Effects of yacon (*Smallanthus sonchifolius*) tuber on physiology, fermentation products, and intestinal microbial communities in rats

(ヤーコン(*Smallanthus sonchifolius*) の根がもたらす効果

－生理機能、発酵生産物、ラットの腸内微生物叢の変化－）

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The beneficial health effects of prebiotics have led to increasing studies of microbial ecology in human and animal guts to better understand the links among food, gut microbiota and overall health. Two approaches are being used to include prebiotics in the diet. One approach is to add prebiotic compounds to commonly consumed foods. The other is to include functional foods, food naturally containing high concentrations of prebiotics, in the diet. Yacon (*Smallanthus sonchifolius)* tuber was examined with regard to its prebiotic effects compared with commercialized fructo-oligosaccharides (FOS). We conducted two different studies to assess the prebiotic effects of yacon tuber. First study, a single blind, placebo-controlled, randomized cross-over study, was undertaken to determine the potential benefits of cookies contain yacon tuber on the human physiology and intestinal microenvironment such as short chain fatty acid fermentation product, fecal microbial communities, serum lipid, and glucose profile. In addition, bowel habit, stool condition and food intake were kept in daily diary by the subjects. The second study was conducted in rats with the aim to compare the physiological and microbiological effects of yacon tuber with commercialized FOS.

Both of our results demonstrated that by consuming 10% of yacon tuber powder and cookies contain 14.75% yacon tuber (equivalent to 5% and 9.5% of FOS, respectively) every day for 28 days resulted in certainly changes in the bowel habit, cecum size (of rats), SCFAs production and intestinal microbial community significantly different from the consumption of commercialized FOS and placebo cookies.

Regarding of the physiological effects, significant changed in bowel habit or stool condition such as increasing of stool quantity, defecation frequency, softer stool, and changed

of color to be yellowish shown that yacon tuber can be acted as an anti-constipation. A significant increased in concentration of acetate, propionate and butyrate (SCFAs) was observed following yacon-cookies consumption in human compare with placebo cookies and in rats fed yacon tuber compare with those of fed FOS. It indicates that yacon tuber was undergoing abundant fermentation in human colon and rat's cecum, resulted in the acidic fecal and cecal pH, produced a selective environment for the intestinal microbiota. Eventhough the amount of yacon tuber in cookies almost similar with rat's diet but the result of SCFAs was different. Acetate, propionate and butyrate concentration of rat's cecal was higher than human fecal. The different result might come from the different site of fermentation and SCFAs absorption while passed the large intestine. Fermentation of non-digestible saccharides was occurred in human colon while in rats it was occurred in cecum. SCFAs were directly absorbed in the large intestine, especially butyrate. During their transport to the anal, some SCFAs were absorbed and the concentration might be decrease when it reached the end of the colon. It means that the fecal SCFAs might not represent the actual intestinal fermentation condition and pH. The pH values were lower than those reported previously in others study using lower yacon-FOS concentrations in animal models whereas few human studies have been done. However, in our result of pH human fecal samples were almost similar to pH of rat's cecal samples. In comparison with placebo cookies and commercialized FOS, the acetate concentration was produced higher, followed by propionate and butyrate during yacon-cookies period and yacon tuber groups of rats. The dramatic change of lactate was related to bacteria fermented in yacon tuber or FOS. Protein fermentation is considered to be a non beneficial process within the colon. Following yacon tuber consumption significantly increased of iso-valerate and valerate concentration was observed, which can therefore be viewed as potentially positive effect as these organic acids are associated with protein fermentation.

The MDS scatter plot in rats showed that the microbial communities were different between fecal and cecal samples and between diets. In the human study, microbial communities in fecal was very different between each subject and changes related to diet was seen only in 4 out of 13 subjects. The microbiota in human are more diverse than rats, and this was affected not only by the yacon diet but also their normal diet, while in rats we can restrict the rat's food. Fewer number of bands in the PCR-DGGE profiles in yacon tuber revealed that

fermentation of these substrates was selectively increased microbial communities in feces and cecum.

Although there have been a few studies examined the prebiotic effects of yacon tuber, as our knowledge, only one study was conducted in human trials, while the others were using animal models. For bacterial identification, they used a cultivation approach targeting specific genera such as *Bifidobacteria* and *Lactobacilli*. Whereas in our study, both in human or rats study, we used molecular genetics based method; 16S rRNA gene PCR-DGGE and demonstrated that the prebiotic effect of yacon tuber contain in cookies or in rat's diet clearly different from control or placebo and bacteria with high GC-content; *Bifidobacteria* was increased in yacon diet. Genus *Bifidobacteria* have been known as beneficial bacteria.

We did not detect any *Lactobacilli* and *Barnesiella* spp. in human fecal samples. *Lactobacilli* were less abundant in human intestinal; while in rats *Lactobacilli* were the main beneficial bacteria. *Barnesiella* spp. was found only in rats fed yacon diet; this genus up to now has not been previously associated with yacon or FOS fermentation using the conventional cultivation technique. *Barnesiella* spp. was recently cultivated from the chicken gut, rumen gut, and human stool samples. *Barnesiella* spp. has recently been identified in the ruminal content and the increase of these bacteria was affected by starch addition. Yacon tuber contains resistant starch, the growth of *Barnesiella* spp. might be because of resistant starch. Oligofructose is a dietary fiber with potential for clinical application. FOS resists digestion by gastric acid and pancreatic enzymes in vivo. A key property of these fibers lies in the indigestibility of oligosaccharides, which give rise to fermentation in the large intestine followed by an increase in *Bifidobacteria* and *Lactobacilli*.

In conclusion, yacon tuber as functional food, naturally contain high amount of prebiotic. The gut microenvironment of human and rats fed the yacon tuber diet clearly differed from those fed the FOS and control diets. Yacon tuber exhibited a prebiotic effect by promoting the growth of *Lactobacilli* and *Bifidobacteria*, resulting in a greater concentration of SCFAs and lower pH. Using culture-independent analysis, 16S rRNA gene PCR-DGGE combined with cloning and sequencing, the difference in the prebiotic effect of yacon was substantiated by finding a band with a sequence most closely related to a *Barnesiella* sp. in rat's cecum. This genus has not been reported to be involved in yacon or FOS fermentation using the conventional cultivation technique. Our results revealed that yacon tuber

consumption might play an important and slightly different role in colonic health maintenance compared with other FOS sources. This suggests that dietary advice on the consumption of non-digestible carbohydrates might need to be personalized in the future.