) and found to be 25, 8, and 156/128/202 µg in 1.0 g of the foods, respectively (Table 1 and Supplementary Fig. 4C); thus, the glycosylceramide content was higher in komeko than in the other fermented foods (Table 1). We also calculated the A. oryzae content in commercially available fermented foods based on the glycosylceramide content, and we found that 1.0 g of shiokoji, amazake, and komeko A/B/C contained 53, 17, and 330/270/430 mg of A. oryzae, respectively.
Fig. 3. Effect of *Aspergillus oryzae* administration on dextran sodium sulfate (DSS)-induced colitis. (A) Mice were separated into four groups: (1) mice without DSS treatment (n = 16), (2) mice with DSS treatment (n = 16), (3) DSS-treated mice administered heat-killed *A. oryzae* spores (n = 15), and (4) DSS-treated mice administered *A. oryzae* spore cell wall (n = 15). Colonic inflammation was induced by the 7-day administration of 2.5% (w/v) DSS in drinking water. (B) Mouse body weight was evaluated throughout the experiment, and values are presented as percentages of change relative to the initial value measured before DSS administration. Different letters represent statistically significant differences (p < 0.05). (C, D) Colon length. Colon length was evaluated at the end of the experiment. Different letters represent statistically significant differences (p < 0.05). (E, F) Spleen weight. Spleen weight was evaluated at the end of the experiment. Different letters represent statistically significant differences (p < 0.01). Data are presented as means ± standard error (error bars). Differences were assessed using Tukey’s HSD test with Box-Cox transformed data.
Fig. 1 A. The abundance of amplified fragments of 16S DNA from *A. oryzae* was higher in Japanese people than in people from other countries. *A. oryzae* spores were 2-3 times greater than that in control mice. Moreover, the administration of glucan, galactosaminogalactan, and α-mannan in the cell wall of *A. oryzae* spores prevented weight loss and reduced the severity of colitis, suggesting that the fungal spores alleviated DSS-induced colitis in mice.

**Table 1**

| Foods                          | Glycosylceramide (μg/1 g food) | *A. oryzae* (mg/1 g food) | Estimated intake (g/day) |
|-------------------------------|--------------------------------|--------------------------|---------------------------|
| Shiokoji                      | 25 ± 2                         | 53 ± 4                   | 3.8                       |
| Amazake                       | 8 ± 1                          | 17 ± 1                   | 11.5                      |
| Komekoji                      | 156 ± 5                        | 330 ± 11                 | 0.6                       |
| A                             |                                |                          |                           |
| Komekoji                      | 128 ± 19                       | 271 ± 4                  | 0.7                       |
| B                             |                                |                          |                           |
| Komekoji                      | 202 ± 18                       | 430 ± 39                 | 0.5                       |

*A. oryzae* contents and estimated daily intake for humans (weight = 60.0 kg) were calculated from glycosylceramide contents in Japanese fermented foods.

### 4. Discussion

In this study, we analyzed the effects of the administration of heat-killed *A. oryzae* spores on the intrainestinal environment of mice. After spore administration, the population of *B. pseudolongum* was 2-fold greater than that in control mice. Moreover, the administration of *A. oryzae* spores prevented weight loss and reduced the severity of colitis, suggesting that the fungal spores alleviated DSS-induced colitis in mice.

The population of *Bifidobacterium* spp. in the gut microbiota is larger in Japanese people than in people from other countries (Nakayama et al., 2015). Our study revealed that the administration of *A. oryzae*, which is used in the production of traditional Japanese fermented foods, increased the *Bifidobacterium* population in the mouse gut microbiota. Japanese fermented foods, such as shiokoji, amazake, and komekoji, contain *A. oryzae* and its cellular components. The fungal cell wall is primarily composed of chitin, β-(1,3)-glucan, β-(1,3)/β-(1,6)-glucan, galactosaminogalactan, and α-mannan (Beauvais et al., 2014; Gow et al., 2017; Yoshimi et al., 2016), and previous analyses have revealed that the cell wall composition does not differ quantitatively between *A. oryzae* spores and mycelia (Horikoshi and Iida, 1964). The aforementioned polysaccharides are known to promote the growth of *B. pseudolongum* in the gut (Ma et al., 2021, Zheng et al., 2018), which suggests that the polysaccharides present in the cell wall of *A. oryzae* spores and/or mycelia in Japanese fermented foods function as prebiotics. Here, we calculated the *A. oryzae* contents in commercially available fermented foods based on the glycosylceramide content, and we found that the foods contained the fungus in distinct quantities. When the mice in this study (weighing ~40 g) were administered *A. oryzae* spores (0.13 mg/day), the population of *Bifidobacterium* species expanded, and DSS-induced colitis was alleviated. These results indicate that the daily intake of 0.5 g of komekoji C might produce similar effects in humans (weighing ~60.0 kg). Thus, *A. oryzae* is highly suitable for application in the development of new prebiotics that can alleviate colitis.

*Bifidobacterium* species are found in the intestinal flora of animals, particularly mice and rats. Various polysaccharides have previously been reported to function as prebiotics and increase the population of *Bifidobacterium* species in the gut (Ma et al., 2021, Zheng et al., 2018). Here, the administration of *A. oryzae* spores or spore cell wall increased the abundance of *B. pseudolongum* in mice. Because the cell wall of *A. oryzae* is composed of the various aforementioned polysaccharides, *B. pseudolongum* uses the polysaccharides as a carbon source, which helps increase its abundance among the gut microbes. *Bifidobacterium* species produce various enzymes of the glycoside hydrolase family, and three *B. pseudolongum* strains have been registered in the Carbohydrate-Active enZymes Database (Cantarel et al., 2009). These bacteria harbor various β-1,3-glucanases, β-1,6-glucanases, chitinases, and α-mannanases, which are involved in the degradation of the cell wall polysaccharides of *A. oryzae*. These findings indicate that the polysaccharides in the fungal cell wall function as prebiotics.

Unrestricted inflammation at intestinal sites leads to the malabsorption of nutrients, severe abdominal pain, and increased risk of colorectal cancer development (Dyson and Rutter, 2012; Balestrieri et al., 2020). β-(1,3)-/β-(1,6)-glucan, and chitin possess anti-inflammatory properties (Jayachandran et al., 2018; Sun et al., 2019; Wagener et al., 2014), and dietary polysaccharides such as β-(1,3)/β-(1,6)-glucan exert beneficial effects against IBD by blocking dectin-1 downstream signaling (Tang et al., 2015). Here, the administration of *A. oryzae* spores or cell wall extracted from the spores alleviated DSS-induced colitis in mice, suggesting that β-(1,3)-glucan, β-(1,3)/β-(1,6)-glucan, and/or chitin present in the cell wall directly reduced inflammation in intestinal epithelial cells. Recently, galactosaminogalactan present in the cell wall of *A. fumigatus* has also been shown to exert anti-inflammatory effects in mice in an inflammasome-dependent manner (Briard et al., 2020). As noted in the preceding paragraph, polysaccharides might alleviate colitis by increasing the population of *Bifidobacterium* species. However, it is currently unclear whether the polysaccharides ameliorate colitis by directly reducing inflammation by blocking dectin-1 downstream signaling and/or acting in an inflammasome-dependent manner, or by increasing the population of *Bifidobacterium* species.

The involvement of *Erysipelotrichaceae* in chronic intestinal inflammation has been reported previously (Park et al., 2020), and an imbalance between the *Erysipelotrichaceae* and *Lachnospiraceae* populations in the gut microbiota has been reported to cause IBD (Chen et al., 2017). We found here that the relative abundances of family *Erysipelotrichaceae* and family *Erysipelotrichaceae* genus *Allobaculum* were markedly decreased in mice that were administered heat-killed *A. oryzae* spores. These findings suggest that the low levels of colitogenic strains of *Erysipelotrichaceae* resulting from the administration of *A. oryzae* spores are potentially associated with the reduction of DSS-induced damage to colon tissues.

### 5. Conclusion

We have shown here that *A. oryzae* used in the production of Japanese fermented foods functions as a prebiotic, and that the administration of either *A. oryzae* spores or the extracted spore cell wall alleviates DSS-induced colitis in mice. These findings suggest that *A. oryzae* could be useful for commercial application in the production of not only traditional Japanese fermented foods, but also new foods with prebiotic...
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