**Dietary Fructooligosaccharides Reduce Mercury Levels in the Brain of Mice Exposed to Methylmercury**

Masaaki Nagano,*+ Masatake Fujimura, Yuya Tada, and Yoshiyuki Seko

*Department of Basic Medical Sciences, National Institute for Minamata Disease; 4058–18 Hama, Minamata, Kumamoto 867–0008, Japan; b Department of Environment and Public Health, National Institute for Minamata Disease; 4058–18 Hama, Minamata, Kumamoto 867–0008, Japan; and c Mount Fuji Research Institute; 5597–1 Kenmarubi, Kamiyoshida, Fujiyoshida, Yamanashi 403–0005, Japan.

Received October 6, 2020; accepted January 17, 2021

Methylmercury (MeHg) exposure during pregnancy is a concern because of its potential health risks to fetuses. Intestinal microbiota has important roles in the decomposition and fecal excretion of MeHg. We investigated the effect of nondigestible saccharides on the accumulation and excretion of Hg after MeHg exposure. Female BALB/cByJ mice were fed a basal diet or the same diet supplemented with 5% fructooligosaccharides (FOS) or 2.5% glucomannan. Six weeks after feeding, mice were administered MeHg chloride (4 mg Hg/kg, per os (p.o.),) and urine and feces were collected for 28 d. FOS-fed mice had lower total Hg levels compared with controls. The results suggest that FOS enhanced fecal Hg excretion and decreased tissue Hg levels after MeHg administration, possibly by accelerating MeHg demethylation by intestinal bacteria (the candidate genus *Bacteroides*). This demethylation also reduces MeHg absorption in thelarge intestine. In conclusion, daily FOS intake may decrease tissue Hg levels in animals and humans exposed to MeHg.

**INTRODUCTION**

Methylmercury (MeHg) is a ubiquitous environmental pollutant and well-known neurotoxicant. Humans are exposed to environmental MeHg mainly through the consumption of fish, shellfish and sea mammals. Pregnant women are cautioned against consuming seafood in Canada, U.K., U.S.A., Australia, Norway and Japan because the developing fetal brain is highly susceptible to MeHg.

MeHg is absorbed almost completely from the gastrointestinal tract. Then, a portion of absorbed MeHg is released back into the small intestine via bile, and secreted MeHg is in large part reabsorbed. Ingested MeHg is transformed gradually to inorganic Hg in vivo via two degradation processes, by intestinal microbiota and the tissues themselves. MeHg transformation by intestinal microbiota reduces the amount of absorbed MeHg from the intestine because MeHg in intestinal contents is used as a substrate. This transformation would, in turn, lead to a reduction in Hg burden. In addition, MeHg transformation by intestinal microbiota is thought to facilitate Hg excretion into feces because inorganic Hg is poorly absorbed from the gastrointestinal tract. Indeed, increased tissue Hg concentrations and decreased Hg excretion have been reported in germ-free mice and cecum-resected mice after MeHg administration. Thus, intestinal microbiota has important roles in the decomposition of organic Hg and fecal Hg excretion in animals administered MeHg.

Prebiotics, including nondigestible oligosaccharides, change the composition and/or activity of gastrointestinal microbiota, thereby conferring health benefits in hosts. Ingestion of fructooligosaccharides (FOS), typical nondigestible oligosaccharides, increase the numbers of *Lactobacillus*, *Bifidobacterium*, and *Bacteroides* in the intestine. Ingestion of glucomannan (GM), a nondigestible polysaccharide, increases bifidobacteria counts in cecum and feces. In addition, lactobacilli, bacteroides and bifidobacteria have been shown to metabolize MeHg to an inorganic form in vitro. Therefore, daily ingestion of FOS or GM may influence MeHg retention indirectly via bacterial MeHg metabolism in the gut. However, reports on the effect of prebiotics on the retention and excretion of MeHg are rare.

The toxicokinetics of MeHg in humans is closer to that in mice. Here, we investigated the effects of daily feeding of nondigestible saccharides (FOS and GM) on tissue Hg concentrations and intestinal microbiota after MeHg exposure in mice.

**MATERIALS AND METHODS**

**Chemicals** MeHg chloride (MeHgCl) and GM (PROPOL®A) were purchased from Nacalai Tesque (Kyoto, Japan) and Shimizu Chemical Corporation (Hiroshima, Japan), respectively. FOS (Meioligo-P) was provided by Meiji Food Materia Co., Ltd. (Tokyo, Japan). FOS is a mixture of 1-kestose, nystose, and 1F-betafructofuranosylnystose. GM is a polysaccharide composed of glucose and mannose residues linked by β1–4 bonds.

**Animals and Diets** Thirteen BALB/cByJ female mice

---

* To whom correspondence should be addressed. e-mail: MASAAKI_NAGANO@env.go.jp

© 2021 The Pharmaceutical Society of Japan
(aged 3 weeks; CLEA Japan, Inc. Tokyo, Japan) were housed in individual plastic cages and maintained on a 12-h light cycle (07:00 to 19:00) at 25 ± 1°C and humidity of 50 ± 5%. They were fed a basal diet for 1 week, then weighed, and divided into three groups (4 or 5 per group). Diets and tap water were provided ad libitum throughout the experiment. The compositions of the diets are shown in Table S1. The control group was fed a basal diet. The protocol for animal experiments was approved by the Ethics and Safety Committee on Animals at the National Institute for Minamata Disease. All care and the experiment procedures for mice were carried out according to the standards of Ministry of the Environment, Japan (Notice No. 88 of 2006) and the fundamental guidelines of Ministry of Education, Culture, Sports, Science and Technology, Japan (Notice No. 71 of 2006). All efforts were made to minimize animal suffering.

**Exposure to MeHg** Six weeks after feeding, mice were administered a single dose of MeHgCl (4 mg Hg/kg body weight, per os (p.o.)). The dose was based on that described in the reports by Rowland et al. and Uchikawa et al. Mice were housed in metabolic cages (one per cage) for 28 d, and urine and feces were collected every day. Daily food intake was measured for 4 d. Thirty-one days after administration, blood was collected from the heart using a heparinized syringe under isoflurane anesthesia. After perfusion with physiologic (0.9%) saline, the brain, liver, and kidneys were removed for Hg analysis. All samples were stored at −80°C until Hg analysis and DNA extraction from fecal samples.

**Hg Analysis** Total Hg content in each sample was determined by the oxygen combustion–gold amalgamation method using a mercury analyzer (MA3000; Nippon Instruments Co., Tokyo, Japan). Samples for inorganic Hg analysis were prepared and analyzed according to the method described by Yasutake et al.

**DNA Extraction from Fecal Samples and Analysis of Microbial Populations** DNA extraction and analysis of the microbial population on feces 28 d after MeHg administration were carried out by Primary Cell Division of Cosmo Bio Co., Ltd. (Sapporo, Japan). Briefly, DNA was extracted using the QIAamp DNA StoolMini Kit (Qiagen, Venlo, the Netherlands) according to manufacturer instructions. 16S ribosomal RNA (rRNA) gene amplicon libraries were prepared following the 16S Metagenomics Sequencing Library Preparation Protocol from Illumina (San Diego, CA, U.S.A.). Sequencing was undertaken using an Illumina MiSeq sequencer with 2 × 300 cycle MiSeq Reagent Kit v3. Data processing was done using Quantitative Insights into Microbial Ecology (QIIME) v1.8.0. Phylogenetic assignment of each operational taxonomic unit was determined using Greengenes 16S rRNA gene database v13.8. Alpha diversity, beta diversity and principal coordinates analysis (PCoA) plots were also generated using QIIME.

**Statistical Analysis** Data are expressed as the mean ± standard deviation (S.D.). The normality of data distribution was analyzed using the F test or Bartlett’s test. Unless stated otherwise, differences in data between groups were determined using the Student’s t-test or one-way ANOVA with the post hoc Bonferroni test if more than two groups were being compared. p < 0.05 was considered statistically significant.

**RESULTS**

**Growth and Food Intake** In a preliminary experiment, MeHg administration to mice fed a 5% GM diet caused diarrhea. Therefore, 2.5% GM was used in the present study—no groups had diarrhea or drastic change in body weight during the experimental period (Fig. S1). Daily food intake was similar in all three groups (Fig. S2).

**Effects of FOS or GM on Tissue Hg Concentrations after MeHg Administration** FOS-fed mice had lower total Hg concentrations in all tissues than those of control mice (Table 1). In particular, total Hg levels in the brain, liver, and kidneys of FOS-fed mice were lower than those in controls by 20, 26, and 22%, respectively. Dietary GM had no effect on tissue Hg concentrations. No differences in the tissue concentrations of inorganic Hg among groups were found (Table 2).

**Effects of FOS or GM on Hg Excretion after MeHg Administration** During the 27 d after MeHg administration, control mice excreted 19.5 and 18.2% of dosed Hg in feces and urine, respectively. Although no differences in fecal Hg excretion between control and GM groups were observed, fecal Hg excretion in FOS-fed mice was significantly higher than that in controls on day 10 (Fig. 1A). Cumulative Hg amounts in the feces of FOS-fed mice increased thereafter and were approximately 1.5-fold higher than those of controls on day 27. Fecal Hg excretion was highest in the first week after MeHg administration in all groups, and then decreased gradually (Fig. 1B). Fecal Hg excretion at the first week after administration did not differ significantly among diet groups. At the second and third week after administration, fecal Hg excretion was significantly higher in FOS-fed mice, but similar in GM-fed mice, compared with that in controls. In GM-fed mice and control mice, fecal Hg excretion at the second and/or third week after administration was significantly lower than that in controls on day 10 (Fig. 1A).

| Table 1. Total Hg Concentrations in Tissues at 31 d after MeHg Administration in Mice |
|---------------------------------------------------------------|
| **Total Hg concentration (µg/g tissue)**                      |
| Control (n = 5)  | 5% FOS (n = 4)  | 2.5% GM (n = 4)  |
| Blood            | 1.61 ± 0.24    | 1.34 ± 0.18     | 1.66 ± 0.21    |
| Brain            | 1.26 ± 0.10    | 1.01 ± 0.08**   | 1.19 ± 0.09    |
| Liver            | 2.70 ± 0.30    | 2.01 ± 0.30**   | 2.63 ± 0.18    |
| Kidney           | 6.99 ± 0.89    | 5.43 ± 0.81*    | 6.64 ± 0.47    |

Mice were administered a single dose of MeHgCl (4 mg Hg/kg body weight, p.o.). Total Hg contents in the tissues were determined by the oxygen combustion–gold amalgamation method. Data are the mean ± S.D. Significantly different from control (*p < 0.05, **p < 0.01).

| Table 2. Inorganic Hg Concentrations in Tissues at 31 d after MeHg Administration in Mice |
|---------------------------------------------------------------|
| **Inorganic Hg concentration (µg/g tissue)**                  |
| Blood             | ND               | ND               | ND               |
| Brain             | 0.05 ± 0.01      | 0.04 ± 0.01      | 0.04 ± 0.01      |
| Liver             | 0.18 ± 0.03      | 0.17 ± 0.04      | 0.18 ± 0.04      |
| Kidney            | 0.87 ± 0.08      | 0.91 ± 0.06      | 0.84 ± 0.06      |

Inorganic Hg contents in the tissues were determined according to the method of Yasutake et al., ND, not determined. Data are the mean ± S.D.
than that at the first week, whereas no difference was observed in FOS-fed mice. Urinary Hg excretion was similar between control (18.18 ± 1.88%) and FOS-fed mice (18.26 ± 3.51%). Urinary Hg excretion in GM-fed mice (12.69 ± 0.61%) was significantly lower (p < 0.05) than that in controls. However, there was no difference in the sum of fecal and urinary Hg excretions between control (37.65 ± 2.11%) and GM groups (34.37 ± 2.48%), which was consistent with the result of total Hg concentration in tissues.

To elucidate the mechanism of FOS-induced enhancement of fecal Hg excretion, cumulative Hg amounts in feces were analyzed in detail. Cumulative Hg amounts in feces were similar between the two groups on days 1–3, but thereafter were higher in the FOS group than those in the control group (Fig. 2A). In particular, fecal Hg excretion on days 11–14 and 18–21 was increased significantly in FOS-fed mice compared with that in controls. As a representative example, the amounts of inorganic Hg in feces on days 1–3 and 11–14 were measured. Although no significant difference was observed between the groups on days 1–3, the proportion of inorganic Hg to total Hg in feces on days 11–14 was significantly higher in FOS-fed mice than that in controls (Fig. 2B).

Effects of FOS on Fecal Microbiota  We assessed the changes of alpha diversity between control and FOS groups using the Shannon Index (an indicator of diversity) and Chao1 Index (an indicator of species richness). The Shannon Index of the FOS-fed group was lower than that of controls, but the difference was not significant (Fig. S3A). There was no significant difference in the Chao1 Index between the FOS-fed group and controls (Fig. S3B). To examine the shift of the community structure in terms of taxonomic composition, we calculated the beta diversity between the groups using the Bray–Curtis Dissimilarity Index and the weighted UniFrac distance. The PCoA plots of weighed UniFrac distances clearly separated the FOS group from the control group (Fig. 3A), but the Bray–Curtis Dissimilarity Index was similar between the groups (p = 0.109, R = 0.889 by analysis of similarity test). Conversely, weighted metrics demonstrated a different microbial structure in the FOS group compared with that in the control group (Fig. 3B).

Analysis of phylum-level distribution revealed Bacteroidetes and Firmicutes to be the major bacterial phyla in the gut microbiota of the two groups (Fig. 4). Actinobacteria to which Bifidobacterium belongs were not detected in either group. Compared with controls, FOS-fed mice showed an increase in Bacteroidetes (p = 0.065) and a decrease in Firmicutes (p = 0.066) populations. At the genus level (Fig. 5), the FOS-fed mice showed an increase in Bacteroides (p = 0.062) and a decrease in both Lactococcus and Streptococcus (p < 0.05 and p < 0.05, respectively) populations.

The three dominant members in the phylum Bacteroidetes in both groups were Bacteroides, two unknown genera from the family Rikenellaceae, and the candidate family S24-7 (Figs. 5, S4). Bacteroides abundance was enriched in the
FOS group, whereas the relative abundance of *Bacteroides* in the control group was similar to that of the candidate family *S24-7*. Compared with the basal diet, the FOS diet led to higher abundances of *Bacteroides* and the family *Rikenellaceae* (*p* = 0.062 and *p* = 0.065, respectively), and a significantly lower abundance of the candidate family *S24-7* (*p* < 0.05).

**DISCUSSION**

We demonstrated enhanced Hg excretion in feces and decreased Hg accumulation in tissues in FOS-fed BALB/cByJ female mice after a single oral dose of MeHg. Notably, FOS-fed mice had significantly lower Hg concentrations in the brain than those of basal diet-fed mice. Thus, the toxicity of MeHg to the central nervous system might be reduced by dietary administration of FOS.

Demethylation of MeHg in tissues occurs mainly in the liver, and then inorganic Hg produced by this demethylation is transported to the kidneys *via* the circulation.19 We did not find significant differences in tissue concentrations of inorganic Hg among groups. In addition, urinary Hg excretion was similar between control and FOS groups. These results indicate that FOS had no effect on MeHg demethylation in the tissues of mice.

Cumulative amounts of Hg in feces of the FOS group were similar to those in controls until 3 d after MeHg administration but increased gradually thereafter. Therefore, it is unlikely that decreased tissue Hg concentrations in FOS-fed mice were associated with inhibition of MeHg absorption by FOS (including binding with FOS). Indeed, FOS-fed mice had a higher proportion of inorganic Hg in feces than that in controls, with a significant increase in fecal Hg excretion. This result suggests that increased fecal Hg excretion by FOS may be due to MeHg demethylation by intestinal microbiota, because FOS had no effect on MeHg demethylation in the tissues of mice. In comparison with MeHg, inorganic Hg is poorly absorbed from the intestine. Consequently, the demethylation by intestinal microbiota, which converts MeHg to inorganic Hg, increases Hg excretion and decreases tissue Hg concentrations.7,8) These reports suggest that this demethylation reduces the amount of absorbed MeHg from the intestine because MeHg in the intestinal content is used as a substrate. Those reports and our results suggest that FOS accelerated MeHg demethylation by intestinal microbiota in mice, and that FOS-accelerated MeHg demethylation led to enhanced fecal
Hg excretion and to reduced MeHg absorption and tissue Hg concentrations. However, the hypothesis that FOS accelerates MeHg demethylation by intestinal microbiota should be investigated using the feces or intestinal contents of FOS-fed mice under anaerobic conditions.

Non-digestible saccharides, such as FOS and GM cannot be degraded by digestive enzymes and are utilized by intestinal bacteria in the large intestine.20) Thus, they modulate the composition of intestinal microbiota. PCoA of the weighted UniFrac distance revealed significantly distinct fecal microbial communities (beta diversity) between control and 5% FOS-supplemented mice. It has been reported that male C57BL/6J mice fed a 10% FOS diet21) or male BALB/c mice fed a 3% mixture of galactooligosaccharides and FOS diet22) showed lower species richness compared with that of control mice, as well as different beta diversity. In our study, the FOS group showed no significant difference in species richness compared with that in controls. However, the FOS group showed a decreasing trend in microbial diversity, indicating that FOS led to increases in certain phylogenetic lineages in intestinal microbiota. In addition, non-digestible saccharides have been reported to increase the densities of cecal and fecal saccharolytic bacteria, such as bifidobacteria, bacteroides and lactobacilli, in animals12–14) and humans.10,11) Unlike those reports, Hosono23) mentioned that Bifidobacterium was rarely detected in the cecal microbiota analysis of mice orally administered FOS (or their components), while the Bacteroides abundance changed most prominently. Nakamichi et al.24) showed that the number of 16S rRNA genes of Bacteroides was increased significantly in the feces of female BALB/c mice fed 7.5% FOS (or their components). Similarly, we observed that the relative abundance of Bacteroides in the feces of FOS-fed mice was higher than that in controls, but Bifidobacterium was not detected. These results indicate that Bifidobacterium was not involved in MeHg demethylation in the large intestine of mice used in the present study.

Bacteroides is one of the most predominant genera of bacteria in the intestine of humans or mice. The mammalian gut is sterile at birth, but the abundance of strict anaerobes such as Bacteroides spp. increases with weaning, which coincides with the time of acquisition of the demethylating activity of MeHg.25) In addition, some Bacteroides spp. are known to metabolize MeHg to inorganic Hg in vitro.26) Hence, Bacteroides may have been involved in MeHg demethylation in the large intestine in the present study. Conversely, Bacteroides spp. have also been reported to metabolize FOS and their components in vitro.27) Therefore, further studies are needed on the metabolism of MeHg by intestinal bacteria. This study may have been limited by its small sample size. However, this is the first report to describe the effect of FOS on tissue Hg concentrations and intestinal microbiota after MeHg exposure in mice. Taken together, FOS enhanced fecal Hg excretion and reduced MeHg absorption, possibly by accelerating MeHg demethylation by bacteria in the large intestine after MeHg exposure. Consequently, FOS decreased tissue Hg concentrations, including those in the brain. Therefore, FOS could reduce the neurotoxic effects of MeHg. These results also suggest that other nondigestible oligosaccharides may have the same effects as those of FOS.

Acknowledgments We are grateful to Noriko Tanaka, Yumi Hirasaki and Kyoko Mentani for assistance in the laboratory.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

REFERENCES

1) National Research Council (U.S.) Committee on the Toxicological Effects of Methylmercury. Toxicological Effects of Methylmercury. National Academies Press (U.S.), Washington DC, pp. 1–344 (2000).
2) Sakamoto M, Kakita A, Wakabayashi K, Takahashi H, Nakano A, Akagi H. Evaluation of changes in methylmercury accumulation in the developing rat brain and its effects: a study with consecutive and moderate dose exposure throughout gestation and lactation periods. Brain Res., 949, 51–59 (2002).
3) Norseth T, Clarkson TW. Intestinal transport of [203]Hg-labeled methyl mercury chloride. Role of biotransformation in rats. Arch. Environ. Health, 22, 588–577 (1971).
4) Rowland IR, Davies MJ, Grasso P. Metabolism of methylmercuric chloride by gastro-intestinal flora of the rat. Xenobiotica, 8, 37–43 (1978).
5) Ishihara N, Suzuki T. Biotransformation of methylmercury in vitro. Tohoku J. Exp. Med., 120, 361–363 (1976).
6) Yasutake A, Hirayama K. Evaluation of methylmercury biotransformation using rat liver slices. Arch. Toxicol., 75, 400–406 (2001).
7) Nakamura I, Hosokawa K, Tamura H, Miura T. Reduced mercury excretion with feces in germfree mice after oral administration of methyl mercury chloride. Bull. Environ. Contam. Toxicol., 17, 528–533 (1977).
8) Seko Y, Miura T, Takahashi M. Reduced decomposition and faecal excretion of methyl mercury in caecum-resected mice. Acta Pharmacol. Toxicol. (Copenh), 50, 117–120 (1982).
9) Seko Y, Takahashi M, Hasegawa T, Miura T. Intestinal absorption of mercury in vitro from intestinal contents of methylmercury administered mice. J. Health Sci., 47, 508–511 (2001).
10) Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr., 125, 1401–1412 (1995).
11) Hidak A, Tashiro Y, Eida T. Proliferation of bifidobacteria by oligosaccharides and their useful effect on human health. Bifidobacteria and Microflora, 10, 65–79 (1991).
12) Jinno S, Nakamura Y, Nagata M, Takahashi T. 1-Kestose consumption during pregnancy and lactation increases the levels of IgA in the milk of lactating mice. Biosci. Biotechnol. Biochem., 78, 564–566 (2014).
13) Chen HL, Fan YH, Chen ME, Chan Y. Unhydrolyzed and hydrolyzed Konjac glucomannans modulated cecal and fecal microbiota in Balbc mice. Nutrition, 21, 1059–1064 (2005).
14) Yeh SL, Lin MS, Chen HJ. Inhibitory effects of a soluble dietary fiber from Amorphophallus konjac on cytotoxicity and DNA damage induced by fecal water in Caco-2 cells. Planta Med., 73, 1384–1388 (2007).
15) Nielsen JB, Andersen O. Methyl mercury chloride toxicokinetics in mice. I: effects of strain, sex, route of administration and dose. Pharmacol. Toxicol., 68, 201–207 (1991).
16) Rowland IR, Mallett AK, Flynn J, Hargreaves RJ. The effect of various dietary fibres on tissue concentration and chemical form of mercury after methylmercury exposure in mice. Arch. Toxicol., 59, 94–98 (1986).
17) Uchikawa T, Yasutake A, Kumamoto Y, Maruyama I, Kumamoto
S, Ando Y. The influence of *Parachlorella beyerinckii* CK-5 on the absorption and excretion of methylmercury in mice. *J. Toxicol. Sci.*, **35**, 101–105 (2010).

18) Yasutake A, Nagano M, Nakano A. Simple method for methylmercury estimation in biological samples using atomic absorption spectroscopy. *J. Health Sci.*, **51**, 220–223 (2005).

19) Hirayama K, Yasutake A. Effects of reactive oxygen modulators on *in vivo* demethylation of methylmercury. *J. Health Sci.*, **45**, 24–27 (1999).

20) Nakamura S, Kondo N, Yamaguchi Y, Hashiguchi M, Tanabe K, Ushiroda C, Kawahashi-Tokuhisa M, Yui K, Miyakoda M, Oku T. Daily feeding of fructooligosaccharide or glucomannan delays onset of senescence in SAMP8 mice. *Gastroenterol. Res. Pract.*, **2014**, 303184 (2014).

21) Liu TW, Cephas KD, Holscher HD, Kerr KR, Mangian HF, Tappenden KA, Swanson KS. Nondigestible fructans alter gastrointestinal barrier function, gene expression, histomorphology, and the microbiota profiles of diet-induced obese C57BL/6J mice. *J. Nutr.*, **146**, 949–956 (2016).

22) Szklany K, Wopereis H, de Waard C, van Wageningen T, An R, van Limpt K, Knol J, Garssen J, Knippels LMJ, Belzer C, Kraneveld AD. Supplementation of dietary non-digestible oligosaccharides from birth onwards improve social and reduce anxiety-like behavior in male BALB/c mice. *Nutr. Neurosci.*, **23**, 896–910 (2020).

23) Hosono A. Immunomodulation by *Bacteroides* species. *Journal of Intestinal Microbiology.*, **27**, 203–209 (2013).

24) Nakanishi Y, Murashima K, Ohara H, Suzuki T, Hayashi H, Sakamoto M, Fukasawa T, Kubota H, Hosono A, Kono T, Kamiyogawa S, Benno Y. Increase in terminal restriction fragments of *Bacteroidetes*-derived 16S rRNA genes after administration of short-chain fructooligosaccharides. *Appl. Environ. Microbiol.*, **72**, 6271–6276 (2006).

25) Rowland IR. Factors affecting metabolic activity of the intestinal microflora. *Drug Metab. Rev.*, **19**, 243–261 (1988).

26) Endo H, Tamura K, Fukasawa T, Kanegae M, Koga J. Comparison of fructooligosaccharide utilization by *Lactobacillus* and *Bacteroides* species. * Biosci. Biotechnol. Biochem.*, **76**, 176–179 (2012).