SCFA Profile of Rice RS Fermentation by Colonic Microbiota, *Clostridium butyricum* BCC B2571, and *Eubacterium rectale* DSM 17629

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Abstract

Resistant starch type 3 (RS3) produced from high amylose food sources through retrogradation or enzymatic process is known to have physiological function as dietary fiber. Fermentation of RS3 by colonic microorganisms produced SCFA (acetate, propionate, and butyrate), maintained the health of colon, balance of gut microbiota, preventing inflammatory bowel diseases (IBD) and colon cancer. RS3 in this study was produced from IR-42 and Inpari-16 broken rice by enzymatic treatment (combination of amylase-pullulanase). The Resistant Starch was fermented for 12 and 24 h by colonic microbiota (extracted from healthy human subject), *Clostiridium butyricum* BCC-B2571, or *Eubacterium rectale* DSM 17629. SCFA produced was analyzed by gas chromatography. Treatment by amylase-pullulanase combination was advantageous to increase their RS3 content. The result showed that after enzymatic process, the RS3 content of IR-42 (41.13%) was not significantly different (p < 0.05) from that of Inpari-16 (37.70%). High concentration of acetate (82.5 mM) and propionate (7.5 mM) were produced by colonic microbiota after 12 h fermentation and best concentration of butyrate (6.8 mM) was produced by colonic microbiota after 24 h fermentation. It is clear that utilization of colonic microbiota rather than single strain was better in the production of SCFA.

Keywords

Resistant Starch, Colonic Microbiota, *Clostridium butyricum* BCC-B2571, *Eubacterium rectale* DSM 17629, SCFA

1. Introduction

Healthy digestive system is increasingly important, in line with changes in diet

and lifestyles. Imbalanced diet, such as not enough consuming dietary fiber, can harm the colon health that can lead to colon cancer. In healthy individuals, composition of the gut microbiota is very diverse, which is beneficial for colonic health. However, a loss of diversity combined with emerging imbalances between the proportions of bacterial strains can have severe consequences. Disruption of the equilibrium is called dysbiosis, associated with diarrhea, inflammatory bowel diseases (IBD), colorectal cancer as well as certain liver diseases and allergies, and nutrition-related conditions such as obesity, type 2 diabetes and celiac disease. Altered compositions of intestinal microbiota also affect the central nervous system as gut and brain are connected by a multitude of communication pathways used by bacterial metabolites and transmitters [1]. So, it is not surprising that even mental and neuro-developmental disorders, for example depression, anxiety and autism, could be linked to dysbiosis of the gut microbiota.

Dietary fiber intake can reduce risk of inflammatory bowel disease, cardiovascular disease, colon cancer, obesity and diabetes [2] [3]. In 2012, colorectal cancer (CRC) covered approximately 1.4 million people [4] andthere are about 80% of CRC cases related to diet, 15% of which are caused by genetic, while the rest comes from other factors, including environment [5]. Food product with high dietary fiber such as Resistant Starch type 3 (RS3) can be used to prevent that.

Resistant starch (RS) refers to starch and starch degradation products that escape from digestion in the small intestine of healthy individuals. Resistant starch, not digested in the small intestine, has physiological function as dietary fibers.Some types of resistant starch (RS1, RS2, and RS3) are fermented by the colonic microbiota and produce metabolite such as short chain fatty acids (SCFA): acetate, propionate, butyrate, and lactate. SCFAs is involved in many factors related to the health of colon,including the composition of gut microbiota, regulation of the immune system, inhibition of pathogens, intestinal motility, energy recovery, metabolic syndrome, bowel disorders, and colon cancer [6] [7] [8]. In colon, fermentationis carried by microbes such as the genus *Eubacterium*, *Peptostreptococci*, *Clostridia*, *Roseburia* spp, and *Butyrofibriofi brisolven*, these microbesexcrete starch degrading enzymes [9].

Applications of resistant starch in food products as prebiotics and food ingredients and their consumption are expected to maintain the health of colon, balance of gut microbiota and prevent colon cancer. Various studies had been conducted to produce RS flour. Basically, RS3 can be produced from high starch materials such as rice, sweet potato, banana, cassava, etc. Rice is food source, largely composed of starch. Rice milling will produce broken rice at considerable amount and currently, broken rice utilization is still limited, even regarded as waste or consumed as animal feed. Potential of the broken rice's to be developed as RS, can be the solution to increase its economical value. Brokenrice production in Indonesia reached about 16% of MPD (milled rice), or about 11.4 million tons annually [10]. Purwani *et al.* [11] reported that RS3 content of rice was higher when produced using combination of amylase-pullulanase by 27%, com-

pared with the treatment of these two enzymes individually. Tan [12] reported RS3 content of the rice was increased to 49.7%, when produced using combination of amylase-pullulanase. Guraya *et al.* [13] reported that RS3 content of rice was increased by 13% when produced using pullulanasefollowed by heating at a temperature of 121°C, 30 min, cooling at 1°C and followed by freeze drying. Kim *et al.* [14] reported that RS3 of rice starch was increased when produced using α-amylase followed by a combination of heat treatment at 121°C, 15 min-then cooled at 4°C for 24 h, resulting in RS3 content of approximately16%.

Zhao and Lin [15] reported that RS3 from corn starch previously hydrolyzed with citric acid, improved the liquid infant's stools, and that fermentation of RS3 at 37°C for 0, 12, 24 h increased butyric acid in line with the fermentation time. Sharp and Macfarlane [16] reported that RS could stimulate the growth (*in vitro*) of butyrate-producing bacteria *Clostridia*. Colonic microbial composition and production of SCFA through fermentation of RS was also reported by other researcher [17].

In our study, RS3 was made through combination of retrogradation (interaction between amylose fractions) and enzymatic hydrolysis (amylase-pullulanase). Then, RS3 was fermented by either colonic microbiota, or individual bacteria: *Clostridium butyricum* BCC-B2571, and *Eubacterium rectale* DSM 17629. The aim of this research were to find out the effect of these bacterial fermentation on the SFCA compositions.

2. Materials and Methods

2.1. Rice and Chemicals

Broken rice IR-42 and Inpari-16 were obtained from the Indonesia Center for RiceResearch, Sukamandi, Indonesia. Two types of starch degradation enzymes were from Novozymes. Enzyme used were: alpha-amilase (Liquozymes® Supra) 135 KNU/g and Pullulanase (Dextrozymes® DX 1.5X) 510 NPUN/g.

2.2. Bacterial Strain and Culture Media

Colonic microbiota was extracted from feces of healthy adult subject, 30 - 50 years, who did not take antibiotics for at least 3 months and had no history of gastrointestinal disease. Feces (10 g) as dissolved in 90 mL of BPW was vortexed 30 seconds, then it was filtered. The filtrate was distributed into serum bottle (contained 100 mL of medium) and flushed with CO_2. Pureculture of *C. butyricum* BCC-B2571 was obtained from Culture Collection of Indonesia Research Center for Veterinary Sciences (IVETRI), Indonesia. *Eubacterium rectale* DSM 17629 was obtained from DSMZ, Germany. The basal medium for colonic microbiota and *C. butyricum* BCC-B2571 consist of (g/L): yeast extract 3, beef powder 10, peptone 10, glucose 5, soluble starch 1, NaCl 5, Na-acetate 3 and cysteine hydrochloride 0.5. The pH was adjusted to 6.8. The basal medium for *Eubacterium rectale* DSM 17629 contained (g/L): tryptone 5, bacteriological peptone 5, yeast extract 10, beef extract 5, glucose 5, Tween 80 1 mL, resazurin 0.001, $CaCl_2$ 0.01, $MgSO_4$ 0.02, K_2HPO_4 0.04, KH_2PO_4 0.04, $NaHCO_3$ 0.4, NaCl

0.08, Vitamin K1 0.0002. The pH was adjusted to 7.0.

2.3. Production of Resistant Starch

Rice was extracted byalkaline solution [18] as follow: rice flour (500 g) was mixed with 0.045 M NaOH 1L, stirred constantly for 1 hour, filtered with 2 layers of filter cloth. The filtrate was collected, and centrifuged (1500 g, 4°C, 7 min). The supernatant was discarded, the upper sediment (protein) was separated from the bottom sediment (starch). Starch fraction was mixed with 0.045 M 1 L NaOH, centrifuged, suspended in H_2O 250 mL, and neutralized twice with 1 M HCl. The starch collected was dried in 40°C oven for approximately 18 h, milled and stored at 4°C until use.

Rice starch was processed into RS3 following Kim *et al.* [14] with modifications. Starch (50 g) was suspended in 200 mL H_2O, boiled (100°C, 10 min), removed to room temperature. The gel was vacuum sealed in a retort pouch and autoclaved at 121°C, 15 psi for 1 h, and stored at 4°C for 12 - 14 h, to induce retrogradation. Retrograded starch was suspended in 1 L of H_2O and blended high speed for 2 min. The starch suspension was enzymatic hydrolyzed, by 1 mL α-amilase for 3 h at 85°C, continued with 1 mL of pullulanase for 3 h at 55°C. The hydrolyzed starch was centrifuged (1500 g). The residue was collected and stored at 4°C for 16 - 18 h, suspended in H_2O 250 mL and homogenized for 2 min by homogenizer. The suspension dried with a spray dryer, with inlet temperature 160°C.

2.4. Analysis of Rice Starch

Moisture, ash, and crude fat were analyzed following AOAC [19], whereas the amylose contentwere analyzed using colorimetric methods [20].

2.5. Determination of Resistant Starch

RS3 content was analyzed according to Goni *et al.* [21]. As much as 50 mg of RS3 was dispersed in 5 mL KCl-HCl pH 1.5, and incubated with 4400 units of pepsin solution at 40°C in shaker incubator for 1 hour to remove the proteins. Tris maleate buffer 0.1 M pH 6.9 (4.5 mL) was added and incubated with amylase (100 units) for 16 h at 37°C to hydrolyze the digestible starch. Sample was then centrifuged (1000 g, 15 min) twice. The supernatant was discarded while the residue was moistened with 1.5 mL H_2O and dissolved with 1.5 mL KOH 4M. RS solution was mixed with HCl 2 M and Na-acetate buffer 0.4M pH 4.75, then incubated with 100 units of amyloglucosidase at 55°C for 45 min. The suspension was centrifuged (1000 g, 15 min) and the supernatant was collected. Glucose in the supernatant was measured by phenol-sulfuric acid method [22]. RS was calculated as glucose × 0.9 and expressed as percent of RS in sample.

2.6. *In Vitro* Fermentation

Growth medium 20 mL, with RS3 (2%), was distributed in the serum bottles

flushed with CO_2, sealed with a rubber and sterilized at 121˚C 15 min. The medium was inoculated with 1 mL of 24 h pre-cultured bacterial strain (at about 10^9 CFU/mL), and incubated under anaerobic condition at 37˚C in water bath. Fermentation was carried out for 12 and 24 h, (three replications). In another in vitro fermentation, glucose (control) was used as the only carbon source (concentration 2%).

2.7. Gas Production and pH Measurement

Gas production (mL) was measured by channeling the gas in the serum bottle to expand into glass syringe. The pH of the cultures was determined by pH meter.

2.8. Analysis of Short Chain Fatty Acid

The fermentation media was centrifuged (3000 g, 10 min), the supernatant was filtered with a membrane (0.45 μm) and stored at 4˚C until use. Samples (1 mL) was injected into gas chromatography (Agilent Technologist, 7890A GC System) equipped with a flame ionization detector (FID) and HP Innowax 19091-136 column (60 m × 0.250 mm). The carrier gas (H_2) was run at speed 1.8 mL/min. The oven temperature was maintained at 90˚C for 0.5 min, and then increased to 110˚C at a rate of 10˚C/min, increased to 170˚C at a rate of 5˚C/min and finally increased to 210˚C at a rate of 20˚C/min. Injector and detector temperatures were 275˚C. SCFA mixture containing acetate, propionate and butyrate at specific concentration were used as standard.

2.9. Statistical Analysis

All data were expressed as means ± SE from three independent trials. Differences between the mean values of multiple groups were analyzed by one-way analysis of variance (ANOVA). Duncan test was carried out to compare the data between treatments, independent t-test. Pearson correlation coefficients, $p < 0.05$ was considered a significant different, and SPSS 22 software was applied to analyze the data.

3. Results and Discussion

3.1. Chemical Composition of Rice Starch

Chemical composition of the extracted rice starch is shown in Table 1. The amylose contentwas 34.09% (IR-42) and 28.28% (Inpari-16). Previous report pointed that the amylose content of IR-42 and Inpari 16 were 26.70% [23] and 22.7% [24]. IR-42 was classified as high amylose and Inpari-16 as intermediate amylose rice. Our result shows higher amylose content than previously reported; this may be due to the genetic make up of the local (West java) rice cultivar. The temperature during grain ripening and nitrogen fertilization have also been shown to affect the rice amylose contant. In this case the local people seemed to apply local wisdom for ripening and fertilization which happen to be the right

Table 1. Composition of rice starch.

Chemical composition	IR-42	Inpari-16
Amylose (%)	34.09 ± 0.17	28.28 ± 0.13
Moisture (%)	8.28 ± 0.15	6.99 ± 0.25
Ash (%)	0.15 ± 0.05	0.09 ± 0.001
Crude Fat (%)	0.29 ± 0.04	0.26 ± 0.02

methods for maintaining high amylose content. The high starch (amylose) content of rice is considered to have better oppurtunity to be processed into RS3.

3.2. Resistant Starch Content

In the production of Resistant starch, therice starch was gelatinized and retrograded before hydrolyzed by enzymes. Gelatinization change the granular structure so that the starch became more accessible to the enzyme action. Storage at 4°C induced retrogradation, crystallization and formation of the starch matrix which had undergone gelatinization. Alpha-amylase hydrolyzes $(1,4)$-α-D-glycosidic bond of the rice starch and produces linear oligosaccharide, maltose and glucose. The short linear oligosaccharide, maltose and glucose were removed during RS process, while the rest of its α-dextrin will be used for RS formation. Pullulanase hydrolyzes $(1,6)$-α-D-glycosidic of the amylopectin and produces linear oligosaccharides, maltose and glucose.Most oligosaccharides are able to form RS3 structure [25].

RS content of the starch extracted from rice IR-42 was higher than that of Inpari-16 (Figure 1). Amylase-pullulanase treatment increased the RS content: IR-42 (41.13%) and Inpari-16 (37.70%), but with no significant difference between these two levels. The amylose content of the starch had significant contribution toRS formation.Compared to the extracted starch, with no enzyme treatment, enzyme addition increased clearly the RS content. Interaction between amylose chains could form double helical structure, stabilized by hydrogen bonds and become moreresistant to the amylase. Higher amylose content could produce RS3 with high RS content. Amylopectin hydrolyzed by pullulanase produced linear oligosaccharides and is expected to increase the double helical structure. The results showed higher resistant starch content than that reported earlier by Purwani *et al.* [11].

3.3. Effect of Resistant Starch on pH and Gas during *In Vitro* Fermentation

The effect of resistant starch on pHs during in vitro fermentation by 12 and 24 his shown in Figure 2. The pH values decreaseddown to 4 to 4.5 in all treatments, compared with initial medium, for each microbe. However, pH after 12 and 24 hwas not significantly different ($p < 0.05$). In many cases of fermentation using colonic bacteria, t appeared that decreasing pHs occured during initial fermentation time (the first 12 hours). Earlier report by Purwani *et al.* [11]
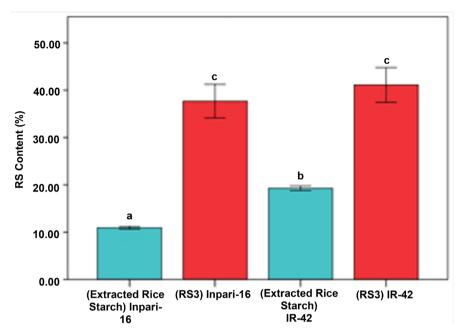
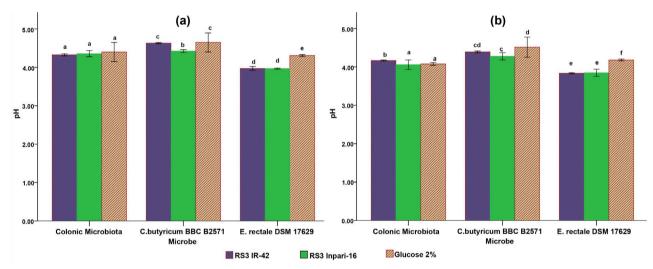**Figure 1.** Resistant starch content of native starch and RS3 of rice.

Figure 2. Profile of pH in the presence of colonic microbiota, *C. butyricum* BCC-B2571 or *E. rectale* DSM 17629 in different media after (a) 12 h and (b) 24 h fermentation. Mean values above bar followed by the different letters represent significant different ($p < 0.05$).

showed that fermentation of RS rice (1%) treated by amylase and pullulanase, resulted in pH 4.5 after 48 h fermentation when *C. butyricum* BCC-B2571 or *E. rectale* DSM 17629 were used. Acetate, propionate, and butyrate suppressed both growth and toxin production by C. *difficile* at concentrations as low as 10 mM, and these effects are pH dependent [26] [27].

The effect of resistant starch on gas produced after 12 and 24 h fermentation is shown in **Figure 3**. Gas production was different after 12 and 24 h fermentation, except when *E. rectale* 17629 was used. Purwani *et al.* [11] reported also that RS rice at 1%, produced 8.70 mL gas by *C. butyricum* BCC-B2571 and 10.60 mL gas by *E. rectale* DSM 17629 after 48 h fermentation.
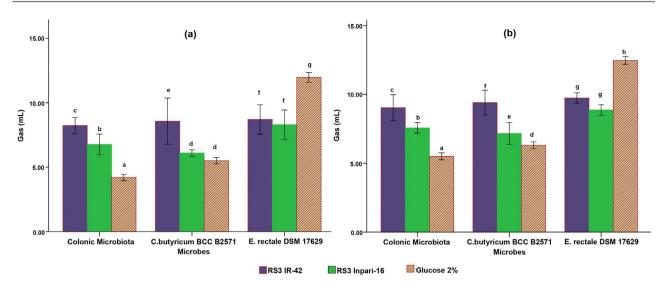
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3.4. Production of Short Chain Fatty Acids during *In Vitro* Fermentation

SCFA profile resulted from 12 and 24 h fermentation by different microbes is shown in Figure 4. The main products after 12 h fermentation of RS3 for each microbe were acetate (18.68 to 82.47 mM), propionate (1.95 to 7.45 mM) and butyrate (0.89 to 6.78 mM). Molar (mM) of acetate:propionate:butyrate after 12 h fermentation by colonic microbiota were 82.47:7.45:6.44 in medium supplemented with RS3 IR-42 and 32.04:2.45:0.89 in medium supplemented with RS3 Inpari-16. Molar (mM) of acetate:propionate:butyrateafter 12 h fermentation by *C. butyricum* BCC-B2571 were 74.93:6.10:6.78 in medium supplemented with RS3 IR-42 and 19.18:1.95:2.51 in medium supplemented with RS3 Inpari-16. Molar (mM) of acetate:propionate:butyrate after 12h fermentation by *E. rectale* DSM 17629, were 21.62:5.33:5.37 in medium supplemented with RS3 IR-42 and 18.68:5.67:5.65 in medium supplemented with RS3 Inpari-16.

The main product after 24 h fermentation of RS3 for each microbe was acetate (18.09 to 63.28 mM), followed by butyrate (4.80 to 6.84 mM) and propionate (3.45 to 6.27 mM). Molar (mM) of acetate:propionate:butyric after 24 h fermentation by colonic microbiota were 63.28:6.27:6.84 in medium supplemented with RS3 IR-42 and 48.64:3.45:4.86 in medium supplemented with RS3 Inpari-16. Molar (mM) of acetate:propionate:butyrate after 24 h fermentation by *C. butyricum* BCC-B2571 were 59.45:4.53:6.39 in medium supplemented with RS3 IR-42 and 35.06:3.98:4.80 in medium supplemented with RS3 Inpari-16. Molar (mM) of acetate:propionate:butyrate after 24 h fermentation by *E. rectale* DSM 17629 were 28.27:5.74:6.48 in medium supplemented with RS3 IR-42 and 18.09:4.97:3.59 in medium supplemented with RS3 Inpari-16.

Our study showed that after 12 h fermentation, *C. butyricum* BCC B2571 produced higher butyrate ($p < 0.05$) in medium supplemented with RS3 IR42
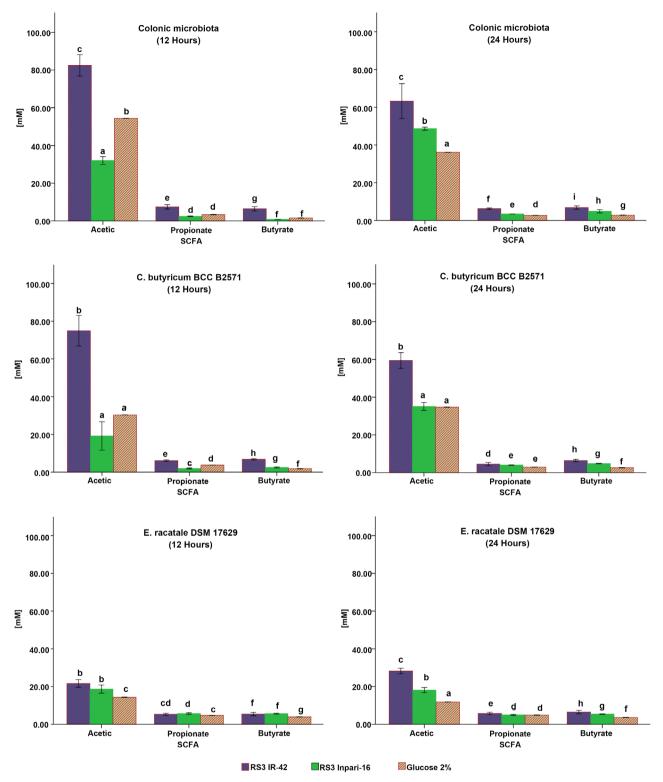
Figure 4. SCFA profile in the presence of colonic microbiota, *C. butyricum* BCC-B2571 or *E. rectale* DSM 17629 in different medium supplemented after 12 or 24 h fermentation. Mean values above bar followed by the different letters represent significant different ($p < 0.05$).

(6.78 mM) than in medium supplemented with RS3 Inpari-16 (2.51 mM). Meanwhile after 24 hfermentation, the colonic microbiota produced butyrate

higher (p < 0.05) in medium supplemented with RS3 IR42 (6.84 mM) than in medium supplemented with RS3 Inpari-16 (4.86 mM).

Table 2 shows SCFA profile after 12 and 24 h fermentation of different RS3. In medium supplemented with RS3 IR-42, acetate production by colonic microbiota, *C. butyricum* BCC-B2571 or *E. rectale* DSM 17629 was different. Molar of acetate produced by colonic microbiota and *C. butyricum* BCC-B2571 were higher after 12 than 24 h fermentation (p < 0.05). Meanwhile *E. rectale* DSM 17629 produced higher acetateafter 24 than 12 h fermentation. RS3 IR-42 fermented by *C. butyricum* BCC-B2571 produced different propionate after 12 and 24 h fermentation, while higher propionate was produced after 12 hfermentation.

In medium supplemented with RS Inapri-16, production of acetate, propionate, and butyrate by colonic microbiota and *C. butyricum* BCC-B2571 showed different molarity after 12 and 24 h fermentation. RS3 Inpari-16 fermented by colonic microbiota and *C. butyricum* BCC B2571, produced higher acetate, propionate, and butyrate significantly after 24 h fermentation compared with at 12 h fermentation.

The result confirmed that proportion and content of SCFA was dependent on the bacterial strain used and type of the resistant starch (RS content). The

Table 2. Profile SCFA by different resistant starch after 12 and 24 h fermentation[a].

Resistant starch	SCFA after fermentation	Colonic microbiota	*C. butyricum* BCC B2571	*E. rectale* DSM 17629
	Acetate (mM) 12 h	82.47	74.93	21.62
	Acetate (mM) 24 h	63.28	59.45	28.27
	p-value	0.037	0.042	0.010
	Propionate (mM) 12 h	6.27	6.10	5.33
RS3 IR-42	Propionate (mM) 24 h	7.45	4.53	5.74
	p-value		0.043	
	Butyrate (mM) 12 h	6.44	6.68	5.37
	Butyrate (mM) 24 h	6.84	6.39	6.48
	p-value			
	Acetate (mM) 12 h	32.04	19.18	18.68
	Acetate (mM) 24 h	48.64	35.06	18.09
	p-value	0.000	0.025	
	Propionate (mM) 12 h	2.45	1.95	5.67
RS3 Inpari-16	Propionate (mM) 24 h	3.45	3.98	4.97
	p-value	0.000	0.000	
	Butyrate (mM) 12 h	0.89	2.51	5.65
	Butyrate (mM) 24 h	4.86	4.80	5.38
	p-value	0.001	0.000	

[a]Only significant independent t-test reported (p < 0.05).

butyrate produced in our study was higher than previously reported (produced by colonic microbiota) [28]. Production of SCFA by microbes in the medium supplemented with RS3 was in general higher than those produced in the medium supplemented with glucose (as the only carbon source), which indicates that RS3 is more effective for SCFA production. Our study also showed that SCFA produced by colonic microbiota in RS3 medium was higher than in the medium supplemented with apple juice extracts [29]. Higher concentration of butyrate was produced after 48 h fermentation of RS3 rice by *C. butyricum* BCC B2571 and *E. rectale* DSM 17629, but lower acetate when *C. butyricum* BCC B2571 was used [11]. Butyrate production by colonic microbiota and *C. butyricum* BCC B2571 was increased after 24 h fermentation in medium supplemented with RS3 Inpari-16.

In our study, accumulation of acetate implied that the butyrate was produced via butyryl-CoA transferase. Miller and Wolin [30] reported the pathway of acetate, propionate, and butyrate synthesis by human colonic microbiota. At the final step of butyrate synthesis, there are two alternatives pathway, butyrate kinase pathway and a butyryl-CoA transferase. Butyryl-CoA transferase pathway is a dominant route for human colonic microbiota for butyrate synthesis [31]. Duncan and Flint [32] reported that during in vitro study, *E. rectale* consumed large amount of acetate to produce butyrate.

The capability of RS3 rice as prebiotic was supported by the fact that RS3 was metabolized by the tested microbes and in the production of SCFA. Resistant starch improve metabolic activity of the gut microbiota by increasing the production of SCFA and, thus supported the growth of beneficial species in the healthy individual [33] and in patients with irritable bowel syndrome or those receiving enteral nutrition [34] [35]. Acetate reduces the appetite by changing the expression profiles of appetite regulatory neuropeptides in the hypothalamus through activation of TCA cycle [36]. Acetate (2 - 10 mM) has been found correlated well with the ability of bifidobacteria to inhibit enteropathogens [8]. In addition, it has been shown that acetate reduces lipopolysaccharide-stimulated tumor necrosis factor (TNF), interleukin (IL)-6 and nuclear factor (NF)-κB level while boosting peripheral blood antibody production in various different tissues [37].

Propionate reduces food intake and increases satiety viaaugmentation of the satiety hormone leptin, and through activation of GPCR [38] [39]. Propionate reduces cholesterol synthesis rate by decreasing the enzyme activity of hepatic HMG-CoA synthase (HMGCS) and HMG-CoA reductase (HMGCR) [40] [41]. Also, propionate reduces human colon cancer cell growth and differentiation viahyperacetylation of histone proteins and stimulation of apoptosis [42] [43]. In addition, propionate also inhibits the production of proinflammatory cytokines (*e.g.*, TNF-*α*, NF-κB) in multiple tissues [44] [45].

Butyrate is an energy source of the intestinal epithelial cells (growth and differentiation) and can increase mucin production which may result in changes on bacterial adhesion [46] and improve tight-junctions integrity [47]. Compared to

acetate and propionate, butyrate exhibits strong anti-inflammatory properties, and this effect is likely mediated by inhibition of TNF-α production, NF-κB activation, and IL-8, -10, -12 expression in immune and colonic epithelial cells [48] [49]. Fu *et al.* [50] reported through differentiation maker (cathepsin C) study, which showed that butyrate, propionate, and acetate could inhibit proliferation and motility of a well-differentiated human colonic cancer cell line. Purwani *et al.* [51] reported that SCFA produced by fermentation of *C. butyricum* BCC B2571 (butyrate of 2.6 - 5.2 mM) or *E. rectale* DSM 17629 (butyrate of 3.6 - 7.2 mM) inhibited proliferation and induce apoptosis of human colorectal cancer cell line HCT-116. Butyrate (0.1 - 10 mM) inhibited proliferation and induce apoptosis of Caco2 [52] [53]. In addition, butyrate and propionate have also been reported to induce differentiation of T-regulatory cells, control intestinal inflammation; and these effects seem to be mediated via inhibition of histone deacetylation [54] [55]. Control of intestinal inflammation shows beneficial health of colon in terms of gut barrier maintenance, reducing the risk of inflammatory bowel disease or CRC.

3.5. Correlation between Microbes and SCFA Concentration

Significant positive correlations were observed between concentrations of SCFA (acetate, propionate, and butyrate) and colonic microbiota or *C. butyricum* BCC-B2571 after 12 h fermentation (Table 3). RS fermentation by colonic microbiota indicated significant positive correlation with propionate concentration after 24 h fermentation. Fermentation of RS3 by *C. butyricum* BCC-B2571 indicated significant positive correlation with propionate and butyrate concentration after 24 h fermentation. In our study, fermentation by *E. rectale* DSM 17629 showed one significant positive correlation with acetate after 24 h fermentation.

4. Conclusion

Treatment with amilase-pullulanase increased RS content of IR-42 from 19% to 41% and Inpari-16 from 10% to 37%. Fermentation of RS3 IR-42 and Inpari-16 by colonic microbiota, *C. butyricum* BCC-B2571, and *E. rectale* DSM 17629, produced SCFA with different molar ratio. Time fermentation affected molar

Table 3. Statistical correlations (Person coefficients) for RS in different microbes versus SCFA concentrations[a].

Microbe	12 h			24 h		
	Acetate	Propionate	Butyrate	Acetate	Propionate	Butyrate
Colonic microbiota	0.991[b]	0.966[b]	0.972		0.986	
C. butyricum BCC-B2571	0.975	0.991	0.991	0.976		0.912
E. rectale DSM 17629				0.975		

[a]Only significant correlations reported (p < 0.05), [b]p < 0.01.

ratio of SCFA production. Fermentation of *C. butyricum* BCC-B2571 in medium supplemented with RS3 IR42 produced molar of acetate:propionate:butyrate, 74.93 mM:6.10 mM:6.78 mM after 12 h fermentation. Fermentation of colonic microbiota in medium supplemented with RS3 IR42 produced molar of acetate:propionate:butyrate, 63.28 mM:6.27 mM:6.84 mM after 24 h fermentation. Both SCFA profile produced high butyrate. Our study showed that RS3 IR-42 had potential in the production of butyrate.

Acknowledgements

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