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# Detection and isolation of typical gut indigenous bacteria in ICR mice fed wheat bran and wheat straw fibre

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ABSTRACT

Wheat bran (WB) and wheat straw fibre (WSF) are by-products of the wheat flour industry. To prove the existence of indigenous gut bacteria responsible for WB and WSF, the Institute of Cancer Research (ICR) mice were fed a diet containing no fibre (CS), 10% WB, or 5% WSF for 14 d. The caecal microbiome was analysed by 16S rDNA (V4 region) amplicon sequencing. Typical colonies were isolated and estimated by 16S rRNA gene BLASTn analysis. The predominant amplicon sequence variants in all diet groups belonged to *Bifidobacterium pseudolongum*- and *Faecalibaculum rodentium*-like bacteria. *Lactobacillus johnsonii*- and *Limosilactobacillus reuteri*-like bacteria were high in the WB group compared with those in the CS group. *Lactobacillus johnsonii* Wheat-1 and *L. reuteri* Wheat-12 strains could be isolated. *Lactobacillus johnsonii* Wheat-1 exhibited good fermentation activity in 10% (w/v) WB suspension. Superoxide anion radical scavenging capacity of the WB suspension was significantly increased by the fermentation.

## 1. Introduction

Various lifestyle diseases, such as obesity, diabetes, cardiovascular diseases, and cancer, in addition to chronic inflammatory diseases, have raised concerns in developed countries, especially because these diseases are among the major causes of deaths in these high-income countries (Minami et al., 2020; Shiels et al., 2021). In general, a healthy human gut (colon) contains several hundred species of bacteria at 11–12 log cells/g. Moreover, it is considered that lifestyle, especially a person's diet, affects the gut microbiome (Wilson et al., 2020). Gut bacteria and metabolism also affect the host's health, including the amelioration and/or promotion of lifestyle and chronic diseases (Ganal-Vonarburg et al., 2020; Yuan et al., 2020). The gut microbiome is rapidly and considerably affected by the type of diet, within a few days to a week, before the appearance of lifestyle disease markers and/or symptoms (David et al., 2014). Dietary fibre is regarded as an important food ingredient that can improve the intestinal environment and prevent food-related lifestyle diseases (Abbate et al., 2020).

Wheat bran (WB) and wheat straw are by-products of the wheat flour industry. Although there are reports of some of the functional properties of WB and wheat straw fibre (WSF) (Neyrinck et al., 2011; Neyrinck et al., 2018; Budhwar et al., 2020; Demuth et al., 2020), large quantities are wasted during the milling process. WB contains approximately 16% w/w proteins, 4.4% lipids, and 65% carbohydrates (of which 43% is dietary fibre); some of these proteins and lipids exhibit unique activities. In particular, the WB lipids exhibit cholesterol-reducing activity, and the WB protein hydrolysates have been reported to have antioxidant and immunomodulation activities (Lei et al., 2018; Zou et al., 2021). Additionally, WB dietary fibre has been found to contain not only cellulose but also a unique fibre called arabinoxylan (Demuth et al., 2020). Furthermore, there are many reports of the inhibitory effects of WB and whole-grain flour on obesity, diabetes, and colitis (Budhwar et al., 2020; Tieri et al., 2020).

Gut-fermentable dietary fibres affect gut indigenous bacteria and thereby the host health. For example, mice that were fed alginate and laminaran, brown algal water-soluble polysaccharides, had a greater abundance of caecal *Bacteroide acidifaciens*- and *Bacteroides intestinalis*-like bacteria compared with those that were not fed dietary fibre (Takei et al., 2020). Additionally, in a previous study, we observed *in vitro* that the antioxidant and immunomodulation activities of *B. acidifaciens* and *B. intestinalis* cultures increased with the fermentable polysaccharides (Harada et al., 2021). It can be considered that the gut indigenous bacteria increased after WB intake and also exerted synergistic effects with the WB compounds. Moreover, we expected that the detection and isolation of the gut responsive indigenous bacteria (RIB) would clarify the synergistic effects of prebiotics and probiotics.

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In the present study, we aimed to detect and isolate RIB from the caecal microbiome of ICR mice fed a corn starch-based diet containing no fibre (CS), 10% WB, or 5% insoluble WSF for 14 d. The microbiota was analysed by 16S rDNA (V4) amplicon sequencing. Typical colonies on agar plates were isolated and estimated by 16S rRNA gene BLASTn analysis. Additionally, the *in vitro* WB fermentation capacity of the isolated RIB and their antioxidant properties were determined.

## 2. Materials and methods

### 2.1. Diet and animal care

Corn starch was purchased from Fujifilm Wako Pure Chemical Co., Ltd. (Osaka, Japan). WB from wheat harvested in Japan was purchased from Life Joy Co., Ltd. (Ama, Japan), and WSF from wheat straw harvested in Germany was purchased from Nichie Co., Ltd. (Nagoya, Japan).

Animal experiments were performed in accordance with the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions, under the jurisdiction of the Ministry of Education, Culture, Sports, Science, and Technology, Japan. The Animal Experiment Committee of the Tokyo University of Marine Science and Technology approved the study protocol (Approval No. H31-5).

Seventeen 5-week-old male ICR mice, purchased from Tokyo Laboratory Animal Science Co., Ltd. (Tokyo, Japan), were housed in metal wire cages and placed at a controlled temperature of 22 ± 2 °C. The mice were allowed to acclimatise to a semi-purified powder diet based on AIN-76 containing 20% (w/w) corn starch (Fujifilm Wako Pure Chemical Co., Ltd.) and 50% sucrose (Mitsui Sugar Co., Ltd., Tokyo, Japan) without cellulose, in addition to distilled water (DW) for drinking, *ad libitum*. After 7 d, the mice were divided into three groups: CS, WB, and WSF ($n$ = 6, 5, and 6, respectively). The CS group mice were fed a diet containing 43% corn starch (Table 1), whereas the WB and WSF group mice were fed a diet containing 10% WB and 5% WSF, respectively, for 14 d. According to the Standard Tables of Food Composition in Japan-2015 (https://www.mext.go.jp/component/english/_icsFiles/afieldfile/2017/12/25/1374049_1r12_1.xlsx), these diets contained approximately 36% carbohydrates from CS, WB, or WSF. During days 11–13 of feeding, the defecation frequency and faecal weight were recorded.

At the end of the experiment, the mice were anaesthetised using isoflurane (Fujifilm Wako Pure Chemical Co., Ltd.) and exsanguinated from the abdominal aorta and vein. Thereafter, the liver, kidneys, spleen, and epididymal fat pads of each mouse were removed and weighed. Ligation of the caecum was performed with yarn and the caecum was excised and placed on ice until microbial analysis.

**Table 1**
Composition of test diets (g/100 g).

| | CS | 10% WB | 5% WF |
|---|---|---|---|
| Corn starch | 43.0 | 35.0 | 38.0 |
| Wheat-bran | | 10.0 | |
| Wheat-insoluble fibre | | | 5.0 |
| Milk casein | 20.0 | 18.4 | 20 |
| DL-Methionine | 0.3 | 0.3 | 0.3 |
| Sucrose | 27.0 | 27.0 | 270 |
| Corn oil | 5.0 | 4.6 | 5.0 |
| Vitamin mix (AIN-76)* | 1.0 | 1.0 | 1.0 |
| Mineral mix (AIN-76) * | 3.5 | 3.5 | 3.5 |
| Choline bitartrate | 0.2 | 0.2 | 0.2 |
| | | | |
| Protein** | 17.3 | 17.6 | 17.3 |
| Lipid** | 5.6 | 5.6 | 5.6 |
| Carbohydrate** | 65.5 | 65.7 | 66.0 |
| Dietary fibre** | | 4.0 | 4.7 |
| Energy** | 398 | 392 | 380 |

*Ingredients for American Institute of Nutrition Rodent Diet.
**According to Standard Tables of Food Composition in Japan-2015.

### 2.2. Bacterial cell count determination and isolation

The caecal contents were diluted with 99 volumes (approximately 5 mL) of phosphate-buffered saline (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), after which, the bacterial cell count was determined using the dielectrophoretic impedance measurement (DEPIM) method (Hirota et al., 2014; Inagaki et al., 2014; Goto et al., 2021) using a bacterial counter DU-AA01NP-H (PHC Holdings Co., Ltd., Tokyo, Japan). The caecal content was serially diluted with 'Dilution A' (4.5 g $KH_2PO_4$, 6 g $Na_2HPO_4$, 0.5 g L-cysteine·HCl·$H_2O$, 0.5 g Tween 80, and 0.75 g/L agar). Aliquots of $10^{-5}$–$10^{-7}$ diluted samples (0.03 mL) were spread on Blood Liver (BL) and Gifu anaerobic medium (GAM) agar (Nissui Pharmaceutical Co., Ltd.) plates containing 5% (v/v) defibrinated horse blood (Nippon Bio-Supply Center, Co., Inc., Tokyo, Japan), and were incubated at 37 °C for 48 h, under anaerobic conditions using AnaeroPack (Mitsubishi Gas Chemical, Tokyo, Japan). Colonies with typical morphologies were isolated using the same agar plates, and the 16S rRNA genes of the isolates were amplified using polymerase chain reaction (PCR), with the 27F and 1492R primers. The amplicons were then sequenced by Macrogen Japan, Corp. (Tokyo, Japan). A homology search was performed using the nucleotide Basic Local Alignment Search Tool (BLASTn; National Center for Biotechnology Information, Bethesda, MD, USA).

### 2.3. Analysis of the caecal microbiota by MiSeq

Amplification and sequencing of the 16S rDNA (V4) amplicon were performed by Fasmac Co., Ltd. (Atsugi, Japan). DNA was extracted from each sample using the Mpure bacterial DNA extraction kit (MP Bio Japan Co., Ltd., Tokyo, Japan), following the manufacturer's instructions. The DNA library was prepared using a two-step PCR (Sinclair et al., 2015) following a previous report (Nakamura et al., 2021). Briefly, the V4 region was amplified using a 23-cycle PCR with the following primers: forward, 515f; reverse, 806r. Next, individual DNA fragments were tagged in an eight-cycle PCR. DNA libraries were multiplexed and loaded onto an Illumina MiSeq system (Illumina Inc., San Diego, CA).

Reads with a mismatched sequence at the start region were filtered using the FASTX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/), and 235–260-base pair reads were selected. Chimeras in the selected reads were identified and omitted using the QIIME2 bioinformatics pipeline (https://qiime2.org/). The feature table was generated using the dada2 denoise-paired option in the QIIME2 plugin (Poncheewin et al., 2020), and the sequences were clustered into amplicon sequence variants (ASVs) using the SILVA 138 database (https://www.arb-silva.de/).

### 2.4. Wheat bran fermentation by the typical bacteria isolated from ICR mice

WB powder (0.5 g) was added to a test tube containing 5 mL of DW and autoclaved at 121 °C for 15 min. *Lactobacillus johnsonii* Wheat-1 (LC586157) and *L. reuteri* Wheat-12 (LC586168) strains isolated from the ICR mic were pre-cultured in 3 mL of GAM broth (Nissui Pharmaceutical Co., Ltd.) at 37 °C for 48 h. The pre-culture (0.05 mL) was then inoculated into the 10% (w/v) WB suspension ($n$ = 3) and incubated at 37 °C for 7 d. The pH of the cultures was measured on days 1, 2, 4, and 7. The fermented sample at 4 days of incubation was filtered through a 0.45-μm pore syringe filter (DISMIC 13CP045AN; Toyo Roshi Kaisha Ltd., Tokyo, Japan) and subjected to high-performance liquid chromatography (HPLC) under the following conditions: column, ICSep ICEORH-801 (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan); operating temperature, 35 °C; elution, 0.01 mol/L sulphuric acid ($H_2SO_4$); and flow rate, 0.8 mL/min. The eluted compounds were detected using a refractive index detector.

Total phenolic compound content (TPC), ferric reducing power, and superoxide anion ($O_2^-$) radical scavenging capacity in WB fermented for 4 days were determined as previously reported (Kuda et al., 2021).

### 2.5. Statistical analysis

The body weight, organ weight, bacterial cell count, and alpha diversity index (diversity within a microbiome) re expressed as mean ± standard error. Analysis of variance and Tukey's and Dunnett's post hoc tests were performed using EXCEL Statistics (Ver. 6; Esumi, Tokyo, Japan), and results with $p < 0.05$ were considered statistically significant. Alpha diversity of the mouse gut microbiome was determined using the Shannon-Wiener (*H'*) and Simpson's (*D*) indices (Kim et al., 2017), whereas distance between groups, based on the differences in the ASVs present in each group is expressed via principal component analysis (PCA), partial least squares-discriminant analysis (PLSDA), and sparse PLSDA (sPLSDA) (Vieira-Potter et al., 2018; Asquith et al., 2019), performed using MetaboAnalyst (https://www.metaboanalyst.ca/MetaboAnalyst/). The 16S rRNA gene-based microbiome taxonomic profiling (MTP) and linear discriminant analysis (LDA) effect size (LEfSe) algorithm, available on the online interface EzBioCloud (https://www.ezbiocloud.net/contents/16smtp), was used to identify the taxa with differential abundance between the CS and WB or WSF groups.

## 3. Results and discussion

### 3.1. Body, faecal, and organ weights

No abnormalities and adverse symptoms were observed in the mice used in this study. A lower body weight gain was observed in mice fed WB, but the difference was not significant (Table 2). Moreover, a high frequency of faecal excretion and increased faecal weights were observed in both WB and WSF groups compared with those in the CS group ($p < 0.05$). The caecal content weight was higher in mice fed WB than in those fed WSF. There were no significant differences among the organ weights of the tested diet groups, although caecal wall weight tended to be higher in the WB and WSF groups. No significant difference was observed in the body and organ weights owing to the short feeding period.

### 3.2. Bacterial cell count and diversities of the caecal microbiome

The direct bacterial cell count in the caecal contents was approximately 11.1–11.5 log cell/g (Table 3). Compared with that in the CS group, the cell number in the WSF group was low. The total read count in the 16S rRNA (V4) amplicon sequencing with MiSeq was 95600–101000. Moreover, the number of ASVs in WB-fed mice ranged from 208 to 290, and it was higher than that of CS-fed mice. Similarly, the alpha diversity indices, namely, the Shannon–Wiener and Simpson's indices, tended to be high in the WB group, although the differences were not significant. Based on the PCA and PLSDA results (Fig. 1A, B), a part of the major caecal bacterial composition of each diet group overlapped, although there was a tendency to be biased for each group in the sPLSDA (Fig. 1C). Furthermore, although the PCA for the caecal microbiome showed overlapping groups, the PLSDA and sPLSDA suggested different microbiome compositions between the CS group and the other two groups (Fig. 1A–C).

Although both WB and WSF are rich in dietary fibres, the results of this study suggest that there were WB-RIBs in the caecum and that there was an increase in alpha-diversity. It has been reported that the intake of dietary fibre increases the ASV number and the alpha diversity of the gut microbiome owing to their beneficial effects, such as anti-obese and anti-type 2 diabetes effects (Menni et al., 2017).

**Table 2**
Body, organ and faecal weights of tested mice.

| CS | 10% WB | | 5% WF |
|---|---|---|---|
| *Body weight (g)* | | | |
| Initial | 37.0 ± 1.0 | 37.1 ± 0.8 | 37.0 ± 1.1 |
| After 14 days | 47.2 ± 1.4 | 44.9 ± 1.4 | 46.3 ± 2.1 |
| Gain | 10.2 ± 1.0 | 7.3 ± 0.9 | 9.3 ± 1.1 |
| *Faeces* | | | |
| Frequency (*n*/day/mouse) | 26 ± 2[a] | 38 ± 2[b] | 40 ± 2[b] |
| Weight (g/day/mouse) | 0.22 ± 0.01[a] | 0.47 ± 0.03[b] | 0.54 ± 0.02[b] |
| Caecal content (g) | 0.189 ± 0.012[a] | 0.317 ± 0.026[b] | 0.261 ± 0.035[ab] |
| *Organ weights (g)* | | | |
| Liver | 2.760 ± 0.208 | 2.685 ± 0.137 | 2.720 ± 0.175 |
| Kidneys | 0.641 ± 0.033 | 0.725 ± 0.029 | 0.651 ± 0.037 |
| Spleen | 0.085 ± 0.007 | 0.103 ± 0.012 | 0.094 ± 0.009 |
| Caecal wall | 0.059 ± 0.005 | 0.087 ± 0.013 | 0.084 ± 0.014 |

Values are presented as the mean ± standard error of the mean (SEM) values ($n = 6$ for CS and WSF; $n = 5$ for WB). [a,b] There was no significant difference between data with the same letters ($p < 0.05$).

**Table 3**
Total bacterial count and alpha diversity indices in caecal microbiome of tested mice.

| | CS | 10% WB | 5% WF |
|---|---|---|---|
| Total bacterial count (Log cells/g) | 11.48 ± 0.12[a] | 11.30 ± 0.08 | 11.06 ± 0.06[b↓] |
| Total read number | 100686 ± 9824 | 95608 ± 3829 | 103579 ± 2909 |
| Number of ASVs | 208 ± 11 | 290 ± 25[↑] | 213 ± 28 |
| Shannon index (*H'*) | 2.911 ± 0.156 | 3.387 ± 0.318 | 2.914 ± 0.442 |
| Simpson diversity (*D*) | 0.861 ± 0.020 | 0.902 ± 0.021 | 0.828 ± 0.045 |

Values are expressed as the mean ± standard error of the mean (SEM) values ($n = 6$ for CS and WSF; $n = 5$ for WB). [a,b] Values with different superscript letters are significantly different, based on the Tukey's method ($p < 0.05$). Up and down arrows indicate higher and lower values than those of CS, respectively, as compared using Dunnett's method ($p < 0.05$).

### 3.3. Caecal microbiome composition

#### 3.3.1. Relative abundance at the phylum, family, and genus levels

The relative abundance at the phylum level revealed that Firmicutes was the predominant phylum in the CS group (65% ± 3%), followed by Actinobacteria (22% ± 2%), Bacteroidota (8.3% ± 2.2%), and Desulfobacterota (3.4% ± 0.5%) (Fig. 1D). While there were no significant ($p > 0.05$) differences among the microbiome compositions of different diet groups, Desulfobacterota tended to be less abundant in the WB group (1.8% ± 0.5%).

With respect to Firmicutes in the CS group, the predominant family was *Erysipelotrichaceae* (38% ± 3%), followed by *Lachnospiraceae* (18% ± 3%), *Lactobacillaceae* (3.2% ± 0.8%), and *Clostridiaceae* (1.7% ± 0.5%) (Fig. 1E). There were no significant differences among these dominant families, but the abundance of *Lactobacillaceae* tended to be higher in mice fed WB (8.5% ± 3.1%). The abundance of *Clostridia*_UCG-014 (1.7% ± 0.7% in WB group) and *Bacilli* (not detected in the CS group; 1.3% ± 0.5% in WB group) was significantly ($p < 0.05$) high in mice fed WB. Almost all strains belonging to Actinobacteriota were classified as *Bifidobacteriaceae* (22% ± 3% in the CS group). Although *Eggerthellaceae* presented a relatively low abundance (0.11% ± 0.03% in the CS group), it was high in the WB group (0.40% ± 0.07%). In Bacteroidota, *Muribaculaceae* was the dominant family (4.7% ± 1.1% in CS group).

With respect to the predominant family *Erysipelotrichaceae* in the CS group, *Faecalibaculum* (23% ± 3%) was the dominant genus, followed by *Allobaculum* (15% ± 3%, Fig. 1F), which was significantly less abundant in WSF-fed mice (1.5% ± 0.7%). *Turicibacter* highly abundant in the WB

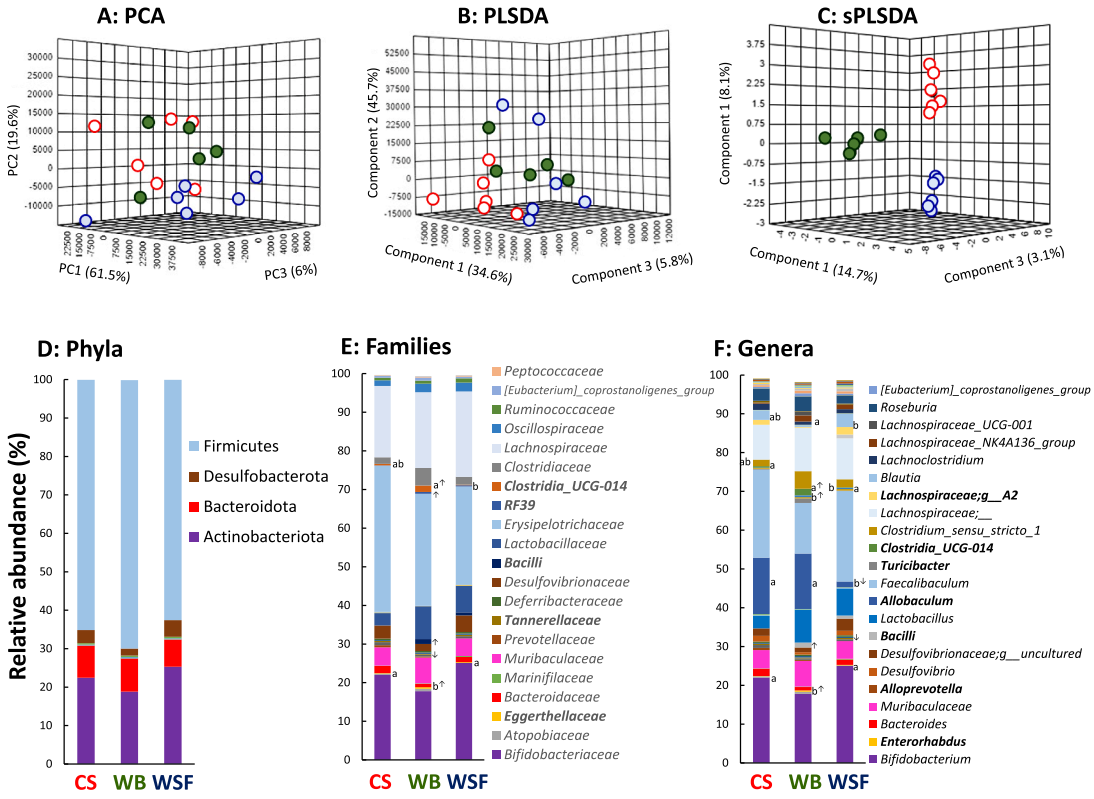


**Fig. 1.** Composition of the caecal microbiome in the tested mice. (a) Principal component analysis (PCA), (b) partial least squares-discriminant analysis (PLSDA), and (c) sparse PLSDA (sPLSDA) of the amplicon sequence variants (ASVs) in the caecal microbiome of mice that were no fed fibre (CS, open circles), 10% (w/w) wheat bran (WB, closed circles), or 5% wheat straw fibre (WSF, semi-closed circles). (d), (e), and (f) show the phylum, family, and genus levels, respectively. Significant differences among the groups were determined using Tukey's test and Dunnett's test. [a, b] Data indicated with the same letter did not differ significantly. Arrows indicate a significant difference from the CS group ($p < 0.05$).

group (1.2% ± 0.4%). With respect to the family *Lachnospiraceae* in the CS group, the two predominant genera were *Roseburia* (3.0% ± 1.5%) and *Blautia* (2.5% ± 0.8%), and these tended to be high (3.7% ± 0.5%) and low (0.43% ± 0.17%), respectively, in the WB group. Most ASVs belonging to *Clostridiaceae* in the CS group were *Clostridium* sensu stricto 1 (1.7% ± 0.5%) that tended to be high in the WB group (4.5% ± 1.9%). Most of the *Eggerthellaceae* family members in the CS group comprised *Enterohabdus* (0.10% ± 0.03%), whose abundance was high in the WB group (0.33% ± 0.06%).

### 3.3.2. *ASV levels*

LDA revealed the existence of *Lactobacillus gasseri* and *L. reuteri* as RIB in WB (Fig. 2A). Other *Lactobacillus* species were also observed as RIB in the WSF groups. Fig. 2B shows a heat-map of the 40 dominant ASVs. Furthermore, defined bacterial names of predominant and typical ASVs having at least 97% similarity (determined using BLASTn) with the de novo sequences (with approximately 250 bp) are shown. The predominant ASVs were predicted to be *Bifidobacterium choeerinum*- or *pseudolongum*-like bacteria (similarity 100%), followed by *Faecalibaculum rodentium*- (100%), *Allobaculum*- (<97%), *L. gasseri*- or *johnsonii*-(100%), and *Ligilactobacillus murinus*- or *animalis*-like (100%) bacteria. In these major ASVs, *Allobaculum*-like bacteria were less abundant in mice fed WSF. *Lactobacillus gasseri*- or *johnsonii*-like bacteria were highly abundant, whereas *L. murinus*- or *animalis*-like bacteria tended to be less abundant in the WB group. Moreover, the abundance of *Turicibacter sanguinis*- (98%) and *L. reuteri*- or *caviae*-like (100%) bacteria was also high in the WB group. Typical *Lacnospiraceae* ASVs were different among the diet groups.

*Allobaculum* ASVs are shown in Fig. 1F. In the case of lactobacilli, although Fig. 1F showed no difference between the WB and WSF groups, different typical lactic acid bacterial species may have been abundant in mice fed WB and WSF. Furthermore, the bacilli shown in Fig. 1 and Fig. 2A were determined to be *L. reuteri*- or *caviae*-like bacteria.

Among the typical bacterial groups in mice fed BW, besides lactic acid bacteria, *Clostridia*_UCG-014 and *Roseburia* are associated with beneficial properties, such as anti-inflammatory and anti-obesity effects (Kasahara et al., 2018; Quan et al., 2018; Yang et al., 2021). *T. sanguinis* might be important for host lipid and steroid metabolism (Hoffman and Margolis, 2020). An increase in the abundance of *Roseburia* and *Enterohabdas* with plant proteins, such as *β*-conglycinin of soybeans, has been reported (Nakamura et al., 2021). A decrease in gut *Allobaculum* with WSF might be due to insoluble dietary fibres, such as cellulose (Arora et al., 2019).

## 3.4. *Typical bacteria isolated and WB fermentation*

On the BL and GAM agar plates, 11 colonies were observed, from which *B. pseudolongum*, *L. johnsonii*, *L. murinus*, *L. reuteri*, *Bacteroides acidifaciens*, *Bacteroides intestinalis*, and *F. rodentium* were isolated and identified by BLASTn. Among these isolates, the typical WB-RIB strains *L. johnsonii* wheat-1 and *L. reuteri* wheat-12, were used for the WB fermentation test.

As shown in Fig. 3A, *L. johnsonii* decreased the pH of the 10% WB suspension from 6.3 to 4.4 and lower, after 48 h of incubation. However, the pH of the suspension with *L. reuteri* was approximately 5.0. Fig. 3B shows the HPLC chromatograph of the supernatant of the fermented WB suspension. A part of the main soluble sugars of WB were converted to lactic acid by *L. johnsonii*. However, in the case of the fermentation setup

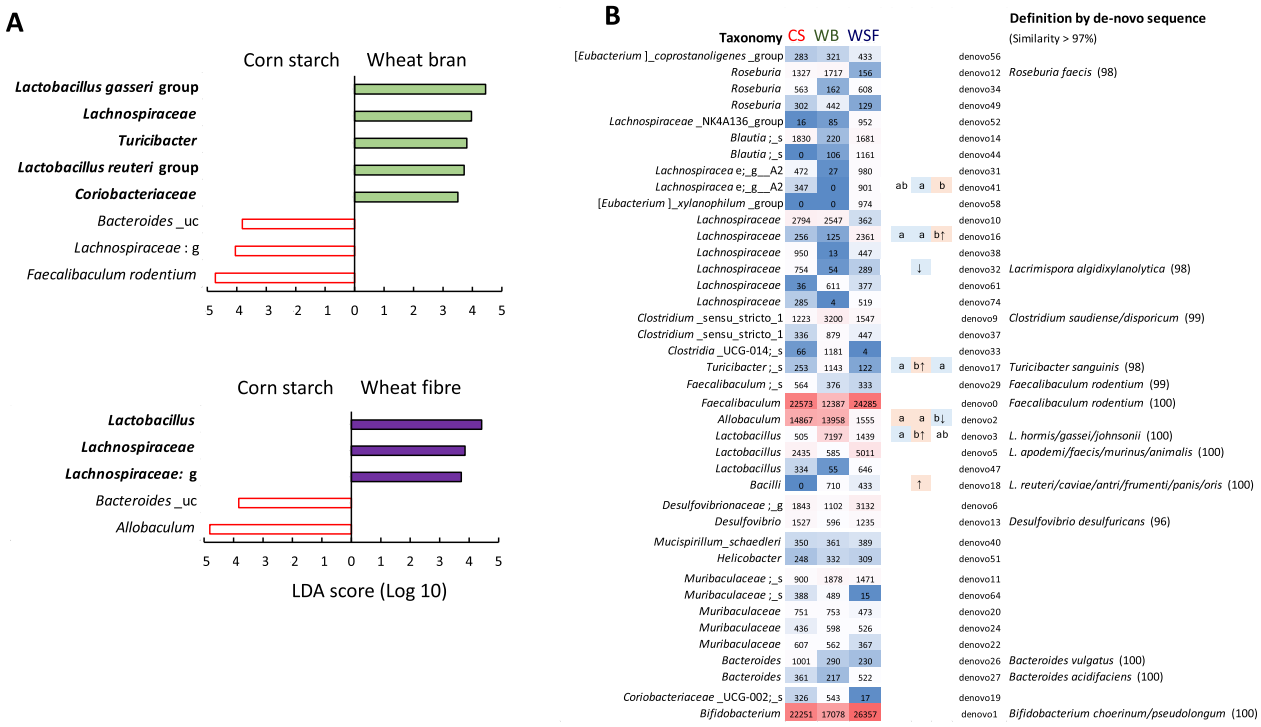


**Fig. 2.** Linear discriminant analysis (LDA) score computed for gut microbiome differentially identified using LEfSe (a), and a heat-map showing the relative abundance of 40 major identified ASVs of the caecal microbiome in mice who were fed CS, 10% (w/w) WB, or 5% WSF (b). [a, b] Data indicated with the same letter did not differ significantly. Arrows indicate a significant difference from the CS group ($p < 0.05$).

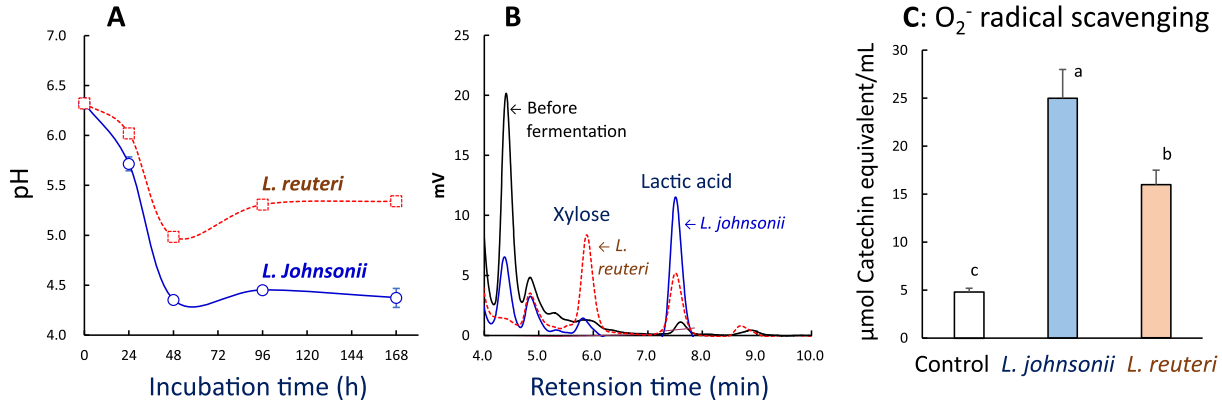


**Fig. 3.** Decreasing pH value (a), the HPLC chromatograph (b), and superoxide anion radical scavenging capacity (c) of 10% (w/v) WB suspension inoculated with *Lactobacillus johnsonii* and *Limosilactobacillus reuteri*. Data are expressed as mean ± standard error of the mean (SEM) values ($n = 3$). [a-c] Data indicated with the same letter did not differ significantly.

with *L. reuteri*, xylose was detected.

The TPC and Fe-reducing power in the WB suspension were approximately 0.57 and 0.16 µmol catechin equivalent/mL, respectively, and were not significantly changed by fermentation (data are not shown). In contrast, $O_2^-$ scavenging capacity was significantly increased by LAB fermentation (Fig. 3C).

Although arabinoxylan and other prebiotic compounds have been reported to increase the growth of *Bifidobacterium* (Paesani et al., 2019), it was not observed in the current study. *Lactobacillus johnsonii* and *L. reuteri* have also been isolated from the human gut, and there are reports of their probiotic capacities (Mu et al., 2018; Davoren et al., 2019). Therefore, it is suggested that the various functional properties of WB, such as hypocholesterolaemia and immunomodulation activities (Demuth, et al., 2020; Zhang et al., 2021), are correlated with the WB-RIBs, and host health status. Furthermore, increasing $O_2^-$ radical scavenging capacity by the fermentation strains (Fig. 3C) might have correlation with the functional properties.

The results of the *in vitro* fermentation experiment elucidated that the fermentation capacity of the isolated *L. reuteri* was weaker than that of *L. johnsonii*, though *L. reuteri* was highly abundant in the gut of WB-fed mice (Fig. 3). To confirm xylose utilisation by these LAB strains, the decrease in the pH in 0.5% (w/v) xylose containing GAM1/4 broth (Harada et al., 2021) was determined and fermentation by *L. reuteri* but not by *L. johnsonii* was observed (data not shown). It can be considered that the fate of these RIBs in the gut environment is affected by other gut commensals, including their metabolites. Furthermore, the role of gluten, a unique protein in WB, was not investigated in this study, although it may have effects on the host's immune system (Wu et al., 2017). Studies on the functional properties of the WB-RIB with or without WB, arabinoxylan, and gluten in the diet are in progress. In our

future study, we will aim to isolate WB-RIB from human faeces.

## 4. Conclusions

To detect and isolate the WB-RIB, the caecal microbiota in ICR mice that were fed a 14-d diet, comprising either no fibre, 10% WB, or 5% WSF, was analysed by 16S rDNA (V4) amplicon sequencing. The predominant ASVs in all diet groups belonged to *B. pseudolongum*- and *F. rodentium*-like bacteria. Among the major ASVs identified, the abundance of *Allobaculum*-like bacteria was lowin mice fed WSF. In contrast, *L. johnsonii*- and *L. reuteri*-like bacteria were relatively more abundant. We isolated *B. pseudolongum, F. rodentium, L. johnsonii*, and *L. reuteri*. Moreover, the isolated *L. johnsonii* exhibited good WB fermentation *in vitro*. In contrast, in *L. reuteri* culture, xylose was generated from WB, whereas arabinoxylan was not. These results suggest that WB-RIBs and other gut bacteria interact with each other in the gut and may have synergistic effects with ingested WB on host health. Further studies on the functional properties of WB-RIB, with or without WB, arabinoxylan, and gluten in the diet, are needed.

## Ethical approval

Animal experiments were performed in accordance with the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions, under the jurisdiction of the Ministry of Education, Culture, Sports, Science, and Technology, Japan. The Animal Experiment Committee of the Tokyo University of Marine Science and Technology approved the study protocol (Approval No. H31-5).

### *CRediT authorship contribution statement*

**Natsumi Takei:** Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft. **Takashi Kuda:** Methodology, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Supervision, Project administration. **Natsumi Handa:** Investigation. **Sae Fujita:** Investigation. **Hajime Takahashi:** Methodology, Supervision. **Bon Kimura:** Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2021.100071.

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