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Inhibitory effects of soybean oligosaccharides and water-soluble soybean fibre on formation of putrefactive compounds from soy protein by gut microbiota

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ABSTRACT

Soybeans are part of the traditional food consumed in Asia countries. In this study, we investigated inhibitory effects of soybean oligosaccharides and water-soluble soybean fibre (Soyafibe) on putrefactive compounds from soy protein by gut microbiota in rats. Caecal microbial fermentation products and microbiota in rats fed 20% soy protein (SP-1) and whole soybean flour (SFL: protein content was 20%) diets were determined. The caecal environment in rats fed 20% soy protein without dietary fibre (SP-2) or with 2% Soyafibe (SFB) was also determined. Compared to SP-1 and SP-2 group, low indole content with high lactic acid was shown in SFL and SFB group, respectively. Using the 16S rRNA genes polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and pyrosequencing. *Prevotella*, Gram-negative anaerobic rods, were detected as dominant in both SFL and SFB groups. Our findings indicated that fermentable polysaccharides in soybeans have inhibitory effects on the formation of putrefactive compounds generated from soy protein by the microbiota.

1. Introduction

The human gut (large intestine) contains 13–14 log bacteria cells that belong to about 1000 species or strains they plays an important role in host health [1]. It is regarded that gut microbiota have beneficial and also harmful effects. Some groups of the residential bacteria, some groups prevent inflammation and stimulate immune responses [2]. On the other hand, protein residues in gut can be converted to putrefactive compounds, such as ammonia, amines, phenols and indoles, by a part of microbiota [3,4]. These putrefactive compounds are considered as putative tumour factors [5,6]. Various factors such as age, stress, climate, pathogens, drugs, and also diet affect the composition of gut microbiota [7]. Furthermore, composition of diet and gut microbiota are dependent on the geographical location and cultural background [8,9].

Soybeans are important protein source in the world. In the case of Japan, soybeans are used in various traditional fermented food products such as soy sauce, *miso,* and *natto*, and processed foods such as *tofu*. Soy protein and peptide are known as functional and beneficial food materials for health. For example, there are reports about their immune activity [10], anti-inflammation activity [11], and improvement of clinical indices in Type 2 diabetes [12]. However, it was shown that the indole content in the gut content of Wistar rats fed soy protein was greater than that in rats fed milk casein [3]. Soy protein is rich in tryptophan, and tryptophan is converted to indoles by the microbiota.

Soybean contains water-soluble dietary fibre composed of galactose, arabinose, galacturonic acid, xylose, fructose, and rhamnose [13], in oligosaccharides, such as raffinose and stachyose [14]. Not only the oligosaccharides, but also the dietary fibre, can be fermented by gut microbiota [15]. We reported that fermentable dietary fibres in brown algae, alginic acid, and laminaran, both viscous and not viscous, could inhibit the putrefactive compounds generated by the gut microbiota by increasing the organic acids [16]. Therefore, it is thought that the fermentable carbohydrates in soybeans affect putrefactive compounds production from soy-protein in the gut, when whole soybeans were administered.

In this study, the inhibitory effects of raffinose, stachyose, and water-soluble dietary fibre from soybeans on the putrefactive compound production by human faecal microbiota in a broth containing soy-protein were examined. The concentration of organic acids and putrefactive compounds, and microbiota in the caecum of rats fed soy-protein, soy-flour (whole soybeans), or the soy-fibre was also determined.

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

Exp. 1			Exp. 2		
	SP-1	SFL		SP-2	SFB
Sucrose	50.0	38.0	Sucrose	50.0	50.0
Soy protein	20.0		Soy protein	20.0	20.0
Soybean flour		41.5			
Corn starch	15.0	6.5	Corn starch	20.0	18.0
Cellulose powder	5.0	4.0	Soy fibre		2.0
Soybean oil	5.0		Corn oil	5.0	5.0
Mineral mix (AIN-76)	3.5	3.5	Mineral mix (AIN-76)	3.5	3.5
Vitamin mix (AIN-76)	1.0	1.0	Vitamin mix (AIN-76)	1.0	1.0
DL-Methionine	0.3	0.3	DL-Methionine	0.3	0.3
Choline bitartrate	0.2	0.2	Choline bitartrate	0.2	0.2

2. Materials and methods

2.1. Carbohydrates

The water soluble soy-fibre (Soyafibe-S-DA100) was kindly obtained from Fuji-Oil (Izumisano, Japan). According to European Commission of Health and Consumer Protection Directorate-General [17], composition of crude protein, crude ash and soluble fibre in the Soyafibe are 6.2, 8.4 and 75.7%, respectively. The Soyafibe is composed of various sugars, such as galactose (43.6%), arabinose (24.8%), galacturonic acid (18.3%), rhamnose (5.2%), xylose (4.1%), fucose (2.5%) and glucose (1.0%). There are three main hemicellulose at masses around 550, 25 and 5 kDa and its viscosity is 62 mPa.s. Raffinose and stachyose were purchased from Wako Pure Chemical (Osaka, Japan).

2.2. In vitro *experiment*

In vitro study was carried out as described previously [6]. Briefly, fresh faecal sample from normal adults male was diluted with nine times volume of a quarter concentration Gifu Anaerobic Medium (GAM 1/4) [4]. The dilutions (0.05 ml) were inoculated in 1 ml of GAM 1/4 containing 30 mg/ml soy-protein (Fuji-Pro-F, Fuji Oil, Izumisano, Japan) with or without 10 mg/ml raffinose, stachyose or Soyafibe ($n = 3$). After 0, 12, 24, and 48 h of incubation at 37 °C under anaerobic condition using the AnaeroPack system (Mitsubishi Gas Chemical, Tokyo, Japan), the pH value was determined with a pH electrode (twin pH, B-211, Horiba, Kyoto, Japan). Ammonia and phenol contents were determined using reagent sets for water analysis (Nos. 7, and 53, respectively, Kyoritsu Chemical-Check, Tokyo, Japan) with a grating microplate reader (SH-1000 Lab; Corona Electric, Ibaraki, Japan). Indole content was determined by a colorimetric assay with Kovac's reagent [4,6].

2.3. Animal care

The animal experiments were performed in compliance with the relevant fundamental guidelines specified by the Ministry of Education, Culture, Sports, Science and Technology in Japan (MEXT), and were approved by the animal experiment committee of Tokyo University of Marine Science and Technology (approval no. H27-4).

In experiment 1 (Exp. 1), four week-old male Wistar rats (Tokyo Laboratory Animals Science, Tokyo, Japan) were housed separately in metal-wire cages and allowed free access to water and food. After acclimation with AIN-76 based diet [16] for 7 days, the animals were divided into two groups ($n = 6$) and provided an experimental diet containing 20% (w/w) of soy-protein (SP-1 in Table 1) or 41.5% (w/w) of soybean flour (SFL) diet for 14 days.

In the case of Exp. 2, after 7 days of acclimation, the animals were divided into two groups ($n = 6$) and provided an experimental diet containing 20% (w/w) of soy-protein without dietary fibre (SP-2) or with 2.0% (w/w) of Soyafibe (SFB) for 14 days. Protein, carbohydrate, and lipid concentration were the same in all of the test diets.

Then, the rats were exsanguinated from the abdominal aorta under anaesthesia with diethyl ether. The organs (liver, kidneys, spleen, thymus, and caecum) were excised and weighed. A portion of the caecal content was used for direct total cell counts by using the Gram staining method [18]. The remaining portion was stored at −80 °C for use in subsequent experiment. Levels of caecal organic acids (lactic acid, acetic acid, propionic acid, and *n*-butyric acid) were determined by high-performance liquid chromatographic (HPLC) methods as described previously [16]. The other caecal metabolites: ammonia, indole and phenols, were determined as described above.

2.4. DGGE analysis

Bacterial DNA extraction and denaturing gradient gel electrophoresis (DGGE) analysis were performed as described previously [3,19]. DNA in caecal contents was extracted using NuCleo Spin Tissue (Takara Bio, Shiga, Japan). For DGGE analysis, primer pairs for the amplification of the V3 region (~220 bp) of the 16S rRNA gene: forward primer with GC clamp GC-399f (5′-CGC CCG CCG CCC GCG CCC GTC CCG CCG CCC CCG CCC GCT CCT ACG GGA GGC ASC AG-3′) and reverse primer V-53r (5′-GTA TTA CCG CGG CTG CTG G-3′) were used. The main 13–15 DGGE bands in each diet group were recovered with ligation, transformation, and sequencing [19]. The sequencing was identified using DNA Data Bank of Japan (DDBJ) database, using the BLASTN program.

2.5. Pyrosequencing

The pyrosequencing of bacterial DNA in caecum was performed as described previously [3,4]. Briefly, the V1–V2 region of the 16S rRNA gene was amplified using bar-coded primers targeting 27–338 bp. The forward primer was 5′-cgtatcgcctccctcgcgccatcag NNNNNNNNNN AGAGTTTGATCCTGGCTCAG-3, where the sequencing of the A adapter is shown in small letters and N···N represents a 10-bp barcode that is unique for each sample [20]. The reveres primer was 5′-ctatgcgccttgccagcccgctcag TGCTGCCTCCCGTAGGAGT-3′, where the sequence of the A adapter is shown in lowercase letters. Pyrosequencing was performed using the Genome Sequencer Junior System (Roche, CT, USA). The pooled DNA samples were adapter ligated with beads and amplified by emulsion PCR using a GS junior Titanium emPCR kit (Lib-A; Roche, CT, USA).

The sequences were analysed with QIIME 1.5.0 pipeline [21], compared with BLAST search, aligned with PYNAST and clustered under 100% sequence identity using UCLUST [22]. The analysed sequences were classified into operational taxonomic units (OTUs) with a 97% threshold identity using UCLUST. The taxonomy of each OTU representative sequence was assigned using the Ribosomal