Physical, Chemical and Physiological Properties of Difructose Anhydride III Produced from Inulin by Enzymatic Reaction

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Abstract: We produced high purity difructose anhydride III (DFA III; α -D-fructofuranose- β -D-fructofuranose 1,2' : 3',2 dianhydride) crystals from the crude inulin extracted from chicory with inulin fructotransferase (depolymerizing) induced by H65-7 strain of *Arthrobacter* sp. The physical, chemical, and physiological properties of DFA III have been investigated in this report. The degradability and Maillard reactivity of DFA III in the low pH solutions were much lower than those of sucrose. DFA III showed no utilization by *Streptococcus mutans* and *Bifidobacterium* *in vitro*. Digestion test with rat intestinal acetone powder and absorption test with everted sacs of rat small intestine showed that DFA III had low digestibility and absorbability *in vitro*. Diet containing 2% (w/w) DFA III increased the cecal short chain fatty acids and induced a shift of the cecal microflora in rats. On the other hand, a daily oral ingestion of 5 g DFA III by healthy adults showed no influence on fecal microflora.

Key words: difructose anhydride III, oligosaccharides, inulin, prebiotics, intestinal microflora

Chicory (*Cichorium intybus* L.) is a perennial plant and is widely cultivated in Europe. Its leaves are used in salads, and its roots are used as the raw material for a coffee substitute. Chicory roots also contain a large amount of inulin that is a linear β -2,1-linked fructan with sucrose residue. Inulin has been utilized as a water-soluble dietary fiber and a substitute for fat.

Producing oligosaccharides from inulin by hydrolyzation has been attempted. For example, fructooligosaccharide has been produced from inulin by hydrolyzation. The enzymes producing difructose anhydride III (DFA III, Fig. 1), inulin fructotransferase (depolymerizing), have also been detected in several kinds of microorganisms.^{1–3)} High purity DFA III crystals were efficiently produced from the crude inulin extracted from chicory by enzymatic reaction (Kikuchi, H. *et al.*, unpublished data). Inulin fructotransferase (depolymerizing) induced by the H65-7 strain of *Arthrobacter* sp.⁴⁾ was used for the production of DFA III.

Oligosaccharides are known to have functions that favorably influence the indigenous intestinal microflora in terms of microbial balance, and thereby, help in improving our health.⁵⁾ This concept is called “prebiotics.” Stimulation of calcium absorption in the large intestine is also

one of the notable functions of oligosaccharides.^{6,7)} Though reports suggested that DFA III efficiently stimulates calcium absorption not only in the large intestine but also in the small intestine,^{8,9)} there are no reports to confirm the properties of DFA III in detail. In this paper, we report the physical, chemical, and physiological properties of DFA III.

MATERIALS AND METHODS

Chemicals. Chicory that contains a large amount of inulin in its root was cultivated on a farm located in Hokkaido, Japan. Inulin was extracted from the harvested chicory roots with hot water. The carbonation juice was obtained from the extract by refining it with the standard liming and carbonation technology of beet sugar factories. DFA III was prepared from the carbonation juice containing 15 kg of inulin with 110,000 U of inulin fructotransferase (depolymerizing) that was induced by *Arthrobacter* sp. H65-7 at 58°C for 24 h. The amount of DFA III was 9.8 kg in the reaction mixture. The mixture was refined with yeast fermentation and ion exchange resin. DFA III crystals (3.0 kg) were crystallized from the refined solution. The purity of the DFA III crystals was 99.7%. The purities of sucrose and raffinose (Nippon Beet Sugar Mfg. Co., Ltd., Tokyo, Japan) were 99.9 and 99.5% respectively. Sorbitol, palatinose, xylitol and fructooligosaccharide were purchased from Wako Pure Chemical Industry (Osaka, Japan).

Animals and diets. Male Sprague-Dawley (SD) rats purchased from Japan SLC (Shizuoka, Japan) were used in the everted sac experiment and animal experiment. The rats were individually housed in stainless steel cages with mesh bottoms at 22±2°C under a 12 : 12-h light: dark

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 Abbreviations: DFA III, difructose anhydride III; PBS, phosphate buffer saline.

Fig. 1. The structural formula and crystals of difructose anhydride III.

photoperiod, and were allowed free access to the basal or experimental diets and tap water. The purified basal diet was composed of 23% (w/w) casein, 61.4% corn starch, 5% cellulose, 5% corn oil, 4% mineral mixture (mixture 2¹⁰), 1% vitamin mixture (AIN-76¹¹), 0.3% DL-methionine, 0.2% choline chloride and 0.1% vitamin E. Experimental diets were prepared by replacing corn starch from the basal diet by adding DFA III at 2.0% (w/w) or raffinose at 5.0%. This study was approved by the Hokkaido University Animal Committee, and animals were maintained in accordance with the Hokkaido University guidelines for the care and use of laboratory animals.

Determination of saccharides. Saccharides were determined by high performance liquid chromatography (HPLC) with a refractive index detector (eluent, water; flow rate, 0.5–1.0 mL/min; column temperature, 80°C; columns, SUGAR KS-801 or SP0810, 8 × 300 mm, Showa Denko K.K., Tokyo, Japan).

Investigation of physical and chemical properties.

(1) **Moisture.** Moisture of DFA III was calculated from the change in weight during heating at 105°C for 5 h.

(2) **Sweetness.** The subjects chose a solution from the sucrose solutions of variable concentrations, which was equivalent to the sweetness of 12% (w/v) solution and 16% (w/v) solution of DFA III, respectively. The temperature of the solutions was maintained at 20°C. For example, if the sweetness of 6% (w/v) solution of sucrose is equivalent to the sweetness of 12% (w/v) solution of DFA III, the sweetness of DFA III will be evaluated as 50%.

(3) **Melting point.** The melting point was measured by a melting point instrument (FP62, Mettler-Toledo International Inc., Greifensee, Switzerland).

(4) **Optical rotation.** The optical rotation was measured by an automatic sugar polarimeter (Sucromat, Dr. Wolfgang Kernchen GmbH, Seelze, Germany).

(5) **Solubility.** The suspension of DFA III or sucrose was stirred at various temperatures over 1 h. The concentration (g/100 g water) of supernatant (saturated solution) was determined by a Brix scale (RX-1000, Atago Co., Ltd., Tokyo, Japan).

(6) **Viscosity.** Viscosity of the solution containing DFA III or sucrose at 40% (w/w) was measured by a viscometer at 24–70°C.

(7) **Hygroscopicity.** DFA III and sucrose were placed under relative humidity of 75 or 94%. The moisture of each saccharide was calculated from the change in weight at a definite time. Humidity for each saccharide sample was obtained from a closed glass case containing the satu-

rated sodium chloride solution (relative humidity, 75%) or the saturated potassium nitrate solution (relative humidity, 94%), which were incubated at 20°C.

(8) Degradability under acidic and heated conditions.

Hydrochloric acid was used to adjust the pH of 1% (w/v) DFA III or sucrose solution from 1 to 5. The solutions were incubated at 100°C for 30 min. Undegraded saccharides were determined by HPLC.

(9) Degradability under acidic conditions for a long term. Citric buffer solution (100 mM, pH 3) containing 12% (w/v) DFA III or sucrose was filtrated, and then incubated at 37°C for 3 months. Undegraded saccharides were determined by HPLC.

(10) Maillard reactivity. The Sørensen phosphate buffer solution (200 mM, pH 3.3–7.4) containing 10% (w/v) DFA III or sucrose and 1% (w/v) glutamic acid was incubated at 120°C for 2 h. The coloring by the Maillard reaction was evaluated from the absorption value at 420 nm.

Investigation of physiological properties.

(1) **Cariogenic test.** One loopful of the *Streptococcus mutans* 6715 or MT8148 was inoculated to Brain Heart Infusion broth (BHI, Difco Laboratories, Detroit Michigan, USA), and subsequently cultivated at 37°C for 20 h. The culture broth was centrifuged, and the cells were washed twice with 5 mM phosphate buffered saline (PBS, pH 7.0) and suspended with PBS. Two milliliters of the suspension was added to 10 mL of 5% saccharide (DFA III, sorbitol, palatinose, xylitol, or sucrose) solutions, and the pH of the solution was measured in due course.

(2) **Digestion test.** The digestibility of DFA III was evaluated using the digestible enzyme of rat intestine as follows: Physiological saline containing 100 mg rat intestinal acetone powder (Sigma Chemical Company, St. Louis, MO, USA) per mL was used as the enzyme solution. Maleate buffer (25 mM, pH 6.0) containing 5 mg test saccharide per mL and 0.5 mL enzyme solution per mL was incubated at 37°C for 1 h. This solution was then heated in boiling water for 10 min to stop the enzyme reaction. Concentrations of fructose and glucose in this solution were determined using commercial kits (fructose; F-kit, Roche Diagnostics K.K., Tokyo, Japan, glucose; GL-5 kainos, Kainos Laboratories Inc., Tokyo, Japan).

The DFA III digestion ratio (%) is given by $100 \times [((\text{fructose in the solution after reaction}) - (\text{fructose in the solution before reaction})) / (\text{DFA III in the solution before reaction})]$.

The sucrose digestion ratio (%) is given by $100 \times 2 \times [((\text{glucose in the solution after reaction}) - (\text{glucose in the solution before reaction})) / (\text{sucrose in the solution be-})]$

fore reaction)].

(3) Absorption test with everted sacs. The intestinal segments of 40 cm dissected from the upper portion of the small intestines of rats (7 weeks old) were everted. The 4-cm segments dissected from the everted segments, were ligated with surgical silk at one end. The other end of each sac was ligated immediately after the instillation of 1 mL of serosal fluid, and the sacs were transferred to individual flasks containing 5 mL of mucosal fluid. After replacing the air in the flasks with oxygen gas, each flask was incubated for 30 min at 37°C with continuous shaking. The serosal fluid consisted of a 100 mM phosphate buffer (pH 7.4), 120 mM NaCl, 3.4 mM KCl, 1.2 mM KH_2PO_4 , 1.2 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.6 mM CaCl_2 . The mucosal fluid consisted of DFA III or sucrose (5 mM) in the serosal fluid. Amount of each saccharide in the mucosal fluids before and after incubation was determined by HPLC.

Absorption coefficient was given by ((total saccharides in the mucosal fluid before incubation) – (total saccharides in the mucosal fluid after incubation).)/(incubation time) × (length of segment)).

(4) In vitro utilization of saccharide by *Bifidobacterium*. In vitro utilization of DFA III, glucose, and raffinose by *Bifidobacterium* was evaluated by a method described previously.¹²⁾ Briefly, a test organism suspension was inoculated into peptone yeast extract Fildes (PYF) broth containing test saccharide at 0.5% (w/v). The inoculated media were incubated anaerobically at 37°C for 48 h by the steel wool method. The degree of utilization was determined by the amount of decrease in pH of the medium. Five strains of *Bifidobacterium* (*B. adolescentis* JCM 1275, *B. longum* JCM 1217, *B. breve* JCM 1192, *B. pseudocatenulatum* JCM 1200 and *B. catenulatum* JCM 1194) were used in this test.

(5) Bacteriological analyses of intestinal and fecal microflora. Bacteriological analyses of the cecal contents in rats or the feces in humans were carried out according to Mitsuoka's method.¹³⁾ Briefly, the fresh samples were diluted in 10-fold steps with anaerobic phosphate buffer. Each dilution (0.05 mL) was inoculated onto three non-selective media (BL, EG and TS agar) and eight selective media (BS, ES, NBGT, mLBS, NN, VS, DHL, TATAC agar; the underlined culture media were used only for human feces). Anaerobic incubation was carried out at 37°C for 48 h by the steel wool method, and the aerobic incubation was carried out at 37°C for 24–48 h. Identification of the bacterial genus was performed with colonial and cellular morphologies, Gram-stainability, spore formation, and aerobic growth.

(6) Animal experiment. Five-week-old rats with the basal diet for a week as an acclimation period were maintained, and then allocated to three groups (n 6 per group) according to body weight. Each group was fed on the basal diet (Control group) or experimental diets (DFA III or raffinose group) for four weeks. Thereafter, the rats were killed, and the ceca were removed for analysis of microflora, organic acids, and pH in the contents. The organic acids and pH were analyzed according to the methods described previously.¹⁴⁾

(7) Administration of DFA III to humans. Seven healthy adults, 27 ± 3 years old, were served DFA III or

raffinose at 5 g/day for two weeks. The feces were collected twice before administration, during administration of DFA III, during the wash-out period, during administration of raffinose, and after administration, respectively. Each feces sample was immediately subjected to analysis of microflora. Analyses of pH and water contents of the feces were carried out according to the methods described previously.¹⁴⁾ The subjects consumed the normal free-choice diet. None of them were given antibiotic treatment, other therapies, food preparations with live microorganism, other bifidus factors, or fermented soybeans. This study has been conducted in accordance with the Helsinki Declaration of 1975.

Statistical analysis. Results are expressed as mean values along with their standard deviations. In the animal experiment, the significance of differences between groups was evaluated by Scheffe's *F*-test. In the clinical experiment, effect of DFA III on humans was examined by one-way ANOVA followed by Tukey's test for multiple comparisons. The frequency of occurrence of intestinal microbes was statistically analyzed with Fisher's exact probability test.

RESULTS AND DISCUSSION

Physical, chemical and physiological properties of DFA III were investigated mainly in order to evaluate its usefulness in processed foods. The structure of DFA III crystals is shown in Fig. 1. These crystals had no water molecules. The melting point was 163.7°C (authentic; 162°C¹⁵⁾). The optical rotation was +134.5° (authentic; +135.64°¹⁵⁾). The solubility (Fig. 2(A)) of DFA III was 90–95% of sucrose. This shows that it is easy to dissolve DFA III. The viscosity of 40% (w/w) DFA III solution was lower than that of sucrose below 50°C (DFA III, 3.91 cP; sucrose, 4.75 cP at 24°C). However, the viscosity of DFA III was almost equal to that of sucrose below 70°C (DFA III, 1.92 cP; sucrose, 1.91 cP at 70°C). The hygroscopicity of DFA III was 28–40% of that of sucrose under a relative humidity of 94% for 28 days (Fig. 2(B)). Moreover, under a relative humidity of 75%, DFA III did not absorb moisture. This property of DFA III suggests that DFA III is suitable for processed foods, such as dry foods, which don't tolerate moisture. DFA III showed much lower degradability at 100°C for 30 min at pH 2 as compared to sucrose (Fig. 2(C)). DFA III was almost undegraded at 37°C and pH 3 after 3 months in comparison with sucrose (no degradation ratio: DFA III 98%, sucrose 9%). Figure 2(D) shows the result of a test conducted on heat coloring in the presence of amino acid. DFA III underwent much less coloring through Maillard reaction than sucrose. The low degradability and Maillard reactivity of DFA III in the low pH solutions suggest that DFA III is a more stable oligosaccharide than sucrose under various conditions. The high stability under acidic conditions suggests that DFA III can be added to acidic beverages, such as fruit juices. DFA III is also suitable for processed foods that don't accept coloring. DFA III has 52% of the sweetness of sucrose, and its quality of sweetness was similar to that of fructose. In the case of using DFA III as a functional food material (e.g., stimulation of calcium ab-

Fig. 2. The physico-chemical properties of DFA III and sucrose.

(A) Solubility, (B) Hygroscopicity, (C) Degradability under acidic and heated conditions, (D) Maillard reactivity. ●, DFA III; □, sucrose.

sorption), this low sweetness suggests that a sufficient amount of DFA III can be added to functional foods, such as supplements, without changing the taste as compared with sucrose.

The results of the cariogenic test with *Streptococcus mutans* MT8148 is given in Fig. 3. *S. mutans* MT8148 did not produce acid from DFA III. The same result was obtained with *S. mutans* 6715. These results show that the cariogenicity of DFA III is low. In comparison to sucrose, DFA III was not degraded by the digestible enzyme of rat intestine *in vitro* (digestion ratio: DFA III below 2%, sucrose 64%). The absorption ratio of DFA III was below $2.0 \mu\text{g}/\text{cm}\cdot\text{min}$ and was much lower than that of sucrose, $14.2 \pm 2.1 \mu\text{g}/\text{cm}\cdot\text{min}$. These results show that DFA III

Fig. 3. The acid production from saccharides by *Streptococcus mutans* MT8148.

The suspension of *Streptococcus mutans* with PBS was added to 5% saccharide aqueous solution, and the pH of the solution was measured over the time course. ○, blank; ●, DFA III; △, sorbitol; ▲, palatinose; □, xylitol; ■, sucrose.

is an indigestible oligosaccharide, similar to many other oligosaccharides.⁵⁾ Glucose and raffinose were utilized by all strains of tested *Bifidobacterium* (*B. adolescentis*, *B. longum*, *B. breve*, *B. pseudocatenulatum* and *B. catenulatum*). The difference in pH between the test and control groups was larger than one. However, DFA III was not utilized by the *Bifidobacterium*. The difference in pH between the test and control was smaller than 0.5.

Basal or oligosaccharide diets containing DFA III or raffinose were fed to the rats for four weeks. No statistical differences were observed in terms of body weight gain ($p=0.64$), feed intake ($p=0.51$) or feed efficiency ($p=0.80$) between the control and oligosaccharide groups (Table 1), and no case of diarrhea were observed in any rats. The weights and the pHs of the cecal contents, and the amount of the cecal organic acids are also shown in Table 1. The weights of the cecal contents of the oligosaccharide groups were significantly increased, and their pHs were significantly decreased as compared with that of the control group. The amount of cecal organic acids of the oligosaccharide groups increased remarkably compared with that of the control group. In particular, the DFA III group showed a higher proportion of acetic acid (acetic acid: propionic acid: butyric acid=13:2:1 vs. control group, 7:2:1, raffinose group, 9:2:1). These results indicate the possibility that DFA III has a fermentative property because of the presence of intestinal bacteria of rats. The counts of Bacteroidaceae, Enterobacteriaceae and Streptococcaceae in the ceca of the oligosaccharide groups significantly decreased in comparison with that of the control group (Table 2). The increase in the number of facultative aerobes, such as Enterobacteriaceae and Streptococcaceae, is reported as a feature of the abnormal flora that is observed in immunodeficiency, surgical operation, aging, etc.¹⁶⁾ It is expected that DFA III and raffinose contribute to the prevention of the abnormal flora. Raffinose diets induce a significant increase in the number of *Bifidobacterium* in rats; however, DFA III diets do not affect the *Bifidobacterium* count. This result is in agreement with *in vitro* carbohydrate utilization tests performed us-

Table 1. Effect of oligosaccharides on the body weight gain, feed intake, cecal content weight, cecal pH and cecal organic acids in rats.

	Control*	DFA III**	Raffinose***
Initial body weight (g)	181±4	181±4	181±4
Body weight gain (g)	147±13	138±24	142±7
Feed intake (g/rat)	516±27	502±14	507±18
Feed efficiency	0.28±0.02	0.27±0.04	0.28±0.01
Cecal contents (g)	2.2±0.2 ^c	4.0±0.5 ^b	6.0±0.6 ^a
pH of cecal contents	7.0±0.1 ^a	6.4±0.3 ^b	5.9±0.4 ^b
Cecal organic acids (μmol/cecum)	234.1±35.1 ^c	496.5±71.6 ^b	811.0±59.2 ^a
Acetic acid	150.7±22.5 ^c	360.5±56.9 ^b	506.4±100.5 ^a
Propionic acid	41.7±5.8 ^b	58.7±11.7 ^b	88.4±24.2 ^a
<i>n</i> -Butyric acid	22.6±4.3 ^b	28.3±7.4 ^b	56.6±20.0 ^a
Formic acid	0.8±0.1 ^b	1.3±0.5 ^b	5.6±3.5 ^a
<i>n</i> -Valeric acid	4.8±1.0	5.3±3.2	1.7±1.1
<i>i</i> -Valeric acid	3.3±0.6	2.7±2.3	6.4±3.2
Succinic acid	9.7±4.2 ^b	33.5±29.1 ^{ab}	50.1±31.3 ^a
Lactic acid	0.6±0.5	6.1±4.6	95.7±137.2

*Control group fed the basal purified diet. **DFA III group fed the basal purified diet containing DFA III at 2% level. ***Raffinose group fed the basal purified diet containing raffinose at 5% level. Values are expressed as mean±SD. Means not sharing a common superscript letter are significantly different ($p < 0.05$).

ing *Bifidobacterium*. The DFA III diet increased *Clostridium* in the ceca, although the species of *Clostridium* spp. has not yet been identified. Though this genus contains harmful species of bacteria such as *Clostridium perfringens*, it has been reported that indigenous *Clostridium* spp. inhibit intestinal colonization of exogenous pathogens such as *Clostridium difficile*¹⁷⁾ and *Escherichia coli*.¹⁸⁾

In healthy humans, daily intake of 5 g DFA III caused no diarrhea and no change in the fecal microflora (Table 3). These results were different from the ones observed in the rats. Raffinose also did not caused any diarrhea, but raffinose intake induced a significant increase in Bifidobacteria compared with the period before administration. There were no changes in the fecal pH or the fecal water contents with or without intake of each oligosaccharide (range of average values across all test periods: pH 6.1–6.3, water content 77.0–80.0%). Though no change of pH in the feces from volunteers given DFA III was observed in this study, it was suggested that DFA III is fermented in large intestines of humans too because DFA III was fermented *in vitro* by the human feces like raffinose (our data unpublished). The usual bacteriological

Table 2. Effect of oligosaccharides on the cecal flora in rats.

	Control* ¹		DFA III* ²		Raffinose* ³	
Total bacteria	10.0±0.1 ^{a,b}		10.1±0.0 ^b		10.3±0.1 ^a	
Bacteroidaceae	10.0±0.1 ^a	(100) ^{*5}	9.6±0.2 ^b	(100)	9.5±0.2 ^b	(100)
	95±5 ^{*6,a}		42±18 ^b		21±10 ^c	
<i>Bifidobacterium</i>	7.3±1.0 ^b	(50)	8.3±0.0 ^b	(67)	9.7±0.6 ^a	(100)
	1±1 ^{*6,b}		1±1 ^b		45±30 ^c	
<i>Eubacterium</i>	7.9±0.3	(50)	8.0±1.2	(67)	8.4±0.1	(83)
	0±0 ^{*6}		2±2		1±1	
Peptococaceae	8.2±0.6	(83)	8.5±0.7	(83)	9.2±0.6	(100)
	3±5 ^{*6}		5±6		16±15	
<i>Clostridium</i>	8.0±0.5 ^b	(33) ^b	9.7±0.2 ^a	(100) ^a	9.1±0.7 ^{ab}	(100) ^a
	0±1 ^{*6,b}		47±17 ^a		13±10 ^b	
<i>Lactobacillus</i>	6.7±0.7	(100)	7.6±1.3	(100)	8.5±0.7	(100)
Total aerobes	7.5±0.4 ^a		6.6±0.3 ^b		6.5±0.5 ^b	
Enterobacteriaceae	7.3±0.3 ^a	(100)	6.2±0.6 ^b	(100)	6.2±0.8 ^b	(100)
Streptococaceae	6.9±0.6 ^a	(100)	6.3±0.2 ^b	(100)	6.1±0.2 ^b	(100)

*¹⁻³See the footnote in Table 1. *⁴Values are expressed as mean±SD of bacterial counts (log NO./g contents of cecum). *⁵Frequency of occurrence. *⁶Values are expressed as mean±SD of percentage of each bacteria group to total counts. Means not sharing a common superscript letter are significantly different ($p < 0.05$).

Table 3. Effect of oligosaccharides on human fecal flora.

	Schedule of administration									
	Before		DFA III* ¹		Wash-out period		Raffinose* ¹		After	
Total bacteria	10.6±0.2 ^{*2}		10.5±0.4		10.6±0.4		10.7±0.2		10.6±0.2	
Bacteroidaceae	10.3±0.3	(100) ^{*3}	10.1±0.5	(100)	10.3±0.4	(100)	10.3±0.3	(100)	10.3±0.2	(100)
	50±15 ^{*4}		47±17		54±13		40±15		50±14	
<i>Bifidobacterium</i>	9.8±0.3 ^b	(100)	9.8±0.3 ^{ab}	(100)	9.8±0.3 ^{ab}	(100)	10.1±0.3 ^a	(100)	9.9±0.3 ^{ab}	(100)
	16±11 ^{*4,b}		24±16 ^{ab}		19±12 ^b		35±19 ^a		20±11 ^{ab}	
<i>Eubacterium</i>	9.8±0.4	(100)	9.5±0.5	(100)	9.7±0.5	(100)	9.8±0.5	(100)	9.8±0.2	(100)
Peptococaceae	9.6±0.6	(100)	9.2±0.8	(100)	9.6±0.3	(93)	9.4±0.4	(100)	9.5±0.5	(100)
<i>Clostridium perfringens</i>	4.7±1.7	(57)	4.5±1.3	(50)	5.6±1.4	(57)	4.3±1.7	(43)	3.9±1.7	(79)
<i>Clostridium</i> -others	8.8±0.5	(64)	8.1±1.1	(43)	8.3±1.3	(43)	8.8±0.6	(29)	8.4±1.1	(43)
<i>Veillonella</i>	6.0±0.8	(71)	5.9±1.4	(71)	6.3±1.5	(71)	6.6±1.4	(71)	6.5±1.3	(64)
<i>Lactobacillus</i>	5.6±1.3	(86)	5.5±1.5	(86)	5.4±1.6	(86)	6.0±1.7	(100)	5.8±1.7	(93)
Curved rods	10.0±0.0	(14)	10.1±0.2	(14)	9.8±0.3	(14)	9.6±0.0	(14)	9.9±0.4	(14)
Total aerobes	7.6±0.6		7.3±0.5		7.8±0.7		7.8±0.7		8.0±0.7	
Enterobacteriaceae	7.0±1.0 ^{ab}	(100)	6.9±0.5 ^b	(100)	7.5±0.6 ^{ab}	(100)	7.5±0.8 ^{ab}	(100)	7.8±0.7 ^a	(100)
<i>Streptococcus</i>	6.3±1.5	(100)	6.7±0.9	(100)	6.9±1.1	(100)	6.7±1.2	(100)	7.0±1.3	(100)

*¹Administration of oligosaccharides at 5 g per day. *²Values are expressed as mean±SD of bacterial counts (log NO./g wet stool). *³Frequency of occurrence. *⁴Values are expressed as mean±SD of percentage of each bacteria group to total counts. Means not sharing a common superscript letter are significantly different ($p < 0.05$).

analysis technique that we have used may be not be able to completely analyze the change in intestinal microflora. Therefore, the identification with biogenetic techniques that detect the targeted genes for identification at species level are required in order to analyze change of intestinal microflora in detail.

An interesting characteristic is that calcium absorption in rats that were fed with DFA III was found to be stimulated not only in the large intestine but also in the small intestine.⁸⁾ However, this calcium absorption is stimulated only in the large intestine when other oligosaccharides were fed.^{6,7)} In the commercialization as a new food material, DFA III is greatly expected to be useful for human health. We have also progressed in the technology for producing DFA III on an industrial scale. In future, we expect that the useful functions of DFA III such as stimulation of calcium absorption will be explained in humans too.

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酵素反応によってイヌリンより生産された ジフルクトース-アンヒドリドⅢの物理的、 化学的および生理学的性質

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我々が、チコリー由来のイヌリンより製造した高純度 difructose anhydride III (DFA III; α -D-fructofuranose- β -D-fructofuranose 1,2': 2,3' dianhydride) 結晶の加工食品における有用性を評価するために、物理的、化学的および生理学的性質を調査した。DFA IIIは水への溶解性が高く、スクロースの90–95%の溶解度を示した。また、DFA IIIの吸湿性はスクロースよりも低く、低pHにおけるDFA IIIの分解性およびメイラード反応性は、スクロースよりもかなり低いことが明らかとなった。DFA IIIは *in vitro* において *Streptococcus mutans* MT8148株および6715株に資化されず、低う蝕性であることが示唆された。またDFA IIIは *in vitro* においてラット消化酵素によって分解されず、ラット小腸を用いた反転腸管サック法においても吸収は認められなかった。これらの結果はDFA IIIが他の多くのオリゴ糖同様に非消化性のオリゴ糖であることを示している。一方、DFA IIIを摂取したラットの盲腸では、短鎖脂肪酸の増大と菌叢の変化が認められたものの、*Bifidobacterium* の増加は認められなかった。1日にDFA III 5gを2週間摂取したヒトでは、糞便菌叢の変化は認められなかった。