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Effects of rice bran and fermented rice bran suspensions on caecal microbiota in dextran sodium sulphate-induced inflammatory bowel disease model mice

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ABSTRACT

Inflammatory bowel disease (IBD) is characterised by bloody stools, a shortened colon, and damage to the colon mucosal layer. Research has shown that the intake of rice bran aqueous extract suspension (RB-AES) *via* drinking water does not ameliorate the effects of IBD induced by 5% dextran sodium sulphate (DSS) in ICR mice. However, RB fermented using *Lactobacillus plantarum* and *Saccharomyces cerevisiae* (FRB) does ameliorate the symptoms of IBD. The ameliorating effect of heat-sterilized FRB (HFRB) is not as pronounced as that of FRB. In the present study, the caecal contents of DSS-induced IBD model mice were sampled. Then the caecal bacterial microbiota were analysed using amplicon sequencing the V4 region of the bacterial 16S rDNA using MiSeq. DSS-RB increased the prevalence of some bacterial groups associated with gut inflammation, such as *Bacteroides* species including *B. acidifaciens*, and Enterobacteriaceae operational taxonomic units. There were no increases in the prevalence of bacterial groups in mice fed FRB and HFRB. Furthermore, the prevalence of the predominant *Lactobacillus;* s2 was only reduced in the DSS-RB group. These results indicate that RB, FRB, and HFRB affected the gut microbiota in different ways. Although RB and the other food materials showed functional effects *in vitro*, such effects are influenced by the comprehensive interaction of the functional materials, the gut microbiota, and the host immune reaction *in vivo*.

1. Introduction

Inflammatory bowel disease (IBD) encompasses two forms of intestinal inflammation: ulcerative colitis (UC) and Crohn's disease (CD). UC is a chronic disease that is characterised by diffuse inflammation of the rectal and colonic mucosa. CD has a broad spectrum of clinical manifestations, and the initial presentation is seldom a good predictor of the clinical course. UC and CD are common in the United States and Western Europe (Kappelman, Moor, Allen, & Cook, 2013), but the number of cases in East Asian countries, such as Korea and Japan, has also increased in recent years (Ishige et al., 2017; Lee et al., 2015). Although the pathogenesis of IBD is not completely understood, it is thought to be due to an aberrant immune response of the epithelial barrier to the intestinal microbiota (Duranti, Ferrario, van Sinderen, Ventura, & Turroni, 2017; Martini, Krug, Siegmund, Neurath, & Becker, 2017). Reports that lactic acid bacteria (LAB) probiotics ameliorate the effects of IBD have increased recently (Kawahara et al., 2015; Nakata et al., 2016; Seo et al., 2017; Yokota et al., 2018).

Rice bran (RB) is a major by-product of rice polishing. It has been reported that the antioxidant properties of some of the chemical compounds found in RB have a beneficial effect on IBD (Islam et al., 2008). RB contains certain oligosaccharides with prebiotic properties (Kurdi & Hansawasdi, 2015). In a previous study, hot aqueous extract suspensions (AES) of RB were successfully using *Lactobacillus plantarum* S-SU8 and *Saccharomyces cerevisiae* Misaki-1 (Kuda et al., 2016; Kuda, Takahashi, Ishizaki, Takahashi, & Kimura, 2017a). RB-AES, fermented RB-AES (FRB), and heat-sterilized FRB (HFRB) had similar antioxidant and anti-inflammatory effects *in vitro* (*i.e.,* inhibition of lipopolysaccharide (LPS)-induced NO secretion in murine macrophage RAW264.7 cells) (Kondo et al., 2016). However, RB-AES had no effect on Institute of Cancer Research (ICR) mice with IBD induced by 5% dextran sodium sulphate (DSS), as indicated using bloody stools, shortened colons, and damage to the colon mucosal layer, although FRB clearly ameliorated the symptoms of IBD. The amelioration effect of HFRB was not as prominent as that of FRB. It is not yet clear what causes the difference in the protective effect between *in vitro* and *in vivo*

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scenarios.

Recent culture-independent next-generation DNA sequencing (NGS) analysis of amplicons of the 16S rRNA gene has shown that the guts of humans and other mammals contain trillions of bacterial cells from 500 to 1000 different bacterial species, and indicate that the gut microbiota is vital to the health of the host (Clavel & Lagkouvardos, 2017; Clavel, Gomes-Neto et al., 2017). A relationship between aging and the gut microbiota was proposed by Metchnikoff over a century ago (Cavaillon & Legout, 2016), and NGS analyses have provided evidence for this hypothesis (Sánchez et al., 2017). The relationships between chronic bowel diseases, the gut microbiota, and certain functional foods including probiotics have been studied extensively in recent years (Gomes, Bueno, de Souza, & Mota, 2014; He & Shi, 2017). The effect of DSS-induced IBD on the gut microbiota has also been reported (Håkansson et al., 2015; Kuda, Yokota, et al. 2017b). In these reports, the prevalence of *Akkermansia muciniphila*, *Bacteroides acidifaciens*, and *Turicibacter* species increased following the administration of DSS. On the other hand, it has been observed that *A. muciniphila* and *B. acidifaciens* induce beneficial immune and metabolic responses of host gut cells, such as increasing of IgA and IgA + B cells, gut barrier, and mucus thickness (Ottman, Geerling, Avlvinl, de Vos, & Belzer, 2017; Yanagibashi et al., 2013).

Recently, Si et al. (2018) reported that fresh RB had no effect on the gut microbiota in high-fat diet induced obese rats at the phylum and genus levels. However, though small (< 0.3%), an increase in *Clostridium leptum,* an obesity related bacteria (Eslinger, Eller, & Reimer, 2014), was observed with the fresh RB. However, the effect of RB on the gut microbiota when IBD is present remains unclear. Therefore, in the present study, to determine the relationships between the gut microbiota and the different effects of RB-AES and FRB on IBD, caecal contents were collected from mice used in a previous study, which had been fed with RB, FRB, or heat-sterilized FRB (HFRB) (the IBD mouse model mentioned above) (Kondo et al., 2016). The bacterial caecal microbiota of the mice using 16S rRNA gene amplicon sequencing using the NGS system (MiSeq) was investigated.

2. Materials and methods

2.1. Preparations of rice bran suspensions

Caecal contents had been collected from mice used in a previous study (Kondo et al., 2016) and stored at −30 °C for the gut microbiota analyses. In the previous study, 100 g of rice bran (RB, Fuji Foods Corp., Yokohama, Japan) was heated at 121 °C with 900 mL of distilled water (DW) for 15 min. The resulting micelle-like supernatant was used as an RB-aqueous extract suspension (AES). The RB-AES (10 mL) were each inoculated with a loop of *Lactobacillus plantarum* Sanriku-SU8 and *Saccharomyces cerevisiae* Misaki-SU1 (Accession No. of GenBank: LC093858 and LC093859) isolated from algal beach casts (Kuda et al., 2016), and incubated at 30 °C for 48 min. Then the pre-incubated RB-AES were inoculated into 1000 mL of RB-AES and incubated at 30 °C for 48 min. This fermented RB-AES (FRB) contained 8 log and 7 log CFU/mL of *L. plantarum* Sanriku-SU8 and *S. cerevisiae,* respectively, these were counted with de Man, Rogosa, and Sharpe (MRS) agar (Oxoid, Basingstoke, UK) and potato dextrose agar (Oxoid) plates. A portion of the FRB was sterilized in boiling water for 20 min (HFRB).

2.2. Animal experiment

Thirty 5-week-old male ICR mice were obtained from the Tokyo Laboratory Animals Science (Tokyo, Japan). The mice were acclimated on a CE2 diet (for rearing and breeding, CLEA Japan, Tokyo, Japan) and DW. After 5 days, the mice were divided into 5 groups ($n = 6$). Two groups (DW and DSS-DW) were administered the same diet and DW. The other groups (DSS-RB, DSS-FRB, and DSS-HFRB) were administered the same diet but with 50% RB-AES, FRB, or HFRB, respectively, instead of drinking water. After 3 days, 5% (w/v) dextran sodium sulphate (DSS; MW = ~5000; Wako Pure Chemical, Osaka, Japan) was added to the drinking water of the DSS-DW, DSS-RB, DSS-FRB, and DSS-HFRB groups. After 6 days of DSS administration, the mice were anesthetized with diethyl ether and exsanguinated by cutting the abdominal aorta. The caecum and other organs were then excised. This animal experiment was approved by the animal experiment committee of the Tokyo University of Marine Science and Technology (Approval No. H27-4).

In this animal experiment, the intake of RB-AES had no effect on ICR mice with IBD induced by treatment with 5% DSS, as indicated by bloody stools, shortened colons, and damage to the colon mucosal layer, although FRB clearly ameliorates the symptoms of IBD (Kondo et al., 2016). The ameliorating effects of HFRB were not as pronounced as those of FRB.

2.3. Analysis of caecal microbiota

2.3.1. Direct cell count

The caecal contents (0.1 g) were suspended in 0.9 mL of DW for direct cell counting using microscopy (Yokota et al., 2018). The suspension (0.01 mL) was spread on a 10-mm^2 square slide glass and Gram-stained with a commercial Favor G kit (Nissui Pharmaceutical Co., Tokyo, Japan) containing Victoria Blue, picric acid/ethanol, and safranin solutions. The number of bacterial cells in 3 fields of view was counted per mice, and the average values were obtained.

2.3.2. Amplicon sequencing using the MiSeq system

Amplicon sequencing of the 16 S rRNA gene was done by Fasmac Co. Ltd. (Atsugi, Japan). Briefly, DNA was extracted from the samples using an MPure bacterial DNA extraction kit (MP Bio Japan, Tokyo, Japan). A DNA library was prepared using a two-step polymerase chain reaction (PCR) method (Sinclair, Osman, Bertilsson, & Eiler, 2015). The V4 region was amplified in a 23-cycle PCR reaction using forward primer 515 f: 5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGCCAGCMGCCGCGGTAA-3′ and reverse primer 806r: 5′-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGACTCHVGGGTWTCTAAT -3′. In the subsequent step, the individual DNA was tagged in an eight-cycle PCR reaction using forward primer: 5′-AATGATACGGCGACCACCGAGATCTACAC-[sequence for individual mouse]- ACACTCTTTCCCTACACCGACGC-3′ and reverse primer: 5′-CAAGCAGAAGACGGCATACGAGAT-[sequence for individual mouse]-CTGACTGGAGTTCAGACGTGTG-3′. DNA libraries were multiplexed and loaded on an Illumina MiSeq instrument according to the manufacturer's instructions (Illumina, San Diego, CA, USA). Reads with the wrong sequence at the start region were filtered using the FASTX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/); reads with quality below 20 and shorter than 40 bases were omitted using Sickle (https://github.com/ucdavis-bioinformatics/sickle). The shortlisted reads were merged using the pair-end merge script FLASH (http://ccb.jhu.edu/software/FLASH/), and 400–500 base length reads were selected. Among the selected reads, chimeras were determined using pipeline QIIME (http://qiime.org/tutorials/chimera_checking.html) and omitted. Sequences were clustered into operational taxonomic units (OTU) according to a 97% identity cut-off using the QIIME workflow script and Greengenes database (http://greengenes.lbl.gov/).

2.3.3. Diversities of the microbiota

The alpha- and beta-diversities of the microbiota in the mice were expressed using the Shannon–Wiener index (H') and principal component analysis (PCA), respectively (Hill, Walsh, Harris, & Moffett, 2003; Kuda, Yokota, et al., 2017b). PCA was done based on the number of OTU from the dominant bacterial genes (filtered by 0.25% counts of whole OTU), and analysed using Easy PCA software (http://hoxom-hist.appspot.com/pca.html).