ISSN (Print) 1225-9918
ISSN (Online) 2287-3406
Journal of Life Science **2012** Vol. 22. No. 11. 1571~1586 DOI : http://dx.doi.org/10.5352/JLS.2012.22.11.1571

- Review -

Use of Cellulose and Recent Research into Butyrate

Tae Jong Yeo[1], In Soon Choi[2]* and Kwang Keun Cho[1]*

[1]*Department of Animal Resources Technology, Gyeongnam National University of Science and Technology, Gyeongnam 660-758, Korea*
[2]*Department of Biological Science, Silla University, Busan 617-736, Korea*

Received October 4, 2012 / Revised October 15, 2012 / Accepted October 19, 2012

On earth, there are about 5,400 kinds of mammals, of which about 1,000 kinds are herbivores. Among herbivores, about 250 kinds are known to be ruminants. As for cattle and sheep, which are ruminants, fermentation takes places mainly in their rumen; in contrast, for pigs and men, which are non-ruminants, fermentation takes place mainly in their caecum, colon, and rectum. As for the kind and dominance of rumen microorganisms, *Bacteroidetes* account for 51% and *Firmicutes* for 43%. As for the dominance of the large intestine microorganisms in men, *Firmicutes* account for 65% and *Bacteroidetes* for 25%. Cell wall components are decomposed by microorganisms, and short chain fatty acids (SCFAs) are generated through fermentation; the ratio of acetate, propionate, and butyrate generate is 60:25:15. Butyrate absorbed through the primary butyrate transporter MCT1 (mono carboxylate transports-1) in the intestines activates such SCFA receptors as GPR43 and GPR41. Butyrate has a strong anti-tumorigenic function. Butyrate is characterized by the fact that it has an effect on many cancer cells, contributes to the coordination of functions in the cells, and induces cancer apoptosis. Butyrate activates caspase but inhibits the activity of HDAC (histone deacetylase), so as to induce apoptosis. In addition, it increases p53 expression, so as to induce cell cycle arrest and apoptosis. Anti-inflammation actions of SCFA include the reduction of IL-8 expression in intestinal epithelial cells, the inhibition of NO synthesis, and the restraint of the activity of NF-kB (nuclear factor kB), so as to suppress the occurrence of cancers caused by inflammation. Butyrate plays an important role in maintaining physiological functions of intestinal mucous membranes and is used as a cure for inflammatory bowel disease (IBD).

Key words : Cellulose, microorganism, butyrate, apoptosis, cancer

Digestive organ of ruminant and mono-gastric animals

Ruminants are referred to as so because of their rumination or special digestive organ (the stomach), in which cud can be chewed. Ruminants are the most advanced animals of ungulates and the most multiplied and prevalent throughout the world. They have a rumination, which is a very peculiar digestive organ and possibly enables them to have survived poor vegetation conditions, allowing them to flourish greatly. The stomach of all ruminants is divided into four compartments including the rumen, reticulum, omasum, and abomasums (of which rumen and reticulum are involved in rumination and called rumen). Rumination is a process in which food, once swallowed, is emitted back unto the mouth for re-chewing; of feeds returned into reticulum via rumen, those of which over a certain size will be returned again into rumen, accumulated, and regurgitated to the mouth. The regurgitated feeds will be smashed finely between molar teeth and mixed with saliva in the mouth, then returned to rumen for fermentation. Ruminants include cattle, sheep, goats, deer, musk deer, giraffes, and impalas.

Pseudo-ruminants have a similar stomach to rumen in which microorganism fermentation takes place, but their stomach is composed of three compartments since omasum and abomasums are not specialized. Pseudo-ruminants include animals that belong to the family of camel (camel, lama, and alpaca) and the family of chevrotain (chevrotain and mouse deer). Non-ruminant herbivores are animals that are herbivores and have not developed rumen (but the caecum and others have), with which fiber materials are digested and fermented for gaining nutriments. They include horse, rabbit, elephant and hippopotamus [94].

When ruminants eat food they chew the cud, which is a process for earning energy from cell wall substances (cellulose) of plants. In other words, cellulose is finely crushed and decomposed through microorganisms in rumen, and

***Corresponding author (Kwang Keun Cho)**
Tel : +82-55-751-3286; Fax : +82-55-751-3689
E-mail: chotwo2@gntch.ac.kr

***Co-corresponding author (In Soon Choi)**
Tel : +82-51-999-5647, Fax : +82-51-999-5644
E-mail: ischoi@silla.ac.kr

it is metabolized to be used as an energy source. Until the present, a focus has been placed only on the fermentation taking place in rumen of ruminants in relation to the digestion process. However, as can be seen in Table 1, much of the fermentation takes place not only in rumen of cattle but also in the caecum, colon, and rectum of pigs and men. An investigation was made into the ratio of intestines versus all digestive intestines where fermentation takes place, and it was found that the ratio is 83% for sheep, 75% for cattle, and 69% and 61% for pig and man, respectively, which are mono-gastric animals. The fermentation takes place mainly in rumen of ruminants like cattle and sheep, while it takes place mainly in the caecum, colon, and rectum of mono-gastric animals such as pigs and men. More than we have conceived, the fermentation takes place in digestive intestines of pig and man. Since a lot of fermentation takes place in men, the concept of dietary cellulose for adjusting the fermentation in intestines appeared long ago to maintain the health of the digestive organs of men, and the importance of the concept is steadily increasing. Many microorganisms exist in each digestive intestine and they have an effect on the health of the intestines including the damage, development, and digesting & absorbing abilities of intestinal epithelial cells; therefore, it is very important to maintain the health of the intestines with the help of intestinal microorganisms [90]. Moreover, obesity may be induced by such microorganisms that are dominant in the intestines. Checking of the kinds of microorganisms existing in the intestines of obese patients, *Firmicutes* accounts for more than 90% and *Bacteroidetes* no more than 3%; *Bacteroidetes* accounts for 30% in men whose body weight is normal. As obese men lose their weight by means of dietary adjustment, intestinal *Bacteroidetes* increases and *Firmicutes* decreases while the intestines of the men resemble those of slender men [55].

Ruminant microorganisms

It is known that more than 200 kinds of rumen bacteria number 10^{10}~10^{11} per ml and account for more than 50% of the total quantity of microorganisms. Rumen bacteria primarily secrete such enzymes that can digest the cellulose of grass feed, and their life is 20 minutes~3 hours. Protozoa are larger than bacteria and exist in gastric juice in the number of 10^{6} per ml, accounting for 50% of the total quantity of microorganisms and 2% of the contents in the stomach. The rumination can be operated without protozoan but function more smoothly with protozoa. Protozoa undertake about 20~40% of starch digestion and are thoroughly anaerobic, with a lifespan of 8~36 hours. Fungi account for 8% of the total quantity of microorganisms and are important microorganisms that stick to plants and decompose cellulose. They disintegrate a small quantity of lignin into small particles, so that bacteria may digest them and have high activity in cellulose and hemicellulose; their germination cycle is 24 hours [94].

In order to identify the kind and dominance of rumen microorganisms, 16 Holstein cows were bred in the same conditions, then DNA was extracted from rumen microorganisms and applied with a pyrosequencing technique in which V2 and V3 regions of 16s rRNA were amplified. As result, it was found that *Bacteroidetes* existed in the ratio of 51%, *Firmicutes* 43%, *Proteobacteria* 5.21%, *Actinobacteria* 0.87%, and *Tenericutes* 0.68% [44].

Microorganisms in the intestines of men

500~1,000 kinds of microorganisms live in the intestines of adult men. From the stomach down to the large intestine, increasingly more anaerobic microorganisms live; from the stomach upwards, increasingly more aerobic microorganisms live. Microorganisms exist in the stomach in the amount of 10^{2} cfu/ml, in the duodenum $10^{1\text{-}3}$ cfu/ml, in the jejunum $10^{3\text{-}4}$ cfu/ml, in the ileum $10^{7\text{-}9}$ cfu/ml, and in the large intestine $10^{10\text{-}12}$ cfu/ml. Fiber material of food taken into the body is fermented in the large intestine and produces SCFAs (Fig. 1) [18,21,28]. As can be seen in Fig. 1, compared to the other digestive organs, microorganisms exist in the large intestine the most. According to a recent study that conducted a biopsy into the fecal and the large intestine of men, 9 kinds of microorganisms (*Firmicutes,*

Table 1. Relative area of digestive intestines where fermentation takes place in each animal (%) [12]

Animal	Rumen	Caecum	Colon/rectum	Total
Cattle	64	5	5~8	75
Sheep	71	8	4	83
Pig	-	15	54	69
Man	-	32	29	61

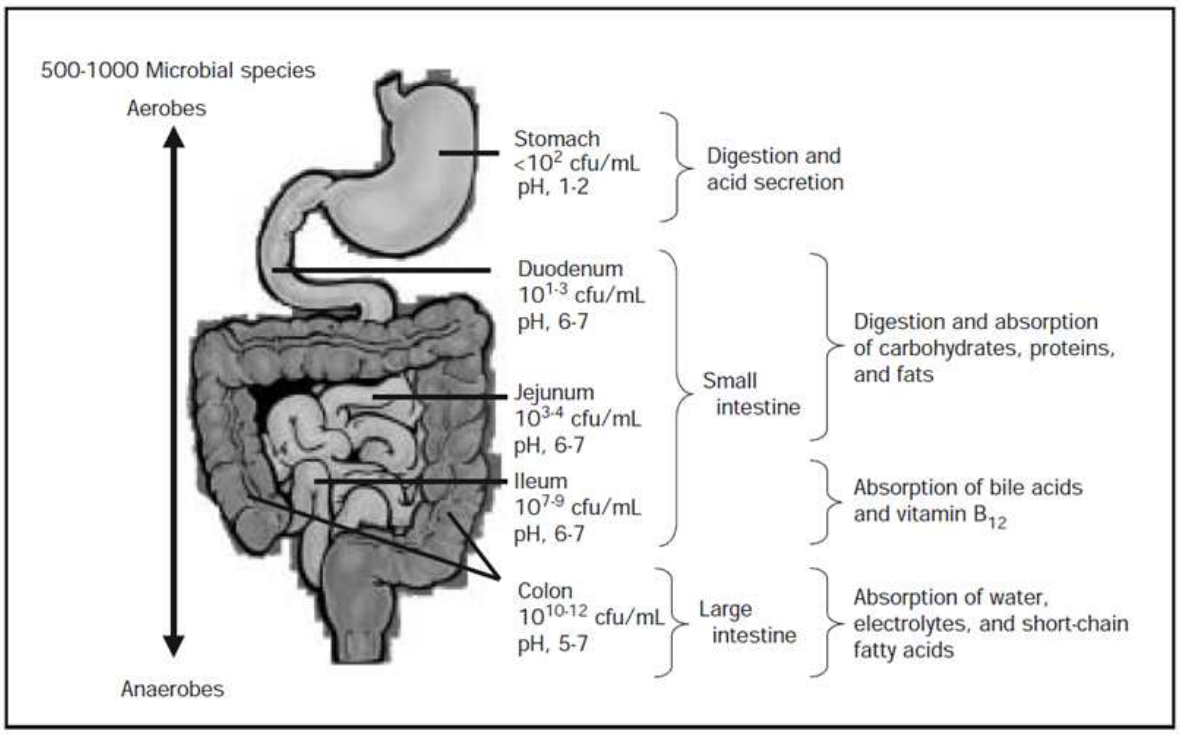

Fig. 1. Key physiologic and microbiological features of the gut [21]. Relative concentrations of bacteria and the pH at various locations within the adult gut are also noted. cfu: colony forming unit.

Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicrobia, Cyanobacteria, Spirochaetes, and *Vadin BE* 97) exist in terms of the phyla [3,18]; and as for the dominance of microorganisms, *Firmicutes* accounts for 65%, *Bacteroidetes* 25%, and *Proteobacteria, Actinobacteria, and Fusobacteria* account for the remaining 10% [1,17,24,85].

Microorganisms that decompose polysaccharide

In omnivorous mammals (i.e., men), less than 10% of energy is generated through the fermentation in the large intestine, but microorganisms in the large intestine play a very important role for the health of omnivorous animals. Microorganisms generate butyrate, which is a SCFA and prevents colon cancer in mice and men [60,68]. Bacteria in the large intestine bring on various metabolic changes and many mutual actions, which involve immune actions of the host [23,38]. In rumen, fungi and protozoa play an important role in decomposing the cellulose of plants; in omnivorous mammals, intestinal microorganisms perform this role. The diversity of microorganisms can be identified against the nucleotide sequence of 16s rRNA. A comparison was made of the number of phylum of microorganisms in the large intestine and rumen of men, pig, and horse; it was found that *Firmicutes* and gram-negative *Bacteroidetes* exist in more than 90% of the phylum [19,79]. A recent study compared intestinal microorganisms in obese men and normal men, and found that the dominance ratio of 2 kinds of bacteria (*Firmicutes* and *Bacteroidetes*) is different; also, when a comparison is made of mice, microorganisms (*Firmicutes*) dominant in obese mice generate more energy from taken feed than microorganisms (*Bacteroidetes*) dominant in normal mice do [55]. Intestinal microorganisms directly decompose non-digested cell walls of plants. Cell walls of plants consist of cellulose, hemicellulose, pectinaceous material, and protein. Bacteria that can decompose cellulose decompose cellulose and bacteria that cannot do so decompose other substances. When feed is taken in, various kinds of polysaccharide move into the intestines and rumen. There are various polysaccharides including oligosaccharide, inulin which is a storage polysaccharide, resistant starch (RS) which is a polymer protecting cell walls, and xylan and pectin which are cell wall polysaccharides. Therefore, there are a greatly increasing number of microorganisms that can decompose polysaccharides. As seen in Fig. 2, it was found

that, when polysaccharides such as cellulose, inulin, resistant starch (RS), and xylan are administered to cattle and men, different microorganisms increase in rumen and intestines depending upon the polysaccharide. Cellulolytic bacteria can decompose such polysaccharides as xylan, mannan, pectin, and cellulose; they do not employ the substances which are melted through decomposition but decompose cellulose so that other microorganisms may use it. For instance, hydrogen generated by polysaccharide decomposition bacteria is used by methanogens and acetogens to generate methane and acetate; when the hydrogen concentration is reduced, the generation of hydrogen is reduced because the amount of hydrogen-generated cellulolytic bacteria is increased. When cellulolytic bacteria like *Ruminococcus* and *Fibrobacter* stick to the surface of plant cell walls and decompose hemicellulose and cellulose to generate solubilized oligosaccharides and polysaccharide, *Butyrivibrio* spp. and *Roseburia* spp. synthesize butyrate. Also, *Bacteroides* spp. and *Prevotella* spp. use solubilized oligosaccharides and polysaccharide to compose propionate (Fig. 3) [11]. *Firmicutes,* which mainly exist in rumen and feces of men, are *Ruminococci* and belong to the *Clostridium leptum* group (*Clostridial cluster IV*); there are many *Firmicutes* in solids and many *Bacteroidetes* in liquids [78,93]. When xylan and resistant starch (RS) are administered, *Butyrivibrio* spp. and *Roseburia* spp. (which are butyrate-generating bacteria) increase. A comparison was made of gram-positive bacterium (*R. flavefaciens*) a polysaccharide decomposition bacterium found in rumen, and gram-negative bacterium (*B. thetaiotaomicron)* a starch decomposition bacterium found in the intestines of men; it was found that *R. flavefaciens* belongs to *Firmicutes* or *Clostridial cluster IV* and is a representative cellulolytic bacterium in rumen that *R. flavefaciens* generates 236 glycoside hydrolases, 15 polysaccharidelyases and 20 carbohydrate esterases, and that the generated enzymes have a complementary part to polymers which exist in cell walls of plants, there with sticking to cell walls and decomposing polysaccharides of cell walls [31]. *B. thetaiotaomicron* is a representative cellulolytic bacterium in intestines of men and generates enzymes such as 236 glycoside hydrolases and 15 polysaccharidelyases, and a starch-utilization gene cluster (sus) consisting of 8 genes. In external cell membranes of

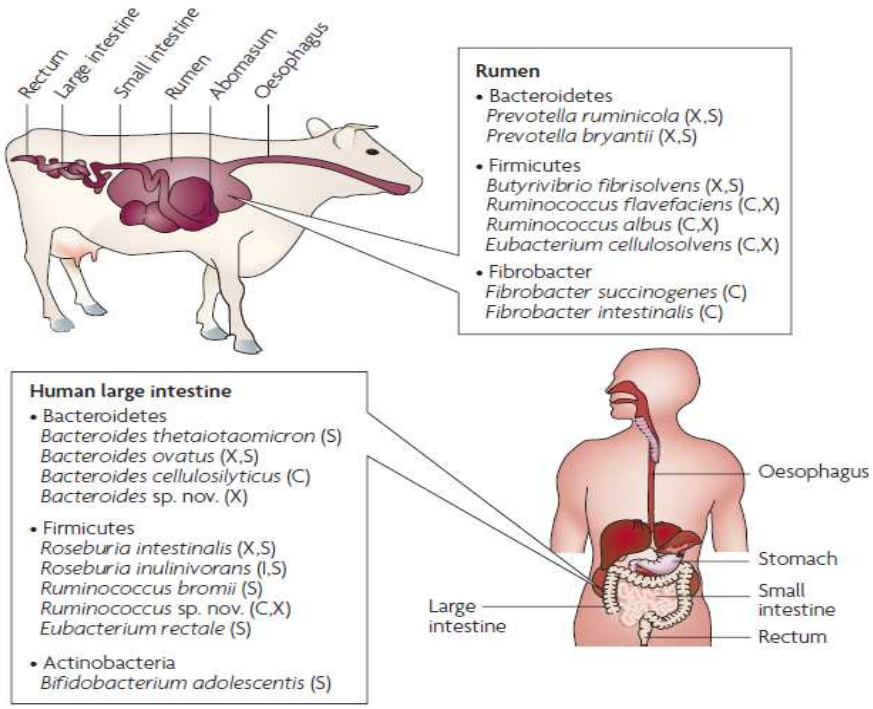

Fig. 2. Polysaccharide-degrading bacteria in the ruminant and human gastrointestinal tracts [27]. The major sites of microbial breakdown of dietary polysaccharides, which also support the highest densities of bacteria, are the rumen in ruminant animals and the large intestine in humans. Examples of cultured polysaccharide-degrading species are shown for these sites, together with the phylum to which they belong (*Firmicutes* or *Bacteroidetes*) and their characteristic polysaccharide-utilizing abilities. Much of the diversity remains undefined, however, and new species of polysaccharide-utilizing bacteria have been described recently in the human colon. C, cellulose; I, inulin; S, starch; X, xylan.

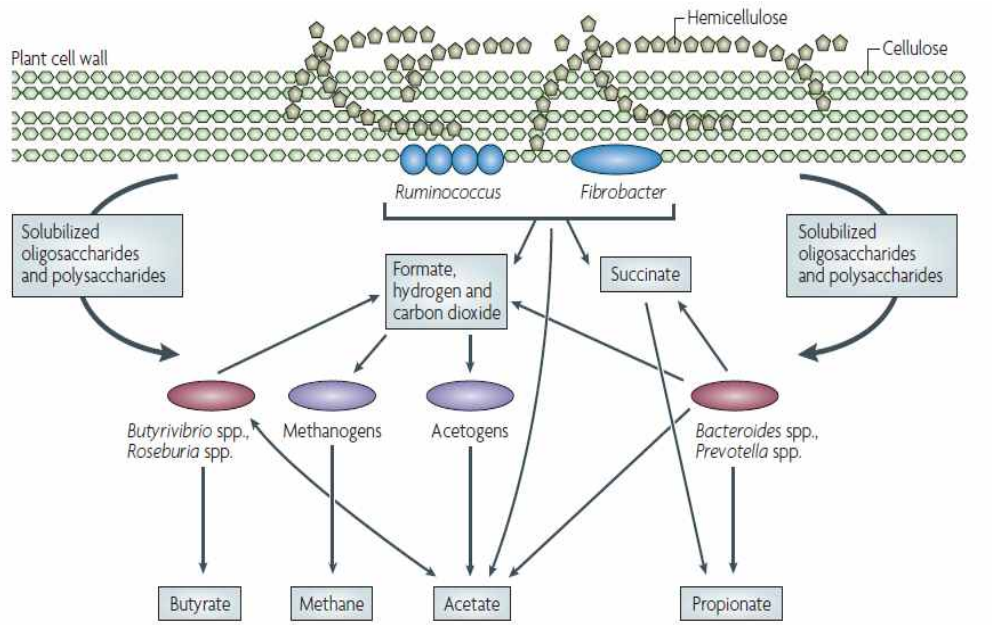

Fig. 3. A simplified schematic illustrating the relationships between primary degraders of insoluble plant fiber and other members of gut microbial commmono-gastricies [27]. Specialist cellulolytic species of *Ruminococcus* and *Fibrobacter* are shown closely attached to the substrate. Other bacteria that is able to use soluble polymers, for example, butyrate-producing *Butyrivibrio* spp. And *Roseburia* spp., succinate-producing *Bacteroides* spp. and *Prevotella* spp., hydrogen-utilizing methanogenic archaea and acetogenic bacteria, are also shown. This diagram is illustrative and not intended to provide a complete description, as the number and diversity of primary degraders, polysaccharide utilizers and other functional groups within different gut communities is still emerging.

B. thetaiotaomicron, there are maltose-inducible outer-membrane proteins such as susC, susD, susE and susF; susC and susD proteins catch starch. susC can hold molecules including maltose (G2) and maltoheptaose (G7), and is decomposed by susG amylase (neopullulanase) and enters into periplasm. Neopullulanase disintegrates amylose, amylopectin and pullulan into an oligosaccharide, which can enter into periplasm. With the help of a transporter, which is decomposed by susA amylase of periplasm and that exists in cytoplasmic membranes, the oligosaccharide enters into cytoplasmic membranes (Table 2) [27].

SCFAs

Ruminants cannot secrete enzymes that disintegrate cell wall components of plants, but instead have a mutual relationship with anaerobic microorganisms that can produce such enzymes. Various kinds of microorganisms completely decompose cell wall components, which are hydrolyzed by enzymes secreted from rumen microorganisms and are converted into monosaccharide. Then, the monosaccharide is again fermented and changed into SCFA's [acetate (C2), propionate (C3), butyrate (C4), and valerate (C5)], which are used as an energy source by ruminants. Within rumen, grass feed that cannot be digested by animals are attacked by various microorganisms and decomposed into a size to be absorbed through the stomach walls of cattle; generated microorganisms again enter into the small intestine, where they are decomposed by protein decomposition enzymes and used as a nutriment. The large intestine of non-ruminant herbivores (horse, rabbit, etc.) is an important portion that is digested by microorganisms. The most outstanding characteristic of herbivores, which use cellulose as an energy source, is that part of a specific intestine that is needed for microorganisms to ferment feeds is enlarged. The digestion of cellulose is totally dependent upon the activities of anaerobic microorganisms in the caecum and the colon. Most of the intestinal contents reach the large intestine within 3 hours after feed is taken; substances that are not digested in the small intestine are decomposed and fermented while they stay in the large intestine for a long time. Yet, since the substances pass through the large intestine faster in ruminants, the ability of herbivores to digest cellulose is inferior to that of ruminants [8,9,13].

Table 2. Genome sequences concerned with plant polysaccharide breakdown in four species of gut bacteria [27]

	Bacteroides Thetaiotaomicron 5482	*Bifidobacterium Longum* NCC 2705	*Ruminococcus Flavefaciens* FD1	*Fibrobacter Succinogenes* S85
Location	Human colon	Human colon	Rumen, cellulolytic	Rumen, cellulolytic
Genome size	6.26 Mb (complete genome)	2.26 Mb (complete genome)	Approximately 4Mb (partial genome; dockerin-encoding genes only)	3.8 Mb (complete genome)
Number of glycoside hydrolases*	236 (40)	47 (17)	65 (14)	104
Number of polysaccharide lyases*	15 (7)	0	12 (4)	4
Number of carbohydrate esterases*	20 (9)	1	23 (5)	14
Number of carbohydrate binding modules*	16 (3)	10 (5)	61 (12)	Limited information available
References	29	49	63	51,58

*The number of enzyme families that are represented is shown in brackets (CAZY (carbohydrate-active enzymes) database; see further information). Mb: megabases.

Almost all SCFA's generated in rumen through the fermentation of carbohydrates are absorbed in rumen by means of simple dissemination, and the remainder is absorbed through reticulum and omasum. About 76% of SCFA's are absorbed in rumen and reticulum, 19% in omasum and abomasums, and the remaining approximate 5% in the small intestine. The speed for SCFA to be absorbed through rumen walls is affected by pH; when pH is low, SCFA is not ionized (HAc) and can be quickly absorbed and easily moved to blood. When pH is normal, the speed for SCFA to be absorbed is the greatest for butyrate, followed by propionate and acetate. When pH is alkaline, the absorption speed is reversed. It is known that, when pH is really high, the absorption speed is the same. SCFA absorbed in such a manner enters into the liver via the 1st gastric vein and the portal vein [12]. Microorganisms prefer carbohydrate fermentation to protein fermentation; carbohydrate fermentation takes place dominantly in proximal colon, and protein fermentation takes place increasingly more as it nears the distal colon [4]. As dietary fiber is less fermented and consumed due to Western diets, if food or drink that include dietary fiber are ingested, the concentration of butyrate increases in the distal colon due to slow bacteria fermentation [91]. SCFA generated in the intestines of men accounts for 5~15% of energy [4], and the concentration of butyrate in feces of men is 11~25 mM [34, 91]. The ratio for acetate, propionate, and butyrate to be generated in the intestines is 60:25:15 [34,84]. Since more than 95% of SCFA is absorbed in the intestines, it is much too difficult to determine the concentration of SCFA [88].

SCFA exists in rumen of ruminants or the large intestine of non-ruminants in the approximate amount of 100mM. SCFA is generated as microorganisms ferment carbohydrates that are not digested from taken-in dietary fiber. SCFA is absorbed into intestinal epithelial cells and has an effect on various functions of gastrointestinal tract. For example, it affects the hematocele of the large intestine, the absorption of moisture/electrolytes, the movements of the large intestine, and the transport of ions [46,82]. SCFA generated via fermentation is absorbed through primary butyrate transporter MCT1 in the intestines, and GPR43 and GPR41 (SCFA receptors) are activated by absorbed SCFA. GPR43 can be found in the intestines of man and mouse, and the expression frequency of GPR43 is the highest in intestinal epithelial cells, which are intestinal mucous membrane tissues [46,47]. Mechanisms for SCFA to inhibit colon cancer are divided into two. When SCFA is at a low concentration, it is combined with GPR43, which is in plasma membrane and instigates multiple cellular signaling events, causing apoptosis. When SCFA is at a high concentration, it brings about direct cell membrane absorption, causing apoptosis (Fig. 4). It can be confirmed that the expression frequency of GPR43 is reduced in cancer cell tissues; it is also confirmed that the expression frequency of GPR43 in malig-

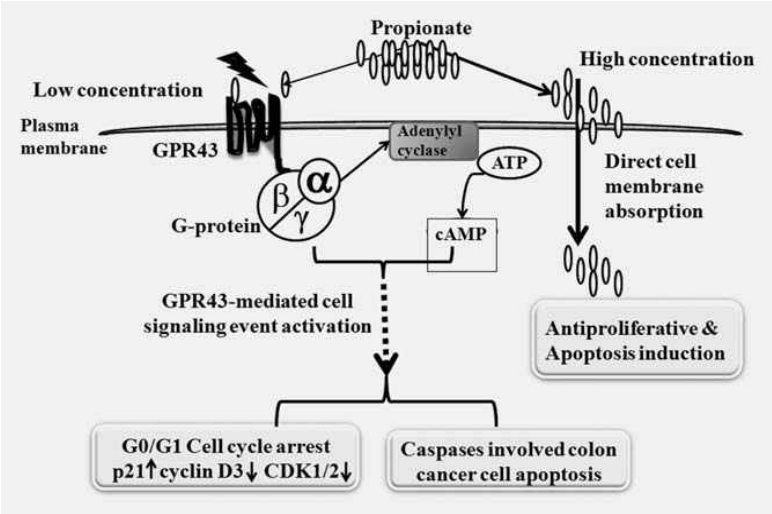

Fig. 4. Schematic representation of the proposed role of GPR43 as a functional tumor suppressor in colon cancer [80]. The bacterial fermentation product of dietary fiber propionate inhibits colon cancer cell proliferation and induces apoptosis by two approaches: one through direct cell membrane absorption and the other through GPR43 activation of multiple cellular signaling events, leading to growth inhibition and apoptosis by influencing several key cell cycle regulators and caspases involved cell apoptosis activation.

nant colon adenocarcinoma tissue, colon hyperplasia and benign colon tumor tissue is reduced to 65% and 80%, compared to common tissues [81]. This result implies that the expression of GPR43 is reduced when colon cancer develops and progresses. Butyrate expresses GPR43, then inhibits the proliferation of colon cancer cell, and brings about apoptosis. GPR41 mainly combines with $G_{i/o}$ protein and GPR43 combines with Gq protein. GPR41 combines more with SCFA receptors in the order of propionate, butyrate and acetate; it is similar with GPR43. GPR43 combines more with acetate while GPR41 combines more with propionate [81,82].

Anti-tumorigenic properties of butyrate

SCFA plays an important role in maintaining physiological functions of intestinal mucous membranes [82]. Although acetate takes the most part in SCFA, butyrate has a strong anti-tumorigenic function [14,84]. When butyrate was *in vivo* and *in vitro* treated in the same concentration, it inhibited the multiplication and specialization of colon cancer cells [64]; when the butyrate concentration was raised and applied to tumor-induced experimental animals, it was confirmed that apoptosis took place partially [14]. Butyrate is characterized by the fact that it has an effect on many cancer cells, contributes to the coordination of functions in cells, and induces cancer apoptosis (Table 3). By means of several proteomic technologies and transcriptomic technologies, it is known that butyrate is related to the death of cancers, that even though there is a sufficient amount of butyrate in some cancer cells, apoptosis is avoided, and that when butyrate resistant cells are treated with butyrate, their growth improves or their aggression is increased [15,30,32,42,57,66,75,79,80]. Yet, through signal transduction mechanism and HDAC inhibition mechanism, fundamental action modes of butyrate more strongly induce the death of cancer cells than the other SCFA's. When treated with butyrate, with the help of the Bcl-2 family that comprises pro-apoptotic protein and anti-apoptotic protein, cytochrome c in mitochondria comes out to cytoplasm and activates caspase to induce apoptosis. It also coordinates the TNF receptor super-family and inhibits the activities of HDAC, which is involved in gene expression, so as to induce apoptosis [2,10,51,66,69]. Relying or not relying on p53, butyrate induces cell cycle arrest and apoptosis. Depending on its concentration, butyrate directly acts on p53 or increases the expression of p53 and induces the expression of target genes (p21, p27, and cyclin dependent kinase) of p53 so as to bring about cell cycle arrest and apoptosis [33,45,61]. Butyrate inhibits the activities of HDAC to increase histone acetylation [89], and it reduces higher order chromatin folding to increase p21 transcription. Also, butyrate reduces the expression of miR-106b and many other miRNAs; since the

Table 3. Summary of the genes and proteins involved in the anti-tumorigenic effects of butyrate [31]

Gene ID	Gene/protein name	Biological role	Effect of butyrate
Apoptosis, cell cycle, stress response			
HDAC	Histone deacetylase	Family of proteins that regulate gene transcription	Butyrate inhibits HDAC activity
CDKN1A	Cyclin-dependent kinase inhibitor 1A, p21	Cell cycle regulator	Expression is induced by butyrate to cause cell cycle arrest
CDKN1B	Cyclin-dependent kinase inhibitor 1B, p27	Cell cycle regulator	Expression is induced by butyrate to cause cell cycle arrest
CDK	Cyclin-dependent kinases	Cell cycle regulator	Expression is induced by butyrate to cause cell cycle arrest
CCND1	Cyclin D1	Cell cycle regulator	Butyrate reduces expression
GADD	Growth arrest and DNA-inducible gene family	Cell stress response	Expression is induced by butyrate
MAPK	Mitogen-activated protein kinase family,includes p38, JNK, ERK	Signalling cascade that mediates the cell stress response	Expression is induced by butyrate
Bcl-2	B-cell CLL/lymphoma 2 protein family	Regulates apoptosis and cell survival. Consists of Bcl2 (anti-apoptosis), Bax, Bak and Bad (pro-apoptotic)	Involved in butyrate-induced apoptosis
HSP27	Heat shock protein 27	Cell stress response, apoptosis	Expression is induced by butyrate
TNFR	Tumour necrosis factor receptor family	Activation by TNF mediates the apoptotic response via the extrinsic pathway	Butyrate sensitises the cell to the apoptotic response occurring via TNFR activation
CYCS	Cytochrome c	Apoptosis	Butyrate induces cytochrome c release into the cytoplasm during apoptosis
TP53	Tumour protein p53	Tumour suppressor and cell cycle regulator	Activation by butyrate is one known mechanism involved in butyrate-induced apoptosis
MYC	c-myc	Transcription factor involved in cell cycle progression and apoptosis	Butyrate reduces expression
Inflammation and the immune response			
NF-kb	Nuclear factor kappa beta family	Transcription factor involved in many cellular processes including inflammation, immune response and apoptosis	Activity of NF-kB is influenced by butyrate to mediate expression of cytokines and chemokines involved in inflammation, e.g. interleukins, MCP1, GM-CSF, VEGF
COX2 (PGH2)	Cyclo-oxygenase-2 (prostaglandin G/H synthase 2)	Involved in inflammation by mediating prostaglandin synthesis	Reduced expression by butyrate in vitro, anti-inflammatory effect
TNF	Tumour necrosis factor family of proteins	Family of pro-inflammatory cytokines, binding to TNFR activates extrinsic apoptotic pathway	Butyrate sensitises cells to TNFR activation to promote apoptosis
IL8	Interleukin 8	Inflammatory response	Butyrate reduces IL8 expression, anti-inflammatory effect

Table 3. Continued

Gene ID	Gene/protein name	Biological role	Effect of butyrate
NOS1	Nitric oxide synthase 1, inducible	Catalyses formation of NO, a free radical involved with many biological processes including inflammation	Butyrate reduces NOS expression, anti-inflammatory effect
TXN	Thioredoxin	Modifies cysteine residue of target proteins via nitrosylation, removes intracellular NO and may play a role in the inflammatory and apoptotic response	Increased expression by butyrate alleviates oxidative stress response
PRDX	Peroxiredoxin family of proteins	Antioxidant enzymes	Increased expression by butyrate alleviates oxidative stress response
GST	Glutathione S-transferase protein family	Cellular detoxification, metabolises toxins via conjugation to glutathione	Increased expression by butyrate, anti-inflammatory effect
Cancer cell metabolism			
HIF-1	Hypoxia inducible factor	Transcription factor involved in energy metabolism, angiogenesis, apoptosis. Facilitates metabolic adaptation to hypoxic environments. Heterodimer consisting of a and b submono-gastrics	Activated under hypoxic conditions, HIF activity is potentially indirectly regulated by butyrate
GLUT1 (SLC2A1)	Glucose transporter type 1	Ubiquitously expressed, transports glucose into the cell. Elevated expression in cancer, including CRC	Not known if butyrate regulates GLUT1 expression directly
Butyrate transporters and receptors			
MCT1 (SLC16A1)	Monocarboxylate transporter 1	Transports monocarboxylates, including SCFA into the cell	Primary butyrate transporter in the colon. Expression is decreased in CRC
SMCT1 (SLC5A8)	Sodium-coupled monocarboxylate transporter 1	Transports monocarboxylates, including SCFA into the cell	Epigenetically silenced in CRC
GPR109A	G-protein coupled receptor 109A	Receptor expressed on plasma membrane of neutrophils and colonocytes	Expression is reduced in CRC and activation by butyrate and other SCFA potentially mediates the apoptotic response
GPR43	G-protein coupled receptor 43	Receptor expressed on plasma membrane of immune cells and possibly colonocytes	Expression is reduced in CRC and activation by butyrate and other SCFA potentially mediates the inflammatory response and induces apoptosis of CRC cells

miR-106b family inhibits p21 translation, after all, inhibiting the expression of miR-106b family will increase p21 translation. Consequently, butyrate generated from dietary fiber inhibits not only inflammation responses but also the generation of cancers (Fig. 5) [39]. Recent cancer research pays increasing attention to cancers generated due to epigenetic changes. In particular, recent research is related to HDACi (histone deacetylase inhibitors) and pays increasingly more attention to not only cancer cures but also chemoprevention (cancer generation prevented through the use of foods and/or drugs). Genetic defects can be restored in epigenetics. HDACi change the acetylation of chromatin and

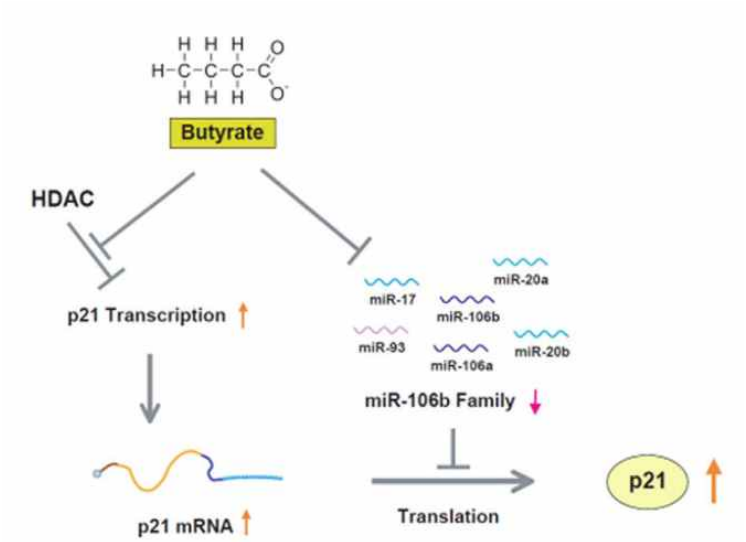

Fig. 5. Butyrate regulates p21 expression via HDAC inhibition and decreased expression of the miR-106b family [39]. Butyrate inhibits HDAC, allowing increased histone acetylation, decreased higher order chromatin folding, and increased transcription of p21. Butyrate also decreases the expression of miR-106b, and several other miRNAs with the same seed sequence region. The miR-106b family inhibits p21 translation, and therefore decreased expression of the miR-106b family leads to increased p21 translation.

other proteins, resultantly alter the expression of genes, induce apoptosis, arrest cell cycle, and inhibit the angiogenesis and transition of cancer cells [57,68]. However, action modes of HDACi at the level of their molecules [51,92] cannot be satisfactorily explained to cancer patients. SCFA (butyrate) activates HDACi even at a low concentration (mM) and inactive genes in cancer cells. Examples are p21, which is a cell cycle inhibitor, and BAK (Bcl-2 homologous antagonist/killer), which induces apoptosis. Butyrate activates these genes in common cells as well [16]. In Table 4, according to the results of most studies conducted on various fatty acids, butyrate induces apoptosis [26].

Regulation of Inflammation by butyrate

Anti-inflammation actions of SCFA include reducing the expression of IL-8 [29] and inhibiting the synthesis of NO in intestinal epithelial cells [77]. Butyrate inhibits the activities of NF-kB, which has an effect on the generation of cancers caused by inflammation. An example is ulcerative colitis, which is an inflammatory bowel disease [34]. Butyrate activates GPR109A, GPR41, and GPR43, which are receptors of medium chain fatty acids [31]. Many studies have found physiological and pathological conditions of intestinal microorganisms and demonstrated that microorganisms and their products play an important role in the gastrointestinal tract, adipose tissue, immune system, and nervous system [20,59,87]. When the distribution of intestinal microorganisms is changed, the concentration of substances that are generated and secreted by microorganisms is changed, which contributes to conditions in which illnesses may break out, such as IBD, colon cancer, obesity, and type 1&2 diabetes[18, 59,87,88]. The SCFA concentration in the gastrointestinal tract and blood is effective to prevent such diseases as IBD, cancer and diabetes [20,34,59,86]. Of SCFA's, butyrate coordinates the proliferation and differentiation of cells and is involved in immunity and inflammation reactions [89]. *In vitro*, SCFA induces the chemotaxsis of neutrophil and activates GPR109A, GPR41, and GPR43, which are receptors [16,43,53,58,76,88]. Anti-inflammation effects of butyrate include reducing the activities of NF-Kb, inhibiting the generation of interferon ɣ, increasing PPARɣ (peroxisome proliferator-activated receptor-ɣ; multiplication and transcription factor for beta cells of the pancreas), inhibiting HDAC, and many others [35]. Butyrate is evaluated as a cure for IBD; in research conducted by Hallert., et al (2003), 20 g of dietary fiber was administered to 22 ulcerative colitis (UC) patients each day for 4 weeks, and it was found that the concentration of butyrate was significantly increased in their feces and the pain of their abdomen was significantly improved [34]. Butyrate facilitates the expression and epigenetic remodeling of pluripotency-associated genes so as

Table 4. Summary of fatty acid induction of apoptosis in CRC cell lines [26]

Author	Fatty acid	CRC cell line	Treatment duration (hr)	Apoptosis detection method	Apoptosis infuced
Hinnebusch, et al. 2002	5 mM Acetic acid	HT-29	24	PI staining via FCMa	NS
	5 mM Propionic acid				NS
	5 mM Na Butg				≈6.5%b*
	5 mMValeric acid				NS
	5 mMCaproate acid				NS
Litvak, et al. 1998	5 mM Na But	Caco-2	24-72	Annexin-V/PI via FCM	13.08±1.409%bc*
Hofmanova, et al. 2009	3 mM Na But	HT-29	24-72	DAPI staining	≈12%bc*
		HCT-116			≈30%bc*
		FHC			≈10%bc*
Pajak, et al. 2009	2 mM Na But+TNFα	COLO 205	24		67.09±6.15bd*
	2 mM Na But		4	Microscopic examination	≈3%bc
	2 mM Na But+TNFα		4	Annexin-V/PI via FCM	≈13%dc
	5 mM Na But		4		≈12%bc
Hýžďalová, et al. 2008	5 mM Na But	HT-29	72	DAPI staining	Detected*
		FHC			DetectedNS
Ruemmele, et al. 2003	20 mM Na But	Caco-2	72	Annexin-V/PI via FCM	90%bc*
Roy, et al. 2009	2.5-10 mM Na But	Caco-2	48	Apoptosis-PI staining-FCM	40-65.7%bc*
				Apoptosis via ELISA	≈150-180%bc*
Lazarova, et al. 2004	5 mM Na But	COLO 201	24	Annexin-V/PI via FCM	6.7±1.2de
		DLD-1			3.5±0.5de
		HCT116			2.5±0.2de
		HT29			1.5±0.2de
		LoVo			2.4±0.1de
		LS174T			3.1±0.7de
		RKO			1.3±0.2de
		SW48			1.7±0.2de
		SW480			2.31±0.3de
		SW620			1.7±0.1de
Buda, et al. 2003	4 mM Na But	SW620	48	Annexin-VPE/7-AAD	9.0 ±0.5bd*
		LS174T			9.3±0.6bd*
		SW1222			2.4±0.2bdNS
Lévy, et al. 2003	5-7.5 mM Na But	Caco-2	72-96	DNA fragmentation	Detected
Domokos, et al. 2010	10 mM Na But	HT29R	48 h	PI staining via FCM	84.6%d*
		HT29-21			8.90%
Narayanan, et al. 2001	5 μg DHAh	Caco-2	24-48 h	DNA fragmentation	Detected
Kato, et al. 2007	125 μM DHA	COLO 205	72 h	Mitochondrial membrane analysis	13.3%d* Compared to Linoleic acid
Schonberg, et al. 2006	70 μM DHA	SW480	96	TUNEL assay	NS
		SW620			NS
Nano, et al. 2003	20 or 100 μM C14-C24 FA's	Caco-2	48	DNA fragmentation	LA*, AA*, EPA*, DHA* (100 μM)
Engelbrecht, et al. 2008	10 μM OAjAAk DHA	Caco-2	48	Caspase 3 activity	DHA*, PMANS, OANS
Toit-Kohn, et al. 2009	10 μM DHA	Caco-2	72	Caspase 3 activity	Detected
				DNA Fragmentation	Detected
Kim, et al. 2002	5 μM CLAl (t10c12)	Caco-2	72	Annexin-VPE/PI Via FCM	≈33%b*

*Significance assumed at $p<0.05$, not significant (NS), [a]flow cytometry, [b]data expressed as mean % compared to control, [c]mean, [d]mean± S.E.M, [e]fold change compared to control, [g]sodium butyrate, [h]docosahexaenoic acid, [i]palmitic acid, [j]oleic acid, [k]arachidonic acid, [l]conjugated linoleic acid.

to enhance the efficiency of induced pluripotent stem cells. Thus, butyrate promotes the reprogramming of somatic cells of men and induces the reprogramming of wrong cells of patients [5].

Conclusions

As mankind changed their lifestyle from a nomadic style to a settled one via agricultural developments, their primary diet also changed from a vegetable-based diet to a meat-based diet. In the process, such geriatric diseases as hypertension and diabetes increased, for which there may be several causes, most likely of which is due to an excessive intake of meat and a reduced intake of vegetables. Various animals on this earth use the cellulose of plants and microorganisms in their gastrointestinal tract to generate short-chain fatty acids. SCFA comprise organic acids like acetate (C2), propionate (C3), butyrate (C4), and valerate (C5), and it is the last product generated when microorganisms ferment dietary cellulose, which is not digested in rumen or intestines of herbivores. Although acetate takes a major part in SCFA, important actions of butyrate include inhibiting the activities of NF-kb and HDAC, and having a strong anti-tumorigenic function. When treated with butyrate, with the help of the Bcl-2 family (which comprises pro-apoptotic protein and anti-apoptotic protein), cytochrome c in mitochondria comes out to cytoplasm and activates caspase so as to induce apoptosis, and also coordinates the TNF receptor super-family and inhibits the activities of HDAC, which is involved in gene expression, so as to induce apoptosis [2,51,66,69]. Relying or not relying on p53, butyrate induces cell cycle arrest and apoptosis. Depending on its concentration, butyrate directly acts on p53 or increases the expression of p53, and induces the expression of the target genes (p21, p27, and cyclin dependent kinase) of p53 so as to bring about cell cycle arrest and apoptosis [32,45,61]. Anti-inflammation actions of SCFA include reducing the expression of IL-8, inhibiting the synthesis of NO in intestinal epithelial cells, and inhibiting the activities of NF-kB, which has an effect on cancer generation caused by inflammation [34,40,77]. Butyrate facilitates the expression and epigenetic remodeling of pluripotency-associated genes so as to enhance the efficiency of induced pluripotent stem cells. Thus, butyrate promotes the reprogramming of somatic cells of men and induces the reprogramming of incorrect cells of patients [5]. In doing so, butyrate protects intestinal mucous membranes from various substances and plays an important role in maintaining intestinal health since it has an oxidative stress reduction function, an anti-cancer action, and an anti-inflammation reaction. Compared to non-vegetarians, vegetarians take in more fruit and vegetables (which contain a lot more dietary fiber) and eat less of sodium, saturated fatty acids and cholesterol; therefore, they have a reduced risk factor for cardiovascular diseases, since a vegetable diet helps prevent and cure cardiovascular diseases [47,71,73,85]. A comparison was made of cancer incidence in vegetarians and non-vegetarians, and it was found that the cancer incidence and BMI (body mass index) is usually much lower in vegetarians than in non-vegetarians [49]. In particular, as obese men take an increasing amount of cellulose, the ratio of *Firmicutes*, which is a microorganism related to obesity, is reduced, and the ratio of *Bacteroidetes* is increased so that body weight is reduced, resembling the structure for the distribution of microorganisms in intestines of slim men. Therefore, it is advisable to reduce the intake of meat and consume a more vegetable-based diet, which contains a lot of cellulose, so as to prevent cancers and other geriatric diseases contracted by modern aged people.

Acknowledgements

This work was supported by a grant from the World Institute of Kimchi (KE0004-1) and local industrial technology development project (project no. R0000861), Ministry Knowledge Economy, Republic of Korea.

References

1. Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., Fernandes, G. R., Tap, J., Bruls, T., Batto, J. M., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M., Kurokawa, K., Leclerc, M., Levenez, F., Manichanh, C., Nielsen, H. B., Nielsen, T., Pons, N., Poulain, J., Qin, J., Sicheritz-Ponten, T., Tims, S., Torrents, D., Ugarte, E., Zoetendal, E. G., Wang, J., Guarner, F., Pedersen, O., de Vos, W. M., Brunak, S., Dore, J., Antolin, M., Artiguenave, F., Blottiere, H. M., Almeida, M., Brechot, C., Cara, C., Chervaux, C., Cultrone, A., Delorme, C., Denariaz, G., Dervyn, R., Foerstner, K. U., Friss, C., van de Guchte, M., Guedon, E., Haimet, F., Huber, W., van Hylckama-Vlieg, J., Jamet, A., Juste, C., Kaci, G., Knol, J., Lakhdari, O., Layec, S., Le Roux, K., Maguin, E., Merieux, A., Melo Minardi, R., M'Rini, C., Muller, J., Oozeer, R., Parkhill, J., Renault, P., Rescigno, M., Sanchez, N., Sunagawa, S., Torrejon, A., Turner, K., Vandemeulebrouck, G., Varela, E., Winogradsky, Y., Zeller, G., Weissenbach, J., Ehrlich, S. D. and Bork, P. 2011. Enterotypes of the human gut microbiome. *Nature* **473,** 174-180.
2. Avivi-Green, C., Polak-Charcon, S., Madar, Z. and Schwartz, B. 2002. Different molecular events account for butyrate-in-

duced apoptosis in two human colon cancer cell lines. *J. Nutr.* **132,** 1812-1818.
3. Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A. and Gordon, J. I. 2005. Host-bacterial mutualism in the human intestine. *Science* **307,** 1915-1920.
4. Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol. Rev.* **70,** 567-590.
5. Berni Canani, R., Di Costanzo, M. and Leone, L. 2012. The epigenetic effects of butyrate: potential therapeutic implications for clinical practice. *Clin. Epigenetics* **4,** 4.
6. Brown, A. J., Goldsworthy, S. M., Barnes, A. A., Eilert, M. M., Tcheang, L., Daniels, D., Muir, A. I., Wigglesworth, M. J., Kinghorn, I., Fraser, N. J., Pike, N. B., Strum, J. C., Steplewski, K. M., Murdock, P. R., Holder, J. C., Marshall, F. H., Szekeres, P. G., Wilson, S., Ignar, D. M., Foord, S. M., Wise, A. and Dowell, S. J. 2003. The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J. Biol. Chem.* **278,** 11312-11319.
7. Buda, A., Qualtrough, D., Jepson, M. A., Martines, D., Paraskeva, C. and Pignatelli, M. 2003. Butyrate down-regulates alpha2beta1 integrin: a possible role in the induction of apoptosis in colorectal cancer cell lines. *Gut* **52,** 729-734.
8. Cho, K. K., Ha, J. K., Choy, Y. H., Han, I. K., Kim, H. S. 1989. Effects of various buffers on rumen pH, VFA, and blood PCV in sheep. *Kor. J. Anim. Feed* **13**, 156-160.
9. Cho, K. K., Ha, J. K., Choy, Y. H., Han, I. K. and Kim, H. S. 1989. Effects of various buffer sources and their levels in the diet on milk production, blood measures and nutrient digestibilities of Holstein dairy cows. *Kor. J. Anim. Feed* **13**, 161-167.
10. Cho, K. K., Kho, Y. J., Lee, D. Y., Ha, S. H., Yin, Y. H., Kim, J. Y., Kim, C. W., Baik, M. G. and Choi, Y. J. 1998. Apoptosis, programmed cell death. *Korean J. Dairy Sci.* **20,** 289-310.
11. Cho, K. K., Kim, S. C., Woo, J. H., Bok, J. D. and Choi, Y. J. 2000. Molecular cloning and expression of a novel family a endoglucanase gene from *Fibrobacter succinogenes* S85 in *Escherichia coli*. *Enzyme Microb. Technol.* **27,** 475-481.
12. Church, D. C. 1979. Digestive physiology and nutrition of ruminants. Oxford press. Portland Oregon.
13. Church, D. C. 1984. Livestock feeds and feedings. Q & B Books Inc. Corvallis. Oregon.
14. Clarke, J. M., Young, G. P., Topping, D. L., Bird, A. R., Cobiac, L., Scherer, B. L., Winkler, J. G. and Lockett, T. J. 2012. Butyrate delivered by butyrylated starch increases distal colonic epithelial apoptosis in carcinogen-treated rats. *Carcinogenesis* **33,** 197-202.
15. Daly, K. and Shirazi-Beechey, S. P. 2006. Microarray analysis of butyrate regulated genes in colonic epithelial cells. *DNA Cell Biol.* **25,** 49-62.
16. Dashwood, R. H. and Ho, E. 2007. Dietary histone deacetylase inhibitors: from cells to mice to man. *Semin. Cancer Biol.* **17,** 363-369.
17. Dave, M., Higgins, P. D., Middha, S. and Rioux, K. P. 2012. The human gut microbiome: current knowledge, challenges, and future directions. *Transl. Res.* **4,** 246-257.
18. De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., Collini, S., Pieraccini, G. and Lionetti, P. 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl. Acad. Sci. USA* **107,** 14691-14696.
19. Dethlefsen, L., Eckburg, P. B., Bik, E. M. and Relman, D. A. 2006. Assembly of the human intestinal microbiota. *Trends. Ecol. Evol.* **21,** 517-523.
20. Diaz Heijtz, R., Wang, S., Anuar, F., Qian, Y., Bjorkholm, B., Samuelsson, A., Hibberd, M. L., Forssberg, H. and Pettersson, S. 2011. Normal gut microbiota modulates brain development and behavior. *Proc. Natl. Acad. Sci. USA* **108,** 3047-3052.
21. DiBaise, J. K., Zhang, H., Crowell, M. D., Krajmalnik-Brown, R., Decker, G. A., Rittmann, B. E. and Rittmann, B. E. 2008. Gut microbiota and its possible relationship with obesity. *Mayo. Clin. Proc.* **83,** 460-469.
22. Domokos, M., Jakus, J., Szeker, K., Csizinszky, R., Csiko, G., Neogrady, Z., Csordas, A. and Galfi, P. 2010. Butyrate-induced cell death and differentiation are associated with distinct patterns of ROS in HT29-derived human colon cancer cells. *Dig. Dis. Sci.* **55,** 920-930.
23. Duchmann, R., May, E., Heike, M., Knolle, P., Neurath, M. and Meyer zum Buschenfelde, K. H. 1999. T cell specificity and cross reactivity towards *Enterobacteria, Bacteroides, Bifidobacterium*, and antigens from resident intestinal flora in humans. *Gut* **44,** 812-818.
24. Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S. R., Nelson, K. E. and Relman, D. A. 2005. Diversity of the human intestinal microbial flora. *Science* **308,** 1635-1638.
25. Engelbrecht, A. M., Toit-Kohn, J. L., Ellis, B., Thomas, M., Nell, T. and Smith, R. 2008. Differential induction of apoptosis and inhibition of the Pi3-kinase pathway by saturated, monounsaturated and polyunsaturated fatty acids in a colon cancer cell model. *Apoptosis* **13,** 1368-1377.
26. Fauser, J. K., Prisciandaro, L. D., Cummins, A. G. and Howarth, G. S. 2011. Fatty acids as potential adjunctive colorectal chemotherapeutic agents. *Cancer Bio. Ther.* **11,** 724-731.
27. Flint, H. J., Bayer, E. A., Rincon, M. T., Lamed, R. and White, B. A. 2008. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat. Rev. Microbiol.* **6,** 121-131.
28. Flint, H. J., Duncan, S. H., Scott, K. P. and Louis, P. 2007. Interactions and competition within the microbial commmono-gastricy of the human colon: links between diet and health. *Environ. Microbiol.* **9,** 1101-1111.
29. Flint, H. J., Martin, J., McPherson, C. A., Daniel, A. S. and Zhang, J. X. 1993. A bifunctional enzyme, with separate xylanase and beta (1, 3-1, 4)-glucanase domains, encoded by the xynD gene of *Ruminococcus flavefaciens*. *J . Bacteriol.* **175,** 2943-2951.

30. Fung, K. Y., Brierley, G. V., Henderson, S., Hoffmann, P., McColl, S. R., Lockett, T., Head, R. and Cosgrove, L. 2011. Butyrate-induced apoptosis in HCT116 colorectal cancer cells includes induction of a cell stress response. *J. Proteome Res.* **10,** 1860-1869.
31. Fung, K. Y., Cosgrove, L., Lockett, T., Head, R. and Topping, D. L. 2012. A review of the potential mechanisms for the lowering of colorectal oncogenesis by butyrate. *Br. J. Nutr.* **5,** 820-831.
32. Fung, K. Y., Lewanowitsch, T., Henderson, S. T., Priebe, I., Hoffmann, P., McColl, S. R., Lockett, T., Head, R. and Cosgrove, L. J. 2009. Proteomic analysis of butyrate effects and loss of butyrate sensitivity in HT29 colorectal cancer cells. *J. Proteome. Res.* **8,** 1220-1227.
33. Gope, R. and Gope, M. L. 1993. Effect of sodium butyrate on the expression of retinoblastoma (RB1) and P53 gene and phosphorylation of retinoblastoma protein in human colon tumor cell line HT29. *Cell Mol. Biol.* **39,** 589-597.
34. Hallert, C., Bjorck, I., Nyman, M., Pousette, A., Granno, C. and Svensson, H. 2003. Increasing fecal butyrate in ulcerative colitis patients by diet: controlled pilot study. *Inflamm. Bowel. Dis.* **9,** 116-121.
35. Hamer, H. M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F. J. and Brummer, R. J. 2008. Review article: the role of butyrate on colonic function. *Aliment. Pharmacol. Ther.* **27,** 104-119.
36. Hinnebusch, B. F., Meng, S., Wu, J. T., Archer, S. Y. and Hodin, R. A. 2002. The effects of short-chain fatty acids on human colon cancer cell phenotype are associated with histone hyperacetylation. *J. Nutr.* **132,** 1012-1017.
37. Hofmanova, J., Vaculova, A., Koubkova, Z., Hyzd'alova, M. and Kozubik, A. 2009. Human fetal colon cells and colon cancer cells respond differently to butyrate and PUFAs. *Mol. Nutr. Food Res.* **53 Suppl 1,** 102-113.
38. Hooper, L. V., Midtvedt, T. and Gordon, J. I. 2002. How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu. Rev. Nutr.* **22,** 283-307.
39. Hu, S., Dong, T. S., Dalal, S. R., Wu, F., Bissonnette, M., Kwon, J. H. and Chang, E. B. 2011. The microbe-derived short chain fatty acid butyrate targets miRNA-dependent p21 gene expression in human colon cancer. *PLoS One* **6,** 16221.
40. Huang, N., Katz, J. P., Martin, D. R. and Wu, G. D. 1997. Inhibition of IL-8 gene expression in Caco-2 cells by compounds which induce histone hyperacetylation. *Cytokine* **9,** 27-36.
41. Hyzd'alova, M., Hofmanova, J., Pachernik, J., Vaculova, A. and Kozubik, A. 2008. The interaction of butyrate with TNF-Alpha during differentiation and apoptosis of colon epithelial cells: role of NF-kB activation. *Cytokine* **44,** 33-43.
42. Iacomino, G., Tecce, M. F., Grimaldi, C., Tosto, M. and Russo, G. L. 2001. Transcriptional response of a human colon adenocarcinoma cell line to sodium butyrate. *Biochem. Biophys. Res. Commun.* **285,** 1280-1289.
43. Itoh, Y., Kawamata, Y., Harada, M., Kobayashi, M., Fujii, R., Fukusumi, S., Ogi, K., Hosoya, M., Tanaka, Y., Uejima, H., Tanaka, H., Maruyama, M., Satoh, R., Okubo, S., Kizawa, H., Komatsu, H., Matsumura, F., Noguchi, Y., Shinohara, T., Hinuma, S., Fujisawa, Y. and Fujino, M. 2003. Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature* **422,** 173-176.
44. Jami, E. and Mizrahi, I. 2012. Composition and similarity of bovine rumen microbiota across individual animals. *PLoS One* **7,** 33306.
45. Janson, W., Brandner, G. and Siegel, J. 1997. Butyrate modulates DNA-damage-induced p53 response by induction of p53-independent differentiation and apoptosis. *Oncogene* **15,** 1395-1406.
46. Karaki, S., Mitsui, R., Hayashi, H., Kato, I., Sugiya, H., Iwanaga, T., Furness, J. B. and Kuwahara, A. 2006. Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and mucosal mast cells in rat intestine. *Cell Tissue Res.* **324.** 353-360.
47. Karaki, S., Tazoe, H., Hayashi, H., Kashiwabara, H., Tooyama, K., Suzuki, Y. and Kuwahara, A. 2008. Expression of the short-chain fatty acid receptor, GPR43, in the human colon. *J. Mol. Histol.* **39,** 135-142.
48. Kato, T., Kolenic, N. and Pardini, R. S. 2007. Docosahexaenoic acid (DHA), a primary tumor suppressive omega-3 fatty acid, inhibits growth of colorectal cancer independent of p53 mutational status. *Nutr. Cancer* **58,** 178-187.
49. Key, T. J., Appleby, P. N., Spencer, E. A., Travis, R. C., Roddam, A. W. and Allen, N. En. 2009. Cancer incidence in vegetarians: results from the european prospective Investigation into cancer and nutrition (EPIC-Oxford). *Am. J. Clin. Nutr.* **89,** 1620-1626.
50. Kim, E. J., Holthuizen, P. E., Park, H. S., Ha, Y. L., Jung, K. C. and Park, J. H. 2002. Trans-10, cis-12-conjugated linoleic acid inhibits Caco-2 colon cancer cell growth. *Am. J. Physiol. Gastrointest Liver Physiol.* **283,** 357-367.
51. Kim, H. J. and Bae, S. C. 2011. Histone deacetylase inhibitors: molecular mechanisms of action and clinical trials as anti-cancer drugs. *Am. J. Transl. Res.* **3,** 166-179.
52. Lazarova, D. L., Bordonaro, M., Carbone, R. and Sartorelli, A. C. 2004. Linear relationship between Wnt activity levels and apoptosis in colorectal carcinoma cells exposed to butyrate. *Int. J. Cancer* **110,** 523-531.
53. Le Poul, E., Loison, C., Struyf, S., Springael, J. Y., Lannoy, V., Decobecq, M. E., Brezillon, S., Dupriez, V., Vassart, G., Van Damme, J., Parmentier, M. and Detheux, M. 2003. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J. Biol. Chem.* **278,** 25481-25489.
54. Levy, P., Robin, H., Bertrand, F., Kornprobst, M. and Capeau, J. 2003. Butyrate-treated colonic Caco-2 cells exhibit defective integrin-mediated signaling together with increased apoptosis and differentiation. *J. Cell Physiol.* **197,** 336-347.
55. Ley, R. E., Turnbaugh, P. J., Klein, S. and Gordon, J. I. 2006. Microbial ecology: human gut microbes associated with obesity. *Nature* **444,** 1022-1023.
56. Litvak, D. A., Evers, B. M., Hwang, K. O., Hellmich, M.

R., Ko, T. C. and Townsend, C. M., Jr. 1998. Butyrate-induced differentiation of Caco-2 cells is associated with apoptosis and early induction of p21waf1/Cip1 and p27kip1. *Surgery* **124,** 161-170.
57. Lopez de Silanes, I., Olmo, N., Turnay, J., Gonzalez de Buitrago, G., Perez-Ramos, P., Guzman-Aranguez, A., Garcia-Diez, M., Lecona, E., Gorospe, M. and Lizarbe, M. A. 2004. Acquisition of resistance to butyrate enhances survival after stress and induces malignancy of human colon carcinoma cells. *Cancer Res.* **64,** 4593-4600.
58. Macfarlane, G. T. and Englyst, H. N. 1986. Englyst, Starch utilization by the human large intestinal microflora. *J. Appl. Bacteriol.* **60,** 195-201.
59. Maslowski, K. M., Vieira, A. T., Ng, A., Kranich, J., Sierro, F., Yu, D., Schilter, H. C., Rolph, M. S., Mackay, F., Artis, D., Xavier, R. J., Teixeira, M. M. and Mackay, C. R. 2009. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* **461,** 1282-1286.
60. McIntyre, A., Gibson, P. R. and Young, G. P. 1993. Butyrate production from dietary fibre and protection against large bowel cancer in a rat model. *Gut* **34,** 386-391.
61. Morrison, M. and Miron, J. 2000. Adhesion to cellulose by *Ruminococcus albus*: a combination of cellulosomes and Pil-proteins. *FEMS Microbiol. Lett.* **185,** 109-115.
62. Nano, J. L., Nobili, C., Girard-Pipau, F. and Rampal, P. 2003. Effects of fatty acids on the growth of Caco-2 cells. *Prostaglandins Leukot Essent Fatty Acids* **69,** 207-215.
63. Narayanan, B. A., Narayanan, N. K. and Reddy, B. S. 2001. Docosahexaenoic acid regulated genes and transcription factors inducing apoptosis in human colon cancer cells. *Int. J. Oncol.* **19,** 1255-1262.
64. Natoni, F., Diolordi, L., Santoni, C. and Gilardini Montani, M. S. 2005. Sodium butyrate sensitises human pancreatic cancer cells to both the intrinsic and the extrinsic apoptotic pathways. *Biochim. Biophys. Acta.* **1745,** 318-329.
65. Nilsson, N. E., Kotarsky, K., Owman, C. and Olde, B. 2003. Identification of a free fatty acid receptor, FFA2R, expressed on leukocytes and activated by short-chain fatty acids. *Biochem. Biophys. Res. Commun.* **303,** 1047-1052.
66. Olmo, N., Turnay, J., Perez-Ramos, P., Lecona, E., Barrasa, J. I., Lopez de Silanes, I. and Lizarbe, M. A. 2007. *In vitro* models for the study of the effect of butyrate on human colon adenocarcinoma cells. *Toxicol. In Vitro* **21,** 262-270.
67. Pajak, B., Gajkowska, B. and Orzechowski, A. 2009. Sodium butyrate sensitizes human colon adenocarcinoma Colo 205 cells to both intrinsic and TNF-Alpha-dependent extrinsic apoptosis. *Apoptosis* **14,** 203-217.
68. Pryde, S. E., Duncan, S. H., Hold, G. L., Stewart, C. S. and Flint, H. J. 2002. The microbiology of butyrate formation in the human colon. *FEMS Microbiol. Lett.* **217,** 133-139.
69. Rincon, M. T., Cepeljnik, T., Martin, J. C., Lamed, R., Barak, Y., Bayer, E. A. and Flint, H. J. 2005. Unconventional mode of attachment of the *Ruminococcus flavefaciens* cellulosome to the cell surface. *J. Bacteriol.* **187,** 7569-7578.
70. Roy, M. J., Dionne, S., Marx, G., Qureshi, I., Sarma, D., Levy, E. and Seidman, E. G. 2009. *In Vitro* studies on the inhibition of colon cancer by butyrate and carnitine. *Nutrition* **25,** 1193-1201.
71. Ruemmele, F. M., Dionne, S., Qureshi, I., Sarma, D. S., Levy, E. and Seidman, E. G. 1999. Butyrate mediates Caco-2 cell apoptosis via up-regulation of pro-apoptotic BAK and inducing caspase-3 mediated cleavage of poly-(ADP-ribose) polymerase (PARP). *Apoptosis Differ.* **6,** 729-735.
72. Ruemmele, F. M., Schwartz, S., Seidman, E. G., Dionne, S., Levy, E. and Lentze, M. J. 2003. Butyrate induced Caco-2 cell apoptosis is mediated via the mitochondrial pathway. *Gut* **52,** 194-100.
73. Sacks Sacks, F. M., Marais, G. E., Handysides, G., Salazar, J., Miller, L., Foster, J. M., Rosner, B. and Kass, E. H. 1984. Lack of an effect of dietary saturated fat and cholesterol on blood pressure in normotensives. *Hypertension* **6,** 193-198.
74. Schonberg, S. A., Lundemo, A. G., Fladvad, T., Holmgren, K., Bremseth, H., Nilsen, A., Gederaas, O., Tvedt, K. E., Egeberg, K. W. and Krokan, H. E. 2006. Closely related colon cancer cell lines display different sensitivity to polyunsaturated fatty acids, accumulate different lipid classes and downregulate sterol regulatory element-binding protein 1. *FEBS J.* **273,** 2749-2765.
75. Serpa, J., Caiado, F., Carvalho, T., Torre, C., Goncalves, L. G., Casalou, C., Lamosa, P., Rodrigues, M., Zhu, Z., Lam, E. W. and Dias, S. 2010. Butyrate-rich colonic microenvironment is a relevant selection factor for metabolically adapted tumor cells. *J. Biol. Chem.* **285,** 39211-39223.
76. Sina, C., Gavrilova, O., Forster, M., Till, A., Derer, S., Hildebrand, F., Raabe, B., Chalaris, A., Scheller, J., Rehmann, A., Franke, A., Ott, S., Hasler, R., Nikolaus, S., Folsch, U. R., Rose-John, S., Jiang, H. P., Li, J., Schreiber, S. and Rosenstiel, P. 2009. G protein-coupled receptor 43 is essential for neutrophil recruitment during intestinal inflammation. *J. Immunol.* **183,** 7514-7522.
77. Stempelj, M., Kedinger, M., Augenlicht, L. and Klampfer, L. 2007. Essential role of the JAK/STAT1 signaling pathway in the expression of inducible nitric-oxide synthase in intestinal epithelial cells and its regulation by butyrate. *J. Biol. Chem.* **282,** 9797-9804.
78. Tajima, K., Aminov, RI. T., Ogata, K., Nakamura, M., Matsui, H. and Benno, Y. 1999. Rumen bacterial diversity as determined by sequence analysis of 16S rDNA libraries. *FEMS Microbiol. Ecol.* **29,** 159-169.
79. Tan, H. T., Tan, S., Lin, Q., Lim, T. K., Hew, C. L. and Chung, M. C. 2008. Quantitative and temporal proteome analysis of butyrate-treated colorectal cancer cells. *Mol. Cell Proteomics* **7,** 1174-1185.
80. Tan, S., Seow, T. K., Liang, R. C., Koh, S., Lee, C. P., Chung, M. C. and Hooi, S. C. 2002. Proteome analysis of butyrate-treated human colon cancer cells (HT-29). *Int. J. Cancer* **98,** 523-531.
81. Tang, Y., Chen, Y., Jiang, H., Robbins, G. T. and Nie, D. 2011. G-protein-coupled receptor for short-chain fatty acids suppresses colon cancer. *Int. J. Cancer* **128,** 847-856.
82. Tazoe, H., Otomo, Y., Kaji, I., Tanaka, R., Karaki, S. I. and Kuwahara, A. 2008. Roles of short-chain fatty acids re-

ceptors, GPR41 and GPR43 on colonic functions. *J. Physiol. Pharmacol.* **59 Suppl 2,** 251-262.
83. Toit-Kohn, J. L., Louw, L. and Engelbrecht, A. M. 2009. Docosahexaenoic Acid induces apoptosis in colorectal carcinoma cells by modulating the Pi3 kinase and P38 Mapk pathways. *J. Nutr. Biochem.* **20,** 106-114.
84. Topping, D. L. and Clifton, P. M. 2001. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol. Rev.* **81,** 1031-1064.
85. Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R. and Gordon, J. I. 2007. The human microbiome project. *Nature* **449,** 804-810.
86. Uronis, J. M., Muhlbauer, M., Herfarth, H. H., Rubinas, T. C., Jones, G. S. and Jobin, C. 2009. Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. *PLoS One* **4,** 6026.
87. Vijay-Kumar, M., Aitken, J. D., Carvalho, F. A., Cullender, T. C., Mwangi, S., Srinivasan, S., Sitaraman, S. V., Knight, R., Ley, R. E. and Gewirtz, A. T. 2010. Metabolic syndrome and altered gut microbiota in mice lacking toll-like receptor 5. *Science* **328,** 228-231.
88. Vinolo, M. A., Ferguson, G. J., Kulkarni, S., Damoulakis, G., Anderson, K., Bohlooly, Y. M., Stephens, L., Hawkins, P. T. and Curi, R. 2011. SCFAs induce mouse neutrophil chemotaxis through the GPR43 receptor. *PLoS One* **6,** 21205.
89. Vinolo, M. A., Rodrigues, H. G., Nachbar, R. T. and Curi, R. 2011. Regulation of inflammation by short chain fatty acids. *Nutrients* **3,** 858-876.
90. Von Soest, P. J. 1982. Nutritional ecology of the ruminant. Q & B Books Inc. Corvallis. Oregon.
91. Wachtershauser, A. and Stein, J. 2000. Rationale for the luminal provision of butyrate in intestinal diseases. *Eur. J. Nutr.* **39,** 164-71.
92. Wagner, J. M., Hackanson, B., Lubbert, M. and Jung, M. 2010. Histone deacetylase (HDAC) inhibitors in recent clinical trials for cancer therapy. *Clin. Epigenetics* **1,** 117-136.
93. Walker, A. W. 2006. Influence of substrate and environmental factors on human gut microbial ecology and metabolism. Thesis, University of Dundee. UK.
94. Wilson, D. E. and Reeder, D. M. 2005. Mammal Species of the world. 3rd eds. Johns Hopkins Univ Pr.

초록 : 섬유소의 이용과 butyrate의 최근 연구

여태종[1] · 최인순[2]* · 조광근[1]*
([1]경남과기대 동물소재공학과, [2]신라대학교 생물학과)

지구상에는 약 5,400여 종의 포유동물이 있고 그 중 약 1,000여 종은 풀을 뜯어 먹고 사는 초식동물이다. 초식동물 중에서 약 250여 종이 반추동물로 알려져 있다. 반추동물인 소와 양은 반추위에서 주로 발효가 일어나지만 비반추동물인 돼지와 사람은 맹장과 결장, 직장에서 주로 발효가 일어난다. 반추위 미생물의 종류와 우점도 *Bacteroidetes* 51%, *Firmicutes* 43% 존재하며, 사람의 대장미생물의 우점도 *Firmicutes* 65%, *Bacteroidetes* 25%로 존재한다. 풀의 세포벽 구성성분은 미생물에 의해 분해, 발효에 의해 SCFA (short chain fatty acid)를 생성하게 되고 acetate, propionate, butyrate 생성비율은 60:25:15이다. 장내 primary butyrate transporter인 MCT1 (monocarboxylatetransports-1)에 의해서 흡수된 butyrate는 SCFA receptor GPR43과 GPR41을 활성화시킨다. Butyrate는 강력한 anti-tumorigenic 기능을 가지고 있다. Butyrate는 다양한 cancer cell에 효과를 나타내며 세포내의 기능 조절에 기여하고, 암세포사멸을 유도하는 특성이 있다. Butyrate는 caspase의 활성화, HDAC (histone deacetylase) 활성을 억제하여apoptosis를 유도하고, p53 발현증가로 cell cycle arrest와 apoptosis를 유도한다. SCFA의 항 염증작용으로는 장 상피세포에서 IL-8 발현 감소, NO합성과 NF-kB (nuclear factor kB)의 활성을 억제하여 염증으로 인한 암 발생을 억제한다. Butyrate는 장 점막의 생리적 기능을 유지하는데 중요한 역할을 하며 IBD (inflammatory bowel disease) 치료법으로 이용되고 있다.