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In Vitro Modulation of Human Intestinal Microbiota by Mannoligosaccharides Synthesized from *Amorphophallus muelleri* Glucomannan

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The corms of *Amorphophallus muelleri* Blume contain a large amount of glucomannan, a kind of polysaccharide that are commonly consumed by people as gelly foods. In order to improve the beneficial properties of glucomannan, we previously have established the enzymatic process to produce the mannoligosaccharides from flour of glucomannan using microbial mannanase. The effects of mannoligosaccharides on the growth modulation of human intestinal microbiota were investigated in this study. A set of *in vitro* single batch culture experiment was conducted to study the effect of mannooligosaccharides on human-origin *Lactobacillus fermentum* AA0014 and *Lactobacillus plantarum* FU0811. A modified MRS medium containing 10% (w/v) sucrose, glucomannan, and mannoligosaccharide was used instead of glucose as carbon source. The results showed the highest growth rate (0.13 h^{-1}) with both *L. fermentum* AA0014 and *L. plantarum* FU0811 in the presence of mannooligosaccharides. We confirmed this result by a similar *in vitro* experiment using human fecal samples of six healthy adults as innocula and analyzed the microbial population by fluorescence *in situ* hybridization (FISH). Lactobacilli were proliferated higher in the presence of mannoligosaccharide than other carbon sources, yielding the microbial proportion as much of 10.9% of total microbiota. Overall, this study demonstrated the potential use of mannoligosaccharides synthesized from *A. muelleri* glucomannan as prebiotic candidate of modulating the beneficial human intestinal microbiota.

Key words: *Amorphophallus muelleri*, growth modulation, human intestinal, mannoligosaccharides, microbiota, prebiotic

Umbi *Amorphophallus muelleri* Blume mengandung sejumlah tinggi senyawa glukomannan, yaitu sejenis polisakarida yang umum dikonsumsi oleh masyarakat sebagai produk pangan jeli. Untuk meningkatkan manfaat glukomannan, penelitian sebelumnya telah dilakukan untuk mendapatkan produk mannooligosakarida hasil reaksi enzimatik tepung glukomannan menggunakan enzim mannanase. Pada penelitian ini, mannooligosakarida hasil reaksi enzimatik dikaji pengaruhnya terhadap modulasi pertumbuhan mikrobiota saluran cerna manusia. Percobaan kultur curah telah dilakukan untuk memahami efek stimulasi mannooligosakarida terhadap bakteri *Lactobacillus fermentum* AA0014 and *Lactobacillus plantarum* FU0811 asal manusia. Medium MRS modifikasi yang mengandung 10% (b/v) sukrosa, glukomannan dan mannoligosakarida digunakan sebagai sumber karbon menggantikan glukosa. Hasil menunjukkan bahwa kecepatan pertumbuhan tertinggi (0.13 jam^{-1}) ditunjukkan oleh *L. fermentum* AA0014 and *L. plantarum* FU0811 pada substrat mannooligosakarida. Konfirmasi dilakukan dengan percobaan *in vitro* serupa, namun menggunakan sampel feses manusia asal enam orang dewasa sehat sebagai inokulum mikrobiota usus. Populasi mikrobiota dianalisis dengan fluorescence *in situ* hybridization (FISH) dan hasil menunjukkan bahwa kelompok lactobacilli dapat digandakan populasinya lebih tinggi dengan substrat mannooligosakarida dibandingkan sumber karbon lainnya, yaitu mencapai 10.9% dari total mikrobiota. Secara umum, penelitian ini menunjukkan potensi penggunaan mannooligosakarida yang disintesis dari glukomannan *A. muelleri* sebagai kandidat prebiotik yang berperan dalam memodulasi pertumbuhan mikrobiota saluran cerna manusia yang menguntungkan.

Kata kunci: *Amorphophallus muelleri*, mannoligosakarida, manusia, modulasi pertumbuhan, mikrobiota saluran cerna, prebiotik

Porang (*Amorphophallus muelleri* Blume) is a robust herbacous plant commonly found in Asia that produces the potential corms, large globulose depressed tubber. The corms contain carbohydrate in which mostly are in the form of mannan (Kay 1973). Depending on the cultivar, the glucomannan contents in the corms of *A. konjac* are 29 to 59% of total dry weight (Chua *et al.* 2010). In several countries, the corms were eaten as vegetable and also used for industrial purposes such as the source of flour and

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mannose (Kay 1973). The corms of *A. konjac* has been used as food and food additives in China and Japan for more than 1000 years. In addition, the potential use of glucomannan derived from konjac tuber have been studied for the application in pharmaceuticals, cosmetics, coating materials, and emulsifier (Aloso-Sande *et al.* 2009; Chua *et al.* 2010; Zhang *et al.* 2005). As food compounds, the glucomannan of *A. konjac* have been investigated previously for anti-obesity, anti-hyperglycemic and hypercholesterolemia, anti-inflammatory, and laxative activities as well as prebiotic properties (Chen *et al.* 2006; Chua *et al.* 2010). Glucomannan was considered to have diet therapy in primary prevention in high risk hypercholesterolemic children (Martino *et al.* 2005).

There is increasing interest in the development of mannan-based prebiotics. The mannanase-producing bacteria were applied to produce prebiotic mannooligosaccharides, using copra as raw materials (Titapoka *et al.* 2008). Basically glucomannan can be hydrolized to produce mannoligosaccharides. In previous study, we have succesfully established the processing methods for synthesizing the mannoligosaccharides from glucomannan by using the mannanase of actinomycetes isolates (Yopi *et al*. unpublished data). Oligosaccharides are important to stimulate efectively the beneficial microbiota in gastrointestinal tract, especially bifidobacteria in human and animal (Dinoto *et al.* 2006a; Dinoto *et al.* 2006b). Lactobacilli was also increased in rat cecal after administering raffinose and encapsulated bifidobacteria (Dinoto *et al.* 2006b). Similary, modulation of beneficial microbes by fructooligosaccharides and galactooligosaccharides were also reported (Rycroft *et al.* 2001). Several oligosaccharides have been investigated intensively in human subject or using human feces as innoculum, including fructooligosaccharides, galactooligosaccharides, maltooligosaccharides, and xylooligosaccharides (Rossi *et al.* 2005; Rycroft *et al.* 2001; Tuohy *et al.* 2005).

The role of mannoligosaccharides in modulation of human intestinal microbiota is not completely understood. Although some information is available on the effects of feeding mannoligosaccharides in the health status of human and animal, there is a limited information about the potential uses *A. muelleri* corms-based mannoligosaccharides for modulating gut microbial population. In this study, we investigated whether mannoligosaccharides influence the microbial population and metabolite profiles in the static batch culture of human intestinal microbiota.

MATERIALS AND METHODS

Strains and Material Preparation. Bacterial strains used in this study are *Lactobacillus fermentum* strain AA0014 and *Lactobacillus plantarum* strain FU0811. Those strains are human-origin and belonging to the collection of Research Center for Biology, Indonesian Institute of Sciences, Indonesia. In this study we used glucomannan flour of *A. muelleri* (provided by PT. Ambico, Indonesia). Mannoligosaccharides sample used in this study was synthesized by the reaction of glucomannan flour and mannanase enzyme of *Saccharopolyspora flava* BTCC ID04-0555, which was kindly provided by the collection of the Biotechnology Culture Collection, Research Center for Biotechnology, Indonesian Institute of Sciences. The liquid products of glucomannan and mannoligosaccharides used in the experiment were adjusted to final concentration of 10 g per liter medium.

Measurement of the Growth Rate. To determine the growth rate of representative probiotic candidate lactobacilli in several carbon sources, a seed culture for *L. fermentum* AA0014 and *L. plantarum* FU0811 growth experiments was grown overnight in MRS medium (de Man *et al.* 1960). At an optical density at OD_{600} = 0.8 of seed culture was transferred to the same MRS-based medium, but containing one of the following carbohydrates (10 g L^{-1}): glucose (Merck), sucrose (Merck), glucomannan, and mannoligosaccharides. Bacterial growth was periodically monitored by determining the OD_{600} of the culture broth. All the cultures were incubated at 37 °C under anaerobic conditions using carbondioxide. Specific growth rates (μ) (in h^{-1}) were calculated during the logarithmic growth phase using the following equation: $\mu = (\ln xt_2 - \ln xt_1)/(t_2 - t_1)$, where xt_2 and xt_1 are the OD_{600} values at times t_2 and t_1, respectively.

Static Batch Culture Fermentations. The experiment was conducted as described by Olano-Martin *et al.* (2000). In autoclaved 50 mL serum bottles containing each substrate (0.5 g), aliquot of 45 mL autoclaved nutrient medium was added by filter sterilization to each bottle. The basal medium contained (g L^{-1}): peptone water, 2; yeast extract, 2; NaCl, 0.1; K_2HPO_4, 0.04; KH_2PO_4, 0.04; $MgSO_4.7H_2O$, 0.01; $CaCl_2.6H_2O$, 0.01; $NaHCO_3$, 2; hemin (dissolved in a few drops of 1 mol l^{-1} NaOH), 0.05; cysteine. HCl, 0.5; bile salts, 0.5; Tween 80, 2 and 10 µL vitamin K_1. The medium was adjusted to neutral (pH 7.0). using 1 mol L^{-1} HCl. Before innoculation, the bottles containing medium were placed inside anaerobic jar (Merck) with

supplementation of Anaerocult (Merck) at 37 °C overnight to prereduce the media. A 10% (w/v) fecal slurry was prepared using fresh feces from six healthy donors (who had not taken antibiotics for 3 months beforehand) and prereduced 0.1 mol L^{-1} phosphate buffer (pH 7.0). Fecal slurry was mixed in a sterilized tube for 2 min. In each serum bottle, 5 mL of the slurry were inoculated, mixed and capped. Samples were removed from the fermenters at 24 h fermentation for enumeration of microbiota, and measurement of SCFA. The fermentation experiments were performed in triplicate for each.

Fluorescence *in situ* Hybridization Analysis. The samples (100 µL) from the static batch culture at 24 h fermentation were removed and washed with phosphate buffered saline (PBS) (130 mM NaCl, 10 mM sodium phosphate buffer; pH 7.2) at 9 000 ×g 4 °C for 2 min. The washed samples were fixed in 4% (w/v) paraformaldehyde in PBS (pH 7.2) for 24 h. Fixed samples were washed once in PBS and stored in a known volume of 50% (v/v) ethanol-PBS at -20 °C until use. FISH analysis was conducted as described by Dinoto *et al.* (2006b). Aliquots (3 µL) of fixed cells were applied to Teflon printed glass slides (ADCELL; 12 wells; diameter, 5 mm; Erie Scientific Company, Portsmouth, N.H.), and air dried. The cells were then dehydrated with a series of solutions containing 50%, 80%, and 99.5% ethanol (3 min for each concentration). The cells fixed on the glass slides were hybridized by addition of 8 µL of hybridization buffer (0.9 M NaCl, 0.01% sodium dodecyl sulfate, 20 mM Tris-HCl, 20% deionized formamide; pH 7.2) with 1 µL of Cy3-labeled oligonucleotide probe (25 ng μL^{-1}; Tsukuba Oligo Service Co., Ltd., Tsukuba, Japan). The 16S rRNA-targeted oligonucleotide probes used for molecular analysis of *Lactobacillus* spp. (Lacb722, S-G-Lacb-0722-a-A-25, YCACCGCTACACAT GRAG TTCCACT) (Sghir *et al.* 1999), *Bifidobacterium* spp. (Bif164m, S-G-Bif-0164-b-A-18, CATCCGGYATTA CCACCC) (Dinoto *et al.* 2006a), *Clostridium coccoides-Eubacterium rectale* group (Erec482, S-*-Erec-0482-a-A-19, GCTTCTTAGTCA RGTACCG) (Franks *et al.* 1998), and *Streptococcus* spp. (Strc493, S-*-Strc-0493-a-A-19, GTTAGCCGTCCCTTTCTG G) (Franks *et al.* 1998). The slides were hybridized at 46 °C for 16 h in a moist chamber. After hybridization, the slides were rinsed with warm hybridization buffer at 48 °C and washed in prewarmed washing buffer (225 mM NaCl, 0.01% sodium dodecyl sulfate, 20 mMTris-HCl; pH 7.2) for 20 min at 48 °C. The washed slides were stained with a DAPI (4',6-diamidino-2-phenylindole dihydrochloride *n*-hydrate) solution for 5 min at room temperature to stain the chromosomes as a control signal. The slides were washed with distilled water for 5 min at room temperature and air dried in the dark. The dried slides were mounted with Vectashield (Vector Laboratories Inc., Burlingame, Calif.) and examined with an Nikon OPTIHOT-2 (Nikon Corporation, Tokyo, Japan) equipped with a Nikon Digital Camera. The DAPI and Cy3 signals were captured in pairs of 10 random microscopic fields (about 500 cells per microscopic field). Hybridization images were manually counted and were colorized when necessary using Adobe Photoshop 7.0 (Adobe Systems Incorporated, San Jose, Calif.). Specific signals from the probes were expressed as average percentages of the total cells visualized by DAPI signals in the same microscopic field.

Short-chain Fatty Acid Analysis and pH Measurement. The measurement of short chain fatty acid was carried out by taking samples from the bottles of the static batch culture at 24 h fermentation. Samples were centrifuged (13 000 ×g for 30 min) and filtered by 0.2 µm syringe to remove particulate material. Ten microliter samples were then injected onto an HPLC system (Model Water 1350T, Biorad, UK). The column used in this analysis was an ion-exclusion Aminex HPX-87H (Bio-Rad) which maintained at 35 °C with a column heater and the pressure of 2071 psi. The eluent, 0.008 N sulphuric acid in HPLC-grade water was pumped through the column at a flow rate of 1.0 mL min^{-1}. Data from the RI detector were integrated and by using calibration curves, acetate, propionate, succinate, and lactate were quantified in the samples. The pH of suspension was determenided by using IQ120 miniLab™ pH meter (Hach Company, Loveland, CO).

Statistical Analysis. The data of total cell counts, microbial proportions, pH, and SCFA concentrations for culture were analyzed statistically using SPSS software version 13.0 (SPSS, Inc., Chicago, IL). Bonferroni tests were performed for pair-wise multiple comparisons of the mean values for the glucose, sucrose, glucomannan, and mannoligosaccharides.

RESULTS

In this study, we determined the specific growth rate of selected *L. plantarum* FU0811 and *L. fermentum* AA0014 as representative human intestinal lactobacilli on the culture containing glucose, sucrose, glucomannan, and mannoligosaccharides as sole carbon source. Under this experimental condition, both

L. plantarum and *L. fermentum* grew faster in the presence of mannoligosaccharides yielding the highest specific growth rate as much of 0.13 h^{-1} (Table 1). We observed that the growth of those strains became slower when they were cultured in the glucomannan medium. Interestingly, *L. plantarum* and *L. fermentum* have lower growth rates in the presence of glucose (0.05 and 0.04 h^{-1}, respectively) as compared to sucrose medium (0.09 and 0.10 h^{-1}), respectively (Table 1).

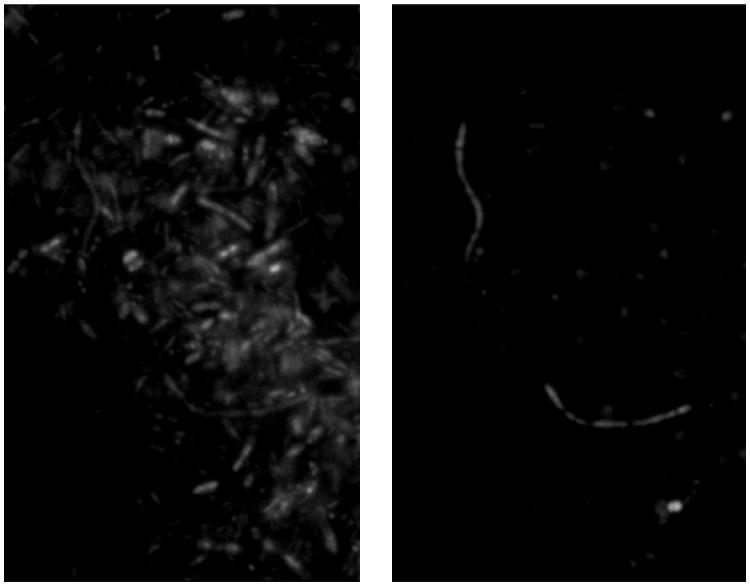
The cell morphology of intestinal microbiota in the batch culture inoculated with human fecal samples of six healthy adults were varied. Based on FISH analysis, we observed many cells were matched with oligonucleotide probe used in this study. The long-rod cells identified as lactobacilli under epifluorescence microscope were clearly distributed among other intestinal bacteria in batch culture (Fig 1). Since we did not monitored the population hour-by-hour, the 24 h observation could be used as representing impact of carbon source on human microbiota. The changes in selected bacterial populations with the carbon sources tested are presented in Table 2. Total cell counts of intestinal microbiota in glucose medium was about 9.43 $\log_{10}$ cells mL^{-1} indicating that microbial cells in fecal slurry proliferated under experimental system. Population of microbes before 6 h fermentation was observed under 7 $\log_{10}$ cells mL^{-1} and it was unsufficient for analysis of FISH. There is different cell number of microbiota when sucrose was used in this experiment (Table 2). With all of the carbon source studied, a large significant increase in numbers of lactobacilli was observed in mannoligosaccharides medium (10.9%), whereas a relatively lower number was monitored in glucomannan medium (3.2%) and sucrose medium (2.6%). The population of lactobacilli in glucose medium was only 0.7% of total microbiota. The highest population of bifidobacteria was found in mannoligosaccharides medium (3.3%) and we also confirmed that this group of bacteria was relatively low in sucrose and glucose media (lower than 0.1% of total microbiota). In contrast, the populations of *Eubacterium*/*Clostridium* showed a lower proportion in mannoligosaccharides medium than in sucrose medium or glucose medium (Table 2). Our data also showed no streptococci were detected in all tested carbon sources, indicating that this group of bacteria was absent or out of detection limit in FISH analysis ($<10^7$ cells). Overall, this experimental system clearly demonstrated that the growth of human intestinal microbiota is much more stimulated by mannoligosaccharides.

There was a change in the pH during fermentation of carbon sources by intestinal microbiota of human origin. We observed that the pH change at the static batch culture of human fecal slurry during 24 h fermentation with various carbon sources used in this study is about 2-3 (Fig 2). In general, carbohydrate was rapidly metabolished by intestinal bacteria to produce organic acids. The occurences of nutrient metabolisms by intestinal microbiota could be simply recognized by the profile of short chain fatty acids (SCFA). SCFA are the products of microbial activities in the fermentations and only produced in appreciable levels in the presence of added carbohydrate. In this study, we observed that only lactate, acetate, and propionate were detected in all samples, whereas succinate was not detected. The

Table 1 The Specific growth rate (μ) of human-origin *Lactobacillus fermentum* AA0014 and *Lactobacillus plantarum* FU0811

	Specific growth rate (h^{-1}) ± SD*
Lactobacillus fermentum AA0014	
Glucose	0.04 ± 0.01
Sucrose	0.10 ± 0.00
Glucomannan	0.01 ± 0.01
Mannoligosaccharides	0.13 ± 0.01
Lactobacillus plantarum FU0811	
Glucose	0.05 ± 0.03
Sucrose	0.09 ± 0.00
Glucomannan	0.02 ± 0.02
Mannoligosaccharides	0.13 ± 0.01

*SD, standard deviation



DAPI Lacb722

Fig 1 Epifluorescence images of microbial cells (stained with DAPI) and *Lactobacillus cells* (hybridized with probe Lacb722) in 24h static batch culture of human fecal slurry with mannoligosaccharides.

Table 2 Microbial populations in the 24 h static batch culture fermentations with the various carbon sources

Population	Stain or probe	Microbial population ± SD*			
		Glucose	Sucrose	Glucomannan	Mannoligosaccharides
Total microbiota (log10 cells.g^{-1})	DAPI	9.43 ± 0.02^{b}	9.40 ± 0.03^{a}	9.47 ± 0.02^{c}	9.47 ± 0.03^{c}
Lactobacillus (%)	Lacb722	0.65 ± 0.59^{a}	2.57 ± 0.67^{ab}	3.18 ± 0.88^{b}	10.90 ± 3.65^{c}
Bifidobacterium (%)	Bif164m	0.89 ± 0.90^{a}	0.10 ± 0.22^{a}	2.46 ± 1.13^{b}	3.28 ± 1.17^{b}
Clostridium/Eubacterium group (%)	Erec482	21.14 ± 5.91^{b}	20.39 ± 5.56^{b}	17.39 ± 4.55^{ab}	13.93 ± 2.02^{a}
Streptococcus (%)	Strc493	ND**	ND	ND	ND

*SD, standard deviation

**ND, not detected

high level lactate were detected in sucrose medium and mannoligosaccharides medium, as much of 36.05 and 34.84 mmol L^{-1}, respectively (Table 3). In contrast, very low level of lactate was observed in glucomannan medium. The culture cultivated in the presence of glucose showed moderate level of lactate (24.06 mmol L^{-1}). The highest concentration of acetate (36.19 mmol L^{-1}) was observed in mannoligosaccharides medium. Glucose medium tend to modulate the microbial community to produce slight acetate in medium. Propionate was detected higher in mannoligosaccharides and glucomannan media (8.54 and 9.58 mmol L^{-1}) than in other substrates. In general, the total detected SCFA in medium was observed in mannoligosaccharides medium (79.57 mmol L^{-1}) indicating that mannoligosaccharides was a substance in which intestinal microbiota prefered to use it as digestible substrate (Table 3).

DISCUSSION

Our data actually support the advantages of mannoligosaccharides as main compound in glucomannan hydrolysate on stimulating intestinal lactobacilli (Table 1, Table 2), in addition, without any significant changes in bifidobacteria and streptococci

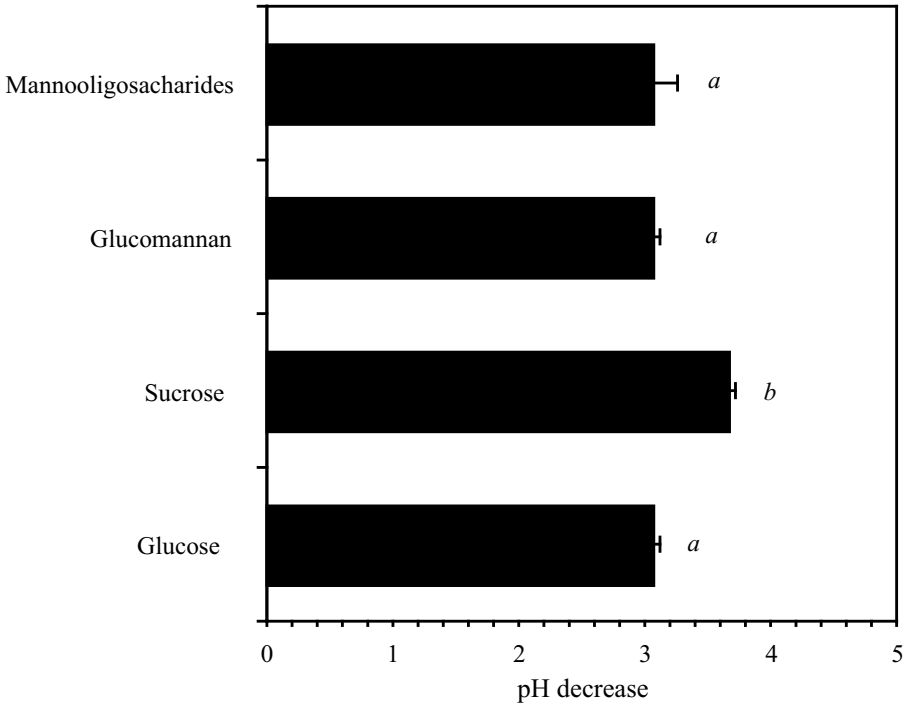


Fig 2 Dicreases in pH value of the static batch cultures of human fecal slurry during 24 h fermentation with various carbon sources.

Table 3 SCFA concentration in the 24 h static batch culture fermentations with the various carbon sources.

	SCFA concentration (mmol L^{-1}) ± SD*			
	Glucose	Sucrose	Glucomannan	Mannoligosaccharides
Lactate	24.06 ± 3.98^{b}	36.05 ± 3.28^{b}	9.93 ± 2.80^{a}	34.84 ± 1.29^{b}
Acetate	11.08 ± 0.72^{a}	17.63 ± 1.73^{a}	18.15 ± 2.30^{a}	24.47 ± 16.57^{a}
Propionate	0.48 ± 0.08^{a}	0.09 ± 0.13^{a}	9.58 ± 0.46^{ab}	5.85 ± 3.79^{b}
Succinate	ND**	ND	ND	ND
Total	35.62 ± 3.34^{a}	53.76 ± 5.13^{a}	37.66 ± 0.95^{a}	65.17 ± 19.07^{a}

*SD, standard deviation

**ND, not detected

(Table 2). We pressumed that mannoligosaccharides hydrolysate is likely preferred by lactobacilli rather than glucomannan itself in the term of simple utilization of oligosaccharides. In animal experiment, the increased population of lactobacilli in dog feces due to diet containing mannoligosaccharides was also reported (Swanson *et al.* 2002). The effects of glucomannan and acid-hydrolyzed mannoligosaccharides of konjac on cecal microbiota in Balb/C mice have been investigated where both glucomannan and mannoligosaccharides were capable of increasing bifidobacteria and oppositely decreasing *Clostridium perfringens* in mice cecum. In addition, mannoligosaccharides caused increases in fecal total anaerobe counts (Chen *et al.* 2005). Similar phenomenon was observed where mannoligosaccharides significantly reduced fecal *C. perfringens* and *Escherichia coli* counts (Elamir *et al.* 2008). The effect of glucomannan hydrolysates added to the ultra-high temperature milk on the growth of lactic acid bacteria was evaluated (Al-Ghazzewi *et al.* 2007). The authors also reported that the glucomannan hydrolysates produced with either mannanase or cellulase enzymes were effective growth promoters (carbon sources) of lactic acid bacteria (Al-Ghazzewi and Tester 2012).

Glucomannan is a kind of polysaccharide of the

glucomannan family. It was composed of β-1,4 linked D-mannose and D-glucose monomers (Maeda *et al.* 1980), although the mannose/glucose monomer ratio may vary depending on the original source of glucomannan. There are several enzymes involving in the complete degradation of the glucomannan including β-mannanase, β-mannosidase, and β-glucosidase (Alonso-Sande *et al.* 2009). Enzyme β-mannanase catalyzed the random cleavage of β-D-1,4-mannopyranosyl linkages to produce mannobiose and mannotriose. The conversion of glucomannan into $_{D}$-mannose is by enzyme β-mannosidase that remove β-mannose residues from β-1,4-linked mannoligosaccharides. Specificaly, the degradation by β-glucosidase occurs only at a terminal glucose unit and stops at terminal oxidized residues or mannose units. The enzyme of β-mannanase and β-mannosidase are present in many microorganisms including bacteria harbored in human gut. In addition, the β-glucosidase genes are widespread among human gut bacteria (Gill *et al.* 2006). A member of human gut lactic acid bacteria, *L. gasseri*, encoded twenty kinds of putative glycosyl hydrolases which mostly glucosidases and galactosidases with predicted substrate specificities for a diversity of di- and trisaccharides. The β-glucosidase of this strain is specific and have no homolog among lactic acid bacteria (Azcarate-Peril *et al.* 2008). The substrate specifity was suggested to be important key in oligosaccharides utilization by lactobacilli.

Although several constraints of this study have included most detected microbes of intestinal samples are culturable and static culture condition may impact to tolerance against acidic condition where organic acid implicate to reduce the pH values, this study could explain the direct impact of prebiotic candidate to the growth selected individual strains (*L. plantarum* and *L. fermentum*). Those strains were previously observed in separate study for reducing the cholesterol in medium after 36 h incubation (Dinoto *et al.* unpublished data). Thus, by understanding the suitable substrate, the development synbiotic product with mannoligosaccharides will be interesting aspect in term of functional foods. In mice experiment, konjac glucomannan and the products of partially-hydrolysed konjac glucomannan was compared. Based on culture-dependent method, the total anaerobes and the populations of lactobacilli, and bifidobacteria were higher in partially-hydrolysed konjac glucomannan indicating the preference of those beneficial bacteria on specific digested product of glucomannan. In addition, it was reported also that partially-hydrolysed konjac glucomannan showed better protective effects on fecal water-induced DNA damage as the prebiotic property than did the unhydrolysed konjac glucomannan (Connoly *et al.* 2010). In conclusion, this study demostrated that mannoligosaccharides synthesized through enzymatic reaction of *A. muelleri* glucomannan have potential properties as prebiotic candidate. The further study could be conducted for clear characterization in order to improve the healthy condition of the host.

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