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Salecan diet increases short chain fatty acids and enriches beneficial microbiota in the mouse cecum

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ABSTRACT

Salecan, a linear extracellular polysaccharide consisting of β-(1,3)-D-glucan, has potential applications in the food industry due to its excellent toxicological profile and rheological properties. The aim of the present study was to evaluate the effects of dietary supplementation with 8% Salecan on the gastrointestinal microbiota in mice. In the Salecan group, the following significant differences ($p < 0.05$) from the cellulose group were found: increased body weight gain, greater mass of cecum and cecal contents, and higher butyrate concentrations in the cecal and colonic contents at wk 4. Moreover, populations of *Lactobacillus* and *Bifidobacterium* increased 3- and 6-fold, respectively, in the cecal contents of mice consuming Salecan. These results suggest that the dietary incorporation of Salecan, by providing SCFAs and increasing beneficial microbiota, may be beneficial in improving gastrointestinal health, and have relevance to the use of Salecan as a dietary supplement for human consumption.

1. Introduction

Human intestinal microflora includes a variety of microorganisms, predominantly bacteria, which colonize the gut of all living organisms (Othman, Agüero, & Lin, 2008). In the adult human intestine, the size of the microbial population can be up to 100 trillion, which far exceeds that of all other microbial communities associated with the body's surfaces and is approximately 10 times greater than the total number of human somatic and germ cells (Bäckhed, Ley, Sonnenburg, Peterson, & Gordon, 2005). This large and dynamic intestinal microflora plays an important role in host health (Guarner & Malagelada, 2003) and has been implicated in diseases ranging from allergies (MacDonald & Monteleone, 2005) to late-onset autism (Finegold et al., 2002), cardiovascular diseases (Thompson-Chagoyán, Maldonado, & Gil, 2005), diabetes (Ley, Turnbaugh, Klein, & Gordon, 2006), inflammatory bowel disease (Hume & Radford-Smith, 2002) and colon cancer (MacDonald & Wagner, 2012). Thus, altering bacterial activities in the gut may promote the general health of humans or provide treatment of specific diseases. Such health-related changes could be achieved via the use of probiotics or prebiotics (Rupa & Mine, 2012; Snart et al., 2006).

A prebiotic is defined as 'a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health' (Gibson & Roberfroid, 1995). Most attention on prebiotic research has been directed toward the use of inulin or fructo-oligosaccharide (Guarner, 2005; Kolida, Tuohy, & Gibson, 2002). Other polymers, such as β-glucans, have been neglected in prebiotic research. β-Glucans from cereals are linear polymers of glucose residues linked by β-(1, 3) or β-(1, 4) glycosidic bonds (Lazaridou & Biliaderis, 2007), and exhibit multiple functions and roles that make them unique as dietary fibers in the food of domestic animals and humans (Wood, 2007). β-Glucans are substrates for fermentation that generate short chain fatty acids (SCFAs) by intestinal bacteria (Wong, de Souza, Kendall, Emam, & Jenkins, 2006) and are beneficial in the prevention of constipation (Lazaridou & Biliaderis, 2007), reduction of the intestinal absorption of cholesterol and bile acid (Reyna-Villasmil et al., 2007), attenuation of the levels of glucose and insulin in circulating blood

Abbreviations: DGGE, denaturing gradient gel electrophoresis; qPCR, quantitative real-time PCR; SCFA, short-chain fatty acid; HE, hematoxylin and eosin; HPIC, high-pressure ionic chromatography; UPGMA, unweighted pair group method with arithmetic averages; SEM, standard error of the mean.

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following a meal (Wood, 2007), and protection against colorectal cancer (McIntyre, Gibson, & Young, 1993).

Salecan is a type of β-glucans produced by *Agrobacterium* sp. ZX09. Its structure consists of the following repeating unit: → 3)-β-D-Glc*p*-(1 → 3)-[β-D-Glc*p*-(1 → 3)-β-D-Glc*p*-(1 → 3)]3-α-D-Glc*p*-(1 → 3)-α-D-Glc*p*-(1 → (Fig. 1 – Supplementary data) (Xiu et al., 2010). In recent years, Salecan has been shown to have multiple biological activities, such as reducing high-fat diet-induced obesity (Zhang et al., 2012) and alleviating the symptoms of drug-induced constipation (Zhou et al., 2013). In addition, a 90-d safety assessment study and rheological study have shown that Salecan is non-toxic with excellent rheological properties and can be utilized in the food industry (Xiu, Zhan, et al., 2011; Xiu, Zhou, Zhu, Wang, & Zhang, 2011). The purpose of this study was to evaluate the in vivo effects of Salecan on food intake and body weight gain, on cecal total and wall weight, on the formation of SCFAs, and on the composition of the microflora.

2. Materials and methods

2.1. Materials

Salecan was extracted from the fermentation broth of *Agrobacterium* sp. ZX09 using centrifugation and ethanol precipitation as previously described (Xiu et al., 2010; Xiu, Zhou, et al., 2011). Commercial Salecan (chemical compositions: sugar 77.13%, protein 6.2%, moisture 5.2%, and ash 10.28%; average molecular weight: 2×10^6; water-soluble) was purchased from Karroten Company (Nanjing, China). Microcrystalline cellulose was purchased from Sigma (St. Louis, USA).

2.2. Animals and experimental design

All animal use procedures were approved by the Institutional Animal Care and Use Committee at Nanjing University of Science and Technology. C57BL/6J male mice were weaned at 3 wk of age. The mice were randomly divided into two groups of six mice each and fed with a diet containing 8% (w/w) Salecan or cellulose (as control) for 4 wk (Table 1 – Supplementary data). This dose was selected based on the previous study (Snart et al., 2006). The mice were housed under a temperature- and humidity-controlled room with a 12/12 h light/dark cycle. Food and water were available ad libitum.

The food intake and growth of the mice were determined every 2 d and weekly, respectively. All feces were collected for 24 h at wk 1–4, and then weighed, freeze-dried (Virtis Benchtop K, USA), and stored at −80 °C until further analysis. The fecal moisture content (%) was calculated as (feces weight before freeze-dried − feces weight after freeze-dried)/feces weight before freeze-dried × 100. After 28 d of treatment, the animals were anesthetized, and the organs (small intestinal, cecum and the rest of large intestine) were aseptically excised. The cecum was dissected free from fat and mesentery, and weighed. Each sample of cecal contents was divided into two equal portions, weighed, and stored at −80 °C until further analysis. Cecal tissue was washed with phosphate-buffered saline (pH 7.4), dried between layers of filter paper, and weighed. The cecal contents were calculated as the weight of the total cecum minus that of the cecal tissue. The colonic contents were also sampled for SCFA analysis.

2.3. Histopathological evaluation

Segments of approximately 0.5 cm were obtained from the midpoints between the point of bile duct entry and the jejunum as well as between the point of proximal cecum and the rectum, and were fixed in 10% neutral buffered formalin, embedded in paraffin, sliced into sections (5 μm thick), and then stained using hematoxylin and eosin (HE) for histopathological examination using light microscopy (Nikon, Japan).

2.4. Determination of lactate and SCFAs

Samples of feces and the contents of the cecum and colon were diluted 10-fold with distilled water, and mixed thoroughly on a vortex-mixer for 2 min. After mechanical shaking (1 h, 4 °C) and centrifugation (10 000 × *g*, 20 min, 4 °C), the supernatants were filtered through a 0.22 μm filter, and the lactate and SCFAs (acetate, propionate and butyrate) were determined using high-pressure ionic chromatography (HPIC) (Dionex ICS-2100, USA) equipped with an IonPac AS11-HC 4 mm × 250 mm column and a CD-25 conductive detector (Robert-Peillard, Palacio-Barco, Dudal, Coulomb, & Boudenne, 2009).

2.5. Extraction of DNA from cecal contents

Bacterial DNA was extracted from cecal contents according to a previously described method (Walter et al., 2000). Briefly, the bacterial cells in the homogenates of cecal contents were mechanically lysed, and the DNA was purified using phenol-chloroform extraction, ethanol precipitation and RNase treatment.

2.6. PCR amplification

Each DNA sample was amplified using primers specific for conserved sequences flanking the variable V3 region of the 16S rDNA in an S1000 Thermal Cycler (Bio-Rad, USA). Primers used in this study were listed Table 2 – Supplementary data. Each 20-μl reaction mixture was prepared with 1 μl (10–50 ng) bacterial DNA and with 20 pmol of each primer (341-F with the GC clamp and 534-R) according to the manufacturer's protocol (TaKaRa, China). The amplification program was 94 °C for 4 min; 30 cycles of 94 °C for 30 s, 64 °C for 30 s, and 72 °C for 30 s; and finally 72 °C for 10 min. The size of the PCR product was confirmed on a 2% agarose gel stained with ethidium bromide.

2.7. Generation of cecal microbiota profiles using DGGE

Generation of denaturing gradient gel electrophoresis (DGGE) was performed using the DCode Universal Mutation Detection System (Bio-Rad, USA) as described previously with slight modifications (Walter et al., 2000). The PCR products were loaded onto 10% polyacrylamide/bisacrylamide (37.5:1) gels in 1× TAE buffer diluted from 50× TAE buffer (2 M Tris base, 1 M glacial acetic acid, and 50 mM EDTA). The gels contained a 40–65% gradient of 40% (vol/vol) deionized formamide and 7.0 M urea, which increased in the direction of electrophoresis. Electrophoresis was performed under a constant voltage of 85 V at 60 °C until the xylene cyanol dye marker reached the bottom of the gel (approximately 12 h). The gels were stained with a 1:10,000 dilution of SYBR Green I dye (Invitrogen, China) and then imaged in the Bio-Rad universal hood II (Bio-Rad Laboratories, Italy).

2.8. Cloning and sequencing analysis

Bands of specific interest were excised from the DGGE gel using a sterile razor, and eluted overnight as described elsewhere (Jensen, Øvreås, Daae, & Torsvik, 1998). The eluate (1 μl) was used as the template DNA in the reamplification PCR. The reactions were performed with primers 341-F and 534-R under the same conditions as described above. Then, the PCR products were gel-purified and inserted into the TA cloning vector pCR2.1 (Invitrogen, China) for sequencing (Invitrogen, China). These sequences were compared

Table 1
Body weight, food intake and fecal excretion for mice fed with experimental diets for 4 wk.

	Cellulose	Salecan
Body weight (g)		
Initial	13.52 ± 0.67	13.18 ± 0.60
Final	22.43 ± 0.74	24.15 ± 0.67
Gain	8.92 ± 0.84	10.97 ± 0.73[a]
Food intake (g/mouse and 24 h)	4.28 ± 0.12	4.54 ± 0.20
Feces (g/mouse and 24 h)		
Wet weight	1.49 ± 0.10	1.60 ± 0.03
Dry weight	0.95 ± 0.05	0.99 ± 0.03
Fecal water content (%)	35.42 ± 1.42	38.80 ± 2.60

Values are expressed as the mean ± SEM ($n = 6$).
[a] Mean values were significantly different from those of the cellulose group ($p < 0.05$).

Table 2
Cecal tissue and contents, and cecal lactate and SCFAs of mice fed experimental diets for 4 wk.

	Cellulose	Salecan
Cecum (g)		
Total weight	0.55 ± 0.05	0.83 ± 0.04*
Wall weight	0.09 ± 0.01	0.16 ± 0.01*
Contents weight	0.46 ± 0.05	0.67 ± 0.04*
SCFA (μmol/g cecal contents)		
Total acids[a]	39.12 ± 3.82	67.44 ± 4.15*
Lactate	1.81 ± 0.27	1.98 ± 0.64
Acetate	26.92 ± 2.98	47.20 ± 3.28*
Propionate	6.32 ± 0.86	8.77 ± 1.07
Butyrate	4.08 ± 0.78	9.50 ± 0.65*

Values were expressed as the mean ± SEM, $n = 6$ for each group.
[a] Total acids is the sum of lactate, acetate, propionate and butyrate.
* Mean values were significantly different from those of the cellulose group ($p < 0.05$).

Table 3
Lactate and SCFAs in colonic contents of mice fed experimental diets for 4 wk.

SCFA (μmol/g colonic contents)	Cellulose	Salecan
Total acids[a]	32.64 ± 5.17	60.05 ± 4.50*
Lactate	1.72 ± 0.22	2.69 ± 0.42*
Acetate	22.19 ± 3.70	43.89 ± 2.81*
Propionate	6.45 ± 1.29	7.12 ± 0.84
Butyrate	2.27 ± 0.43	6.35 ± 1.17*

Values were expressed as the mean ± SEM, $n = 6$.
[a] Total acids is the sum of lactate, acetate, propionate and butyrate.
* Mean values were significantly different from those of the cellulose group ($p < 0.05$).

with those in the NCBI database using the BLASTN algorithms (Altschul, Gish, Miller, Myers, & Lipman, 1990). The closest matched sequences were obtained, and then aligned using the ClustalW function of BioEdit version 7.0.0. The neighbor-joining phylogenetic tree was constructed using Mega version 5.05. The reliability of the tree topology was gauged by performing bootstrap analysis using 1000 replicates (Ellis, Morgan, Weightman, & Fry, 2003).

2.9. Profile analysis of DGGE fingerprints

Analysis of the DGGE profiles was performed according to protocols previously described (McCracken, Simpson, Mackie, & Gaskins, 2001; Ying, Lv, Min, & Cheng, 2008) using Quantity One version 4.6.1 (Bio-Rad). Briefly, clustering analysis was performed using the unweighted pair group method with arithmetic averages (UPGMA) to calculate the dendrogram of the DGGE gel. The DGGE profiles were also compared using the number of bands, evenness index, Shannon index, Simpson index and Sorenson index.

2.10. qPCR analysis

Quantification of the *Lactobacillus*, *Bifidobacterium* and *Clostridium* cluster XIVa was carried out using quantitative real-time PCR (qPCR). qPCR was performed using the ABI 7300 real-time PCR system with bacterial DNA samples and amplified in a 20-μl reaction volume containing 1× SYBR Green PCR master mix (Applied Biosystems, USA). The amplification program consisted of one cycle at 50 °C for 2 min; one cycle at 95 °C for 10 min; 40 cycles at 95 °C for 15 s and 60 °C for 1 min; and finally one cycle for melting curve analysis. All results were calculated relatively as the ratio of the species DNA levels to the V3 region expression levels to correct the data for differences in total DNA concentration between individual samples, as described elsewhere (Licht et al., 2010).

2.11. Statistics

All data were expressed as the mean ± standard errors of the mean (SEM). Student's *t*-test was used to evaluate the differences between the two groups. Differences were considered significant if $p < 0.05$.

3. Results

3.1. Body weight, food intake and feces excretion

The two diets were well accepted by the mice. All mice were generally healthy during the experimental period. Although there was no difference observed in the food intake, mice fed with the Salecan-containing diet showed a significant increase in body weight gain compared to mice fed with the cellulose-containing diet (Table 1). In addition, the fecal output was similar between the two groups (Table 1).

3.2. Histopathological evaluation

No pathological changes were observed macroscopically and microscopically in either the small intestine or large intestine of the mice from the two groups after 4 wk of treatment. No histopathological changes were found in the villi and crypts of the small intestine or in the mucosa of the large intestine in cellulose-fed and Salecan-fed mice (Fig. 1).

3.3. Cecal weight, lactate and SCFAs in the intestinal contents and feces

The total weight, wall weight and contents weight of the cecum were presented in Table 2. The cecal total weight and wall weight were higher ($p < 0.05$) in mice consuming Salecan compared with the cellulose group. In addition, the cecal contents were heavier in mice fed with Salecan, which was most likely due to the presence of a high concentration of viscous Salecan in the diet.

Cecum exhibited the greatest fermentation activity where the highest concentrations of SCFAs were found. In contrast to the cellulose group (~40 μmol total SCFA/g cecal contents), the total SCFA concentration was ~70 μmol/g cecal contents in the Salecan group ($p < 0.05$) (Table 2). Acetate was the predominant SCFA, followed by propionate, butyrate and lactate. The addition of Salecan resulted in higher ($p < 0.05$) concentrations of acetate and butyrate, while the concentrations of lactate and propionate were unaffected. In the colonic contents, with the exception of propionate, both the individual and total SCFAs were greater in mice supplemented with Salecan ($p < 0.05$) (Table 3). The fecal SCFAs were determined every week. The levels of lactate, acetate, propionate, butyrate and the total SCFAs in the feces changed within the two groups during the 4-wk treatment (Table 4). Regarding the comparison between the two

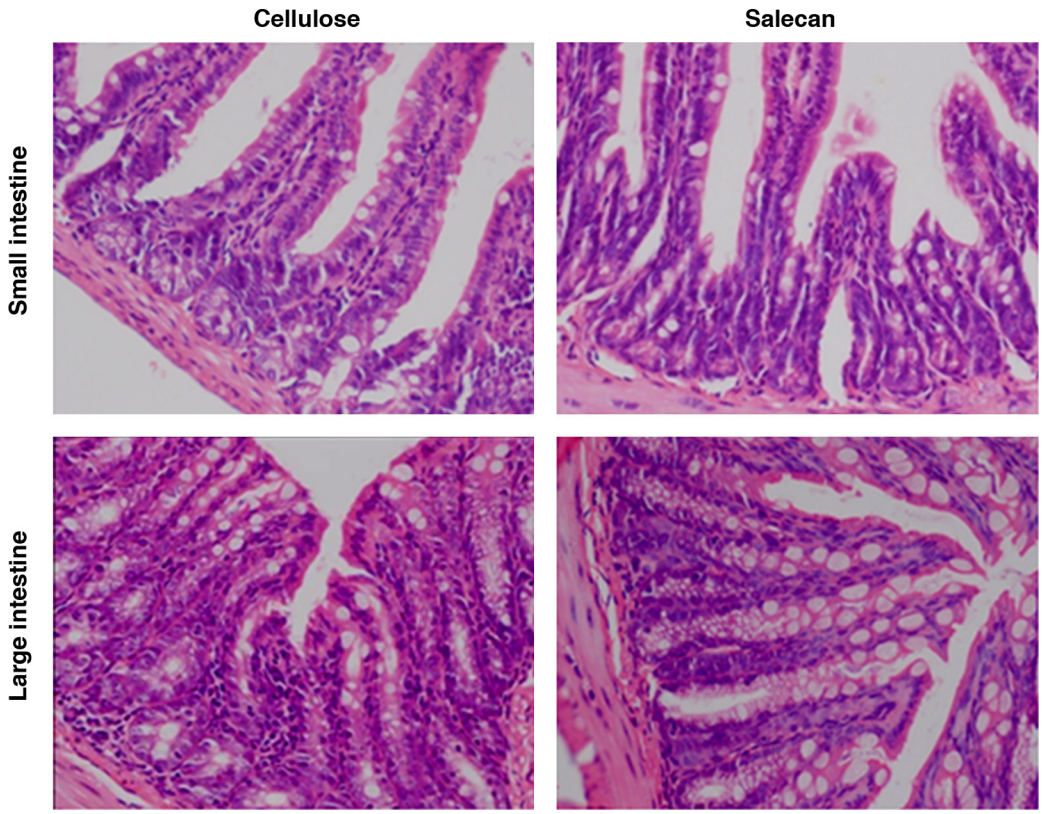

Fig. 1. Photomicrographs of small and large intestine sections stained with HE from mice fed with cellulose or Salecan after 28 d. Original magnification, 400×.

groups, in all sampling points, with the exception of wk 3 and wk 4 for butyrate, the concentrations of total SCFAs, lactate and butyrate were dramatically higher as a result of Salecan consumption.

3.4. Cecal microflora PCR-DGGE profiles

The composition of the cecal microbial community, as reflected using PCR-DGGE profiles generated from the V3 region of bacterial 16S rDNA, differed between the two groups (Fig. 2). The UPGMA dendrogram generated from the DGGE fingerprints revealed clusters with specificity to diet, indicating that feeding of Salecan had an effect on the cecal microflora composition. Pair-wise comparison using the Sorenson index revealed that the cecal microbiota of the mice fed with Salecan was only 72% similar to the control and had a higher degree of uniformity with an average similar of 92% within the individual animals of this group (Table 3 – Supplementary data). The diversity of the microbial population in the cecum was assessed using the number of bands, Shannon index, evenness index and Simpson index. Despite its effect on the composition of the cecal microbiota, Salecan did not conspicuously affect the microbial diversity based on the above four indices (Table 3 – Supplementary data).

3.5. Phylogenetic analysis of nucleotide sequences

Partial 16S rRNA gene sequencing and subsequent phylogenetic analysis revealed that bands 1, 2, 5 and 6 represented species belonging to *Bacteroidetes* and bands 3 and 4 belonging to *Firmicutes* (Fig. 3). Bands 1, 2 and 3, which became more prominent in the Salecan-fed mice, represented the Gram-negative genera of *Parabacteroides* and *Alistipes* and the Gram-positive genus of *Lactobacillus*, respectively, whereas band 4, which was suppressed, was close to the Gram-positive genus of *Coprococcus*.

Table 4
Lactate and SCFAs in feces of mice fed experimental diets for 1–4 wk.

Week	Treatment	Lactate and SCFA concentrations (μmol/g dry feces)				
		Total acids[a]	Lactate	Acetate	Propionate	Butyrate
1	Cellulose	45.25 ± 2.24	4.49 ± 0.36	32.83 ± 1.08	4.93 ± 0.74	2.99 ± 0.64
	Salecan	79.60 ± 6.85*	8.96 ± 1.51*	58.72 ± 4.55*	7.21 ± 1.37	4.70 ± 0.45*
2	Cellulose	47.58 ± 3.94	3.55 ± 0.23	35.58 ± 2.97	4.78 ± 0.64	3.67 ± 0.49
	Salecan	96.59 ± 4.71*	8.98 ± 1.84*	73.97 ± 3.79*	8.21 ± 1.30*	5.43 ± 0.63*
3	Cellulose	36.57 ± 3.18	2.37 ± 0.71	25.50 ± 2.07	4.94 ± 0.57	3.77 ± 0.34
	Salecan	55.97 ± 4.73*	4.52 ± 0.65*	41.14 ± 3.87*	6.50 ± 0.73	3.81 ± 0.29
4	Cellulose	62.89 ± 9.39	5.08 ± 0.72	46.45 ± 8.44	6.14 ± 1.01	5.22 ± 1.04
	Salecan	91.01 ± 9.31*	9.23 ± 1.60*	63.32 ± 6.86	12.39 ± 1.84*	6.07 ± 0.40

Values were expressed as the mean ± SEM of six animals.
[a] Total acids = lactate + acetate + propionate + butyrate.
* Mean values were significantly different from those of the cellulose group ($p < 0.05$).

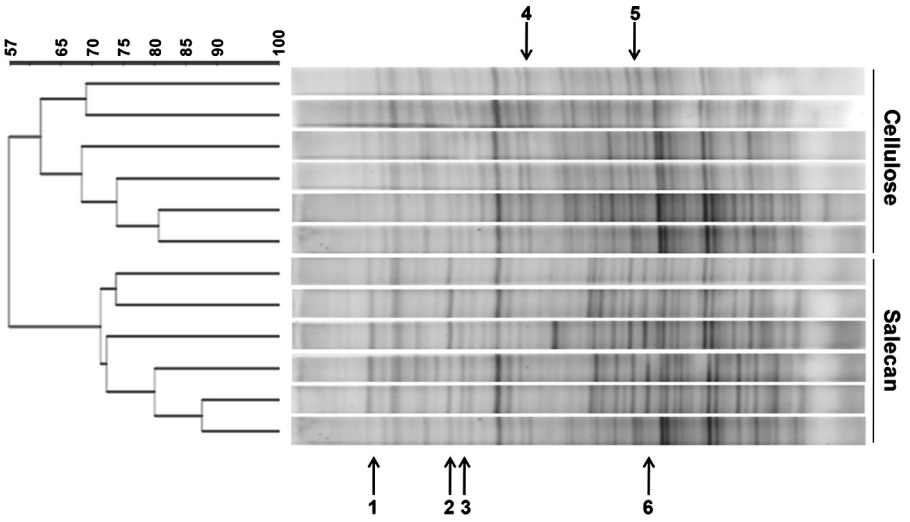

Fig. 2. DGGE profiles of PCR-amplified 16S rRNA gene fragments of the cecal microbiota of mice fed with the cellulose or Salecan diet for 4 wk and a UPGMA dendrogram of the DGGE profiles of the cecal microbiota. Each lane represents the microbial profile of each mouse per group (six animals, respectively). The arrows and numbers indicate the excised and reamplified bands for species identification.

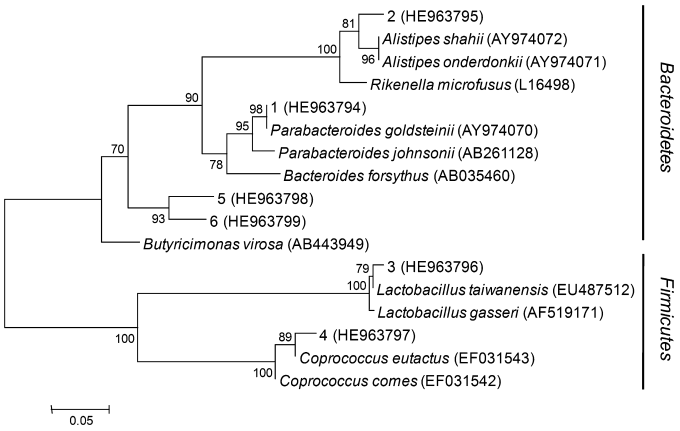

Fig. 3. Unrooted phylogenetic tree constructed using the neighbor-joining algorithms based on fragments of 16S rRNA gene sequences. Reference sequences used in this figure were derived from the NCBI database. Bands 1–6 represent the specimen codes, and the names within the parentheses represent the EMBL accession numbers. The scale bar represented 0.05, estimated nucleotide changes per sequence position. Two different divisions (*Bacteroidetes* and *Firmicutes*) were indicated in the figure.

3.6. Quantification of the bacterial number in cecum

To confirm the effect of Salecan on the cecal microbial community, qPCR was used to determine the levels of *Lactobacillus*, *Bifidobacterium* and *Clostridium* cluster XIVa in the cecal content samples of mice receiving a standard diet supplemented with cellulose or Salecan. The relative amounts of *Lactobacillus*, *Bifidobacterium* and *Clostridium* cluster XIVa, which were presented as a percentage of the control, were shown in Fig. 4. The 16S rRNA gene contents of *Lactobacillus* and *Bifidobacterium* were significantly (3-fold and 6-fold, respectively) higher in mice fed with Salecan (Fig. 4a and b), while there was no statistically significant difference in the *Clostridium* cluster XIVa between the two groups (Fig. 4c).

4. Discussion

β-D-glucans as fermentable dietary fibers have been demonstrated to play an important role in human nutrition. In our previous studies, we showed that Salecan enhances lipid and glucose metabolism (Zhang et al., 2012). In the present study, we evaluated the potential effect of Salecan ingestion on the intestinal tract of mice. We showed that Salecan consumption changed the microbiota composition and raised the SCFA levels in the mouse cecum.

Cellulose was selected as a control due to its very low fermentability. Our study was performed on young rather than adult mice. The Salecan-containing diets did not negatively affect the growth and development of these mice. Interestingly, Salecan consumption resulted in a higher body weight gain without a significant change in food intake. Dongowski, Huth, Gebhardt, and Flamme (2002) reported that body weight was increased more in rats fed with the β-glucan-rich barley-containing diets. The cecal contents and cecal wall weight were remarkably higher in mice fed with Salecan compared to controls, which was consistent with previous studies on fermentable polysaccharides (Brunsgaard, Eggum, & Sandström, 1995; Dongowski et al., 2002). These effects might result from the formation of SCFAs, which are involved in stimulating mucus secretion and normalizing cell proliferation (Campbell, Fahey, & Wolf, 1997; Finnie, Dwarakanath, Taylor, & Rhodes, 1995).

β-Glucans, which enter the cecum are poly- and oligomers that have escaped digestion in the upper part of the gastrointestinal tract. Breakdown of β-glucans in the large intestine is a complex process involving various enzymes from many different species and cross-feeding by the microflora (Cumming & Mcfarlane, 1991). Based on structural analysis, Salecan is a linear β-glucan, which consists of subunits consisting of seven residues of (1, 3)-linked β-D-glucose separated by two residues of (1, 3)-linked α-D-glucose with an average molecular weight of 2×10^6 (Xiu et al., 2010). Similar to other β-glucans, Salecan was not absorbed in the small intestine and was rapidly fermented by the intestinal microflora, resulting in a higher cecal total SCFA concentration in mice fed with Salecan compared to controls. Cecum is the principal site of fermentation in mice (Breves & Stuck, 1995), and the SCFAs are rapidly absorbed from the gut lumen as indicated by the decline in SCFA concentrations during passage through the large bowel. In our study, the SCFA concentration was selected to expressed as μmol/g contents rather than mmol/L to more accurately reflect cecal fermentation, which was also observed by Berggren, Björck, Nyman, and Eggum (1993).

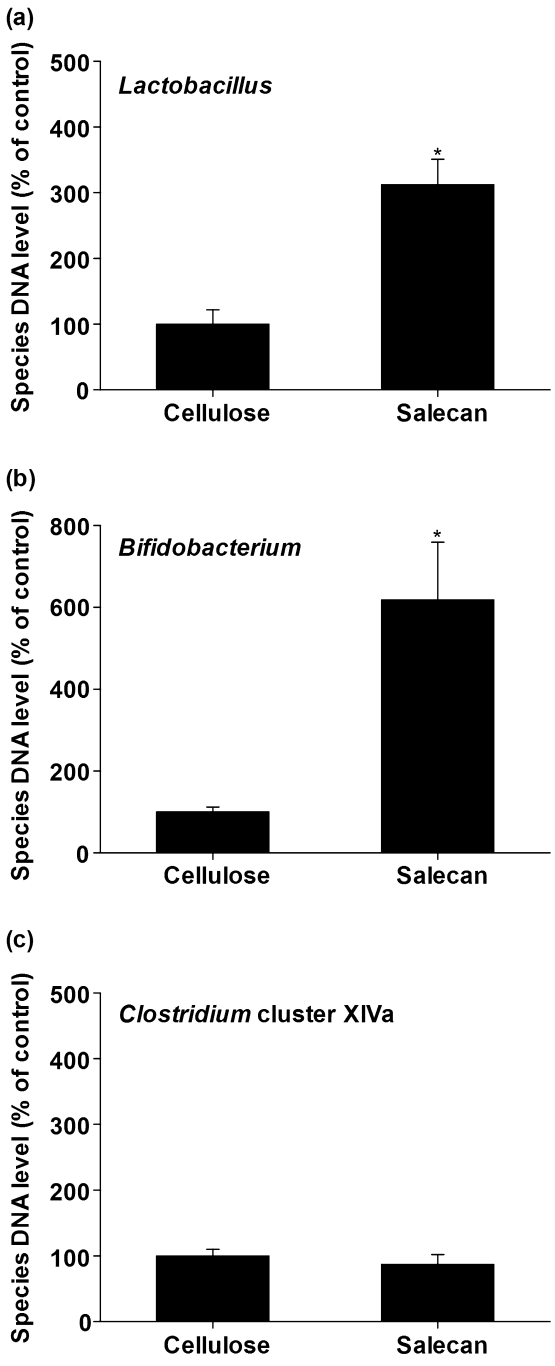

Fig. 4. Relative amount of target gene in the cecum contents of cellulose- and Salecan-fed mice. Target genes encoded either 16S rRNA from *Lactobacillus* (a), *Bifidobacterium* (b) or *Clostridium* cluster XIVa (c). DNA amount in the cellulose group was set to 100%. Data were presented as the mean ± SEM, $n = 6$. $^{*}p < 0.05$.

Importantly, Salecan significantly increased the fecal lactate concentration at wk 1 and this effect was maintained over time, which indicated that lactic acid producers were most likely affected after Salecan intake. Among lactic acid bacteria, the most concerns are focused on *Lactobacillus* and *Bifidobacterium* with regard to the promotion of human health, including the production of vitamins and inhibition of the growth of potential pathogens (Gibson & Roberfroid, 1995). The effects of dietary fibers on the intestinal microflora are dependent on their structural characteristics. Salecan consists of a large amount (~78%) of β-(1, 3)-D-glucosidic linkages in main bone structure. The interesting effect of Salecan consumption was the enrichment of lactobacilli as confirmed using both PCR-DGGE and qPCR, which confirmed previous observations that barley β-glucan with (1, 3) and (1, 4) linkages favored the growth of lactobacilli in the cecum of rats (Snart et al., 2006) and piglets (Pieper et al., 2008). Short-chain β-glucans, which are released from the β-glucans by hydrolysis, are catalyzed by extracellular β-glucanases secreted by the other cecal residents, and are the substrates that produce the 'lactobacillogenic' effect in the cecum (Jaskari et al., 1998; Snart et al., 2006). In our study, at the genus level, *Parabacteroide* and *Alistipes* were also enriched in the Salecan group. Although speculative at this point, Salecan also contains a small proportion (~22%) of α-(1, 3)-D-glucosidic linkages, which may provide growth substrates for some strains displaying α-glucosidase activity, such as *Parabacteroide* (Sakamoto & Benno, 2006) and *Alistipes* (Song et al., 2006). Oligosaccharides released from Salecan by hydrolysis catalyzed by these α-glucosidases, might further provide growth substrates for lactobacilli. In addition, a remarkable increase in *Bifidobacterium* in the cecal contents after 4 wk of Salecan ingestion was also observed in our study. To the best of our knowledge, such an effect of fructo-oligosaccharide has been well established; however, for β-glucans, it has been inconsistent (Hughes, Shewry, Gibson, McCleary, & Rastall, 2008). The unique structure of Salecan might be involved in the factors mediating this effect. The effect on *Bifidobacterium* was not revealed from DGGE profiles, which might be due to the limitations of the PCR-DGGE (Muyzer & Smalla, 1998). Unexpectedly, there was no large difference in the level of cecal lactate between the two groups at the end of the experiment. Dongowski et al. (2002) found a decrease in cecal pH in rats fed with the barley-containing diets as a result of the increased concentrations of SCFAs. A low luminal pH may promote the growth of lactic acid bacteria, including *Lactobacillus* and *Bifidobacterium* (Le Blay, Michel, Blottière, & Cherbut, 1999). However, lactate is recognized as an intermediate in the global fermentation process in microbiota, and is to varying extents metabolized to SCFAs, such as acetate and butyrate, by cross-feeding species in the ecosystem (Macfarlane & Macfarlane, 2003). Potentially, cecal microflora adapted to the continuous intake of Salecan, promoting the conversion of lactate by lactate-utilizers. These lactate-utilizers might progressively adapt to the acidic conditions. Thus, the lactate did not accumulate to a significant extent in the cecal contents of mice fed with Salecan for 4 wk.

Among the SCFAs, butyrate is perceived as a key factor for gut health. In addition to being the preferred energy source for colonocytes, it also promotes apoptosis and inhibits colorectal cancer (Pryde, Duncan, Hold, Stewart, & Flint, 2006). Cecal butyrate was specifically elevated in mice fed with Salecan, which is consistent with previous reports in vivo (Dongowski et al., 2002) and in vitro (Kim & White, 2009). The factors mediating this butyrogenic effect, such as chemical structure, transit time and the release of intestinal mucins, are most likely involved (Le Blay et al., 1999). The growth and activity of butyrate-producing bacteria are likely also promoted by Salecan. These bacteria may utilize Salecan either directly or metabolize lactate produced during Salecan fermentation. On the one hand, butyrate-producing bacteria numerically belong to the *Clostridium* cluster XIVa (Pryde et al., 2006). Metzler-Zebeli, Zijlstra, Mosenthin, and Gänzle (2011) reported that β-glucan decreased the number of the cluster XIVa. However, in our study, Salecan did not affect the relative amount of cluster XIVa gene copies in cecal contents analyzed using qPCR but resulted in a lower level of *Coprococcus*, as indicated using PCR-DGGE. Potentially, Salecan fermentation is more efficient by cluster XIVa due to its unique structure, resulting in a high level of cecal butyrate without changing the level of cluster XIVa. In addition, considering the PCR-DGGE limitations, Salecan might also promote the growth of other butyrate-producing bacteria, which may utilize β-glucan. On the other hand, butyrate production may also occur through lactate conversion by cross-feeding species in the ecosystem (Pryde

et al., 2006). This type of cross-feeding might contribute to the reported butyrogenic effect of specific dietary substrates, such as resistant starch. Similarly, the butyrogenic effect of Salecan is potentially due to promoting the growth or metabolic activity of butyrate-producing bacteria with lactate, as suggested above. Furthermore, to some extent, it may be concluded from the results of cecal butyrate and fecal butyrate after 4 wk of treatment that more butyrate was taken up by the colonocytes in the Salecan group.

5. Conclusions

The data presented in this study clearly demonstrate that Salecan exhibits a variety of beneficial physiological effects in the intestinal tract of mice. The increased SCFA production and the increased cecal weight observed in the Salecan-fed mice indicated increased cecal fermentation, which is considered beneficial for gut health. Moreover, Salecan was preferentially fermented by lactobacilli and bifidobacteria and affected the gut microbiota balance to positively direct metabolic activity toward increased colonic production of butyrate. Overall, this study suggests that Salecan contributes to the health of the individual through dramatic adjustments in the intestinal microbiota and may be used as a functional dietary food supplement. Given that some bacterial enzymes, such as β-glucuronidase, have a close relationship with gastrointestinal health (Licht et al., 2010; Shen, Dang, Dong, & Hu, 2012), further studies may be performed to determine the effect of Salecan on intestinal bacterial enzymes.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.10.091.

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