Title	Elucidating the anti-colitic mechanisms of rice bran supplementation via nutriomics
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Abstract	10%米ぬかを摂取したマウスが大腸炎発症による体重減少を抑制した．腸内細菌叢解析を行った結果，米ぬかの摂取によってClostridiaceaeの割合が高くなったことが明らかになった。無菌マウスへ米ぬかを摂取したマウスの糞便を経口投与して、大腸炎発症による体重減少を抑制した。米ぬかを摂取したマウスの便中のトリプトファン関連代謝物には有意義に変動した。 Our results suggest that colitic mice fed 10% RB supplemented diet had higher abundance of Clostridiaceae. Fecal microbiota transplantation from RB-fed mice suppressed colitis. This strongly suggests that RB suppresses colitis through gut microbiota modulation.The fecal metabolome profile was dynamically changed by RB intake. Enrichment analysis revealed that RB intervention enriched the tryptophan metabolism pathway. Our results revealed that RB improves colitis via gut environment modulation.
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科学研究費助成事業　　研究成果報告書

令和　２ 年　 ６ 月 １８ 日現在

機関番号：３２６１２
研究種目：若手研究
研究期間：2018～2019
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研究課題名（和文）Elucidating the anti-colitic mechanisms of Rice Bran supplementation via nutriomics

研究課題名（英文）Elucidating the anti-colitic mechanisms of Rice Bran supplementation via nutriomics

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交付決定額（研究期間全体）：（直接経費）　　2,600,000円

研究成果の概要（和文）：10%米ぬかを摂取したマウスが大腸炎発症による体重減少を抑制した．腸内細菌叢解析を行った結果，米ぬかの摂取によってClostridiaceaeの割合が高くなったことが明らかになった。無菌マウスへ米ぬかを摂取したマウスの糞便を経口投与して、大腸炎発症による体重減少を抑制した。米ぬかを摂取したマウスの便中のトリプトファン関連代謝物には有意義に変動した。

研究成果の学術的意義や社会的意義
We have provided scientific evidence that rice bran suppresses colitis and we could apply it to recommending IBD patients to consume unpolished brown rice instead of white rice. Our methodology in elucidating the anti-colitic mechanisms of rice bran could also be applied to other food products.

研究成果の概要（英文）：Our results suggest that colitic mice fed 10% RB supplemented diet had higher abundance of Clostridiaceae. Fecal microbiota transplantation from RB-fed mice suppressed colitis. This strongly suggests that RB suppresses colitis through gut microbiota modulation.The fecal metabolome profile was dynamically changed by RB intake. Enrichment analysis revealed that RB intervention enriched the tryptophan metabolism pathway. Our results revealed that RB improves colitis via gut environment modulation.

研究分野：Nutriomics

キーワード：Rice bran　colitis　gut microbiome　metabolome

１．研究開始当初の背景

In recent years, the prevention of life-style related disease such Type 2 Diabetes (T2D) (**Nutr Diabetes.,** 5 (11), e185, 2015), metabolic disease (**Br. J. Nutr.,** 112: 717-719, 2014) and IBD (**Sci. Rep.,** 7, 43993, 2017) via nutritional intervention is popular and consumers are becoming more conscious of their nutritional intake to maintain good health. RB that is contained in brown rice, a type of Japanese food that is commonly consumed, has reported colitis suppression effects, but the molecular mechanisms are not yet understood (**Scand. J. Gastroenterol.,** 46, 40–52, 2011).

２．研究の目的

The purpose and "key scientific questions" that would be answered in this project are (i) **elucidating the molecular mechanisms of colitis suppression by RB and its components via nutriomics analysis.** I am using **an original approach: nutriomics** strives to promote a greater understanding of the ways nutrition influences metabolic pathways and homeostatic control and how this regulation is disturbed in the early stages of a diet-related disease (**Semin. Immunopathol.**, 37 (1), 5-16, 2015). The gastrointestinal tract is a complex ecosystem that harbors various microorganisms which influence many aspects of health including inflammatory bowel disease, cancer and obesity in adults. The intricate interaction among gut microbiota-derived metabolites, the gut microbiota itself and the host immune system is transmitted via a large array of signaling pathways that extend beyond the immune system. These complex inter-relationships come together to form a series of host-microbe metabolic axes. By integrating metagenomic and metabolomics information on a systems biology-wide approach, I would be better able to understand this interplay between RB, gut microbiome, host metabolism and IBD. Through the modelling of metabolic interactions between RB and microbiota, I foresee that integrated omics-based understanding of the gut ecosystem is the new avenue, providing exciting novel therapeutic approaches for personalized host health.

３．研究の方法

We performed 3 animal experiments (2 animal experiments using conventional mice and 1 animal experiment using germ-free mice). The 1st animal experiment was to observe dose dependency of RB on colitis suppression. Following a 1-week acclimatization period, mice were randomly assigned to 4 different groups (n=5 in each group): Control: mice given the AIN-93G basal diet and tap water for 1 week, after which colitis was induced by administering 2.0% (w/v) DSS-containing water for 10 days; 5% RB, 10% RB and 20% RB-supplemented AIN-93G basal diet and tap water for 1 week, then 2.0% DSS-containing water for 10 days respectively. During the experimental period, fecal samples from all of the mice were collected daily for 16S rRNA gene analysis. In addition, during the DSS administration period, severity of colitis would be assessed. On day 10 after DSS administration, all mice would be sacrificed.

The 2nd animal experiment was to investigate fat-soluble/water-soluble components of RB on colitis suppression: Following a 1-week acclimatization period, mice were randomly assigned to 6 different groups (n=5 in each group): Control: mice given the AIN-93G basal diet and tap water for 1 week, after which colitis was induced by administering 2.0% (w/v) DSS-containing water for 10 days; RB, RBN, RBG and RBD: 10% RB, γ-oryzanol and 10% defatted RB-supplemented AIN-93G basal diet and tap water for 1 week, then 2.0% DSS-containing water for 10 days respectively. During the experimental period, fecal samples from all of the mice were collected daily for 16S rRNA gene analysis. In addition, during the DSS administration period, severity of colitis would be assessed. On day 10 after DSS administration, all mice would be sacrificed.

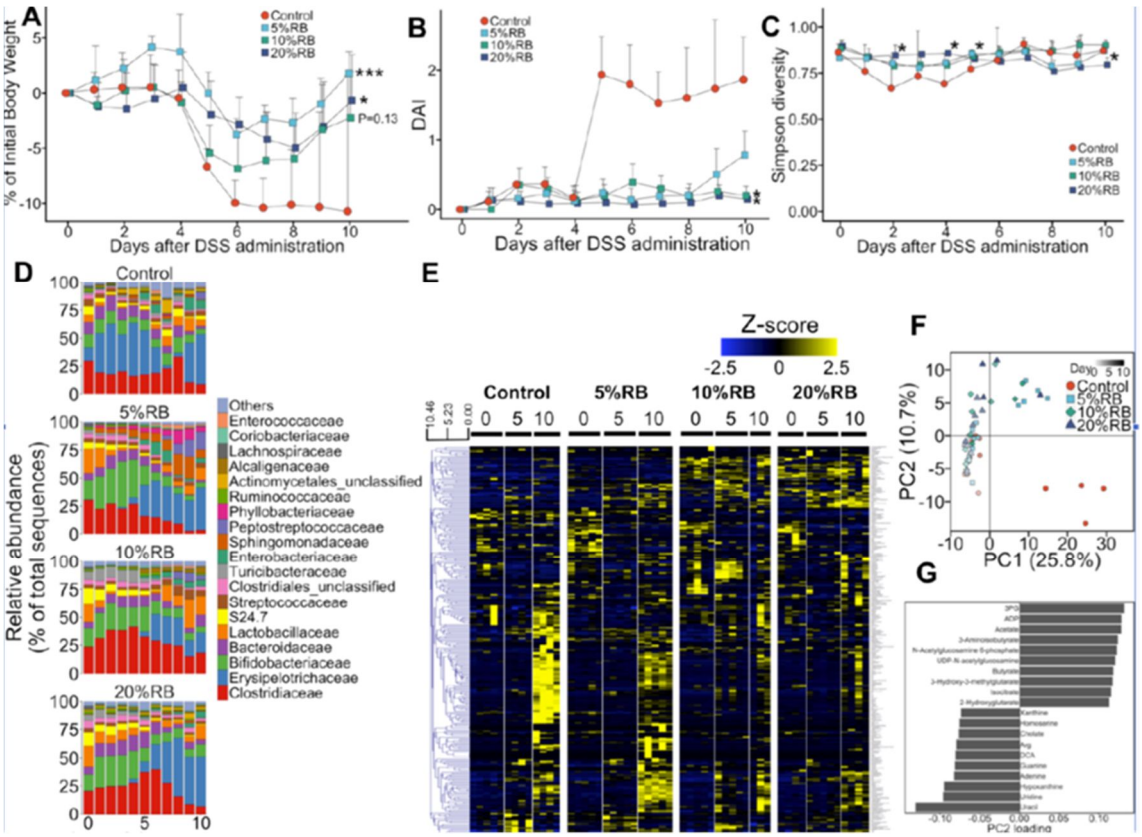
The germ-free murine experiment was to investigate if the colitis suppression effects from RB was solely dependent on gut microbiome modulation. Briefly, 8-week-old specific-pathogen-free male C5BL6J donor mice (n=3 per diet group) were administered with AIN-93G with tap water (CON), 10% supplemented RB diet with tap water (10% RB). After 2 weeks of administration, fecal samples were collected and pooled. 100 mg of fecal sample was resuspended in 1 ml of sterile PBS. The solution was vigorously mixed for 10s using a benchtop vortex (Vortex-Genie 2, Scientific Industries, USA; speed 9), before centrifugation at 800g for 3 min. The supernatant that was prepared on the same day of transplantation within 10 min before oral gavage to prevent changes in microbiota composition was collected and was administered into recipient mice daily (350 uL for each mouse) by oral gavage. Recipient mice were randomly assigned to 2 groups and were fed with AIN-93G basal diet and tap water for 10 days, then administered 2.0% DSS-containing water for 10 days. Additionally, recipient mice were administered supernatant of fresh fecal suspension from CON and 10% RB to their respective recipient groups, respectively, by oral

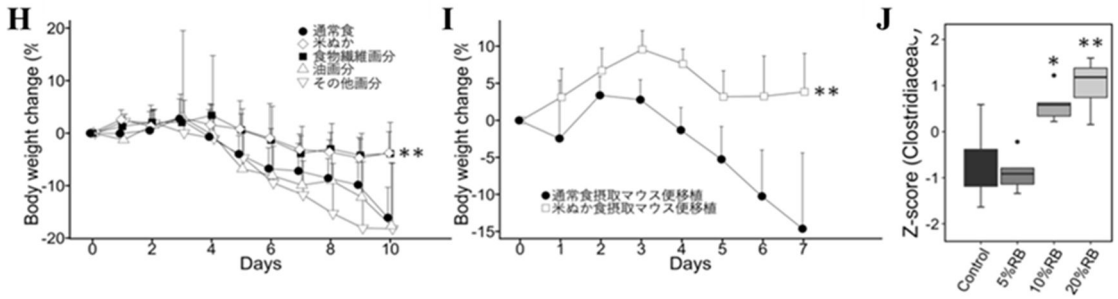
gavage (350 uL for each mouse) daily.

For all animal experiments, we performed the following observations.
Colon observations Length of colon and presence of tumors in colon will be macroscopically inspected. For histopathology analysis, a representative sample from the mid-part of colon will be selected.
Collection of tissues for multi-omics evaluation Fecal samples will be collected on as indicated above for metabolite analysis. Intestinal contents will also be collected from the ileum and colon at time of sacrifice for metabolomics (CE-TOF/MS, Agilent, available at Keio University) and metagenomic (Miseq System, Illumina, available at Keio University) analyses.
Evaluation of omics data for integrated omics analysis One of the core goals of nutritional research is to understand how diet can influence metabolic regulations and how scientists in this field can use this diet to improve health and well-being. I will integrate multiple omics platforms and finally to systems biology by using software like Ingeunity Pathway Analysis (IPA, Qiagen) and MetaCore (Thomson Reuters) to have an overview of integrated omics data to visually compare the responses of multiple metabolic pathways simultaneously. The pathways/genes/proteins/metabolites are activated or repressed among the multiple metabolic pathways can be visually and intuitively found. Gut microbiome will be analysed using the Quantitative Insights Into Microbial Ecology (QIIME) platform.

４．研究成果

In this study, we have shown that 5%. 10% and 20% RB fed mice have reported colitis suppression (A). By using a time-course microbiome and metabolome approach, I aimed to elucidate the dynamics of gut environment, and identified RB-related gut environmental alterations. 20 families, especially Alcaligenaceae and Lactobacillaceae showed significant increases and Enterobacteriaceae was significantly decreased in 10% RB-fed mice (D). Clostridiaceae had significantly higher abundances (J). 41 metabolites, especially N1,N12-Diacetylspermine, Indole-3-ethanol, N1-Acetylspermidine, 5-Hydroxy-indoleacetate and short-chain fatty acids were significantly increased RB-fed mice (E,F). Metabolite set enrichment analysis revealed that Vitamin B6 metabolism, tryptophan metabolism and ubiquinone biosynthesis were significantly increased in RB-fed mice (G). Mice that underwent fecal microbiota transplantation from donor mice fed RB had significantly improved body weight loss (I).

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５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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