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ARTICLE

Fermentation of *Metroxylon sagu* Resistant Starch Type III by *Lactobacillus* sp. and *Bifidobacterium bifidum*

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The in vitro fermentability of sago (*Metroxylon sagu*) resistant starch type III (RS_3) by selected probiotic bacteria was investigated. Sago RS_3 with 12% RS content was prepared by enzymatic debranching of native sago starch with pullulanase enzyme, followed by autoclaving, cooling, and annealing. The fermentation of sago RS_3 by *L. acidophilus* FTCC 0291, *L. bulgaricus* FTCC 0411, *L. casei* FTCC 0442, and *B. bifidum* BB12 was investigated by observing the bacterial growth, carbohydrate consumption profiles, pH changes, and total short chain fatty acids (SCFA) produced in the fermentation media. Comparisons were made with commercial fructo-oligosaccharide (FOS), Hi-maize 1043, and Hi-maize 240. Submerged fermentations were conducted in 30 mL glass vials for 24 h at 37 °C in an oven without shaking. The results indicated that fermentation of sago RS_3 significantly ($P < 0.05$) yielded the highest count of *Lactobacillus* sp. accompanied by the largest reduction in pH of the medium. Sago RS_3 was significantly the most consumed substrate compared to FOS and Hi-maizes.

KEYWORDS: *Metroxylon sagu*; sago; resistant starch; *Lactobacillus*; *Bifidobacteria*; probiotic

INTRODUCTION

Starch, the major dietary source of carbohydrates, is the most abundant storage polysaccharide in plants, occurring as granules in the chloroplasts of green leaves and the amyloplasts of seeds, pulses, and tubers (*1*). Industrial starches are typically derived from cereals (corn, wheat, rice, and sorghum), tubers (potato and sweet potato), roots (cassava), and legumes (bean and green pea), but sago starch is an example of commercial starch derived from the stem of sago palm (*Metroxylon sagu*) (*2*). In the Asia-Pacific region, sago starch is produced at a rate of 300 million tons per year (*3*). The sago industry in Malaysia (in the State of Sarawak) has made sago flour one of the most important export commodities, with a current output of 45,000 tons per year, with revenue expected to increase from US $10.2 million/year to US $0.7 billion/year in 2015 (*4*).

Resistant starch (RS) is a nondigestible starch fraction, recognized for its incomplete digestion and absorption in the small intestine (*5*, *6*). Extensive studies have shown that the physiological functions of RS are similar to those of dietary fiber (*7*, *8*). There is a classification of the various types of RS, food sources, and factors affecting their resistance to digestion in the colon (*9*). RS is grouped into four types: RS_1, RS_2, RS_3, and RS_4. Sago RS in this study is classified as RS_3, which was formed through gelatinization and retrogradation processes of starch granules, which are mainly composed of amylose and amylopectin. During gelatinization, the starch granules are disrupted as water is absorbed (*8*). This causes the leaching of polymer molecules amylose and amylopectin (*8*). Upon cooling or retrogradation, the leached amylose rearrange into double helices structure, stabilized by hydrogen bonds to form RS type III (*10*, *11*). In addition, reassociation of polymers to form insoluble structures during retrogradation is resistant to enzymatic hydrolysis (*12*). RS has been suggested for use as a prebiotic (*9*) in probiotic compositions to promote the growth of beneficial microorganisms (*13*) such as *Bifidobacterium* and *Lactobacilli*. The potential health benefits and functional properties of RS have been comprehensively reviewed (*10*).

A prebiotic is a nondigestible but fermentable food ingredient that confers a health benefit on the host associated with the modulation of microbiota in the colon (*14*). This definition assumes that a prebiotic should increase the number and/or activity of bifidobacteria and/or lactic acid bacteria, as these groups of microorganisms are claimed to have several beneficial effects on the host (*15*). To be classified as a prebiotic, an ingredient should fulfill these criteria (*16*): (a) resistant to gastric acidity, hydrolysis by mammalian digestive enzymes, and gastrointestinal absorption; (b) fermentable by intestinal microflora; and (c) able to promote growth and/or activity of intestinal bacteria that contribute to health and well-being.

As there is a strong and growing demand for sago starch, it is timely that more diversified research on the use of sago starch be undertaken. Therefore, the aim of the present work was mainly to study the in vitro fermentability of sago RS by *Lactobacillus* sp. and *Bifidobacterium bifidum*, with comparison to commercial high amylose content maize and fructooligosaccharides. Through

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this research, sago starch could find another potential use in the food industry that would help to accelerate the development of the sago industry.

MATERIALS AND METHODS

Microorganisms. *Lactobacillus acidophilus* FTCC 0291, *Lactobacillus bulgaricus* FTCC 0411, and *Lactobacillus casei* FTCC 0442 were obtained from the Food Technology Research Center, Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor. *Bifidobacterium bifidum* BB12 was purchased from Chr. Hansen A/S Regional Office (Selangor, Malaysia). Cultures were maintained on de Man, Rogosa and Sharpe (MRS) agar slants at 4 °C and subcultured every month.

Materials. *Carbohydrates*. Four types of carbohydrates were used in these experiments: native sago starch (Nitsei Sago Industries Sdn. Bhd., Malaysia), which was used to produce the RS sample, fructooligosaccharide (FOS; Raftilose P95, Orafti, Belgium), and two high-amylose corn (maize) starches (Hi-maize 1043 and Hi-maize 240, National Starch and Chemical, Australia). Raftilose P95 is a commercial powder produced through enzymatic hydrolysis of chicory inulin. The powder contains oligofructose (93.2%, w/w) with small amounts of glucose, fructose, and sucrose as impurities. Hi-maize 1043 and 240 are corn starches containing resistant starch type II. Hi-maize 1043 and 240 contain 60% and 40% total dietary fiber, respectively.

Enzymes and Chemicals. Pullulanase (Promozyme 500 PUN/mL) was obtained from Novozymes (Bagsvaerd, Denmark) and used as received. Unless otherwise specified, all other reagents and standards were of analytical grade and were purchased from Sigma Chemicals Ltd., St. Louis, U.S.A.

Bacterial Growth Media. All culture experiments were conducted using sterile basal medium (*17*), which contained the following (per L): 10.0 g glucose, 2.0 g peptone, 2.0 g yeast extract, 0.1 g NaCl, 0.04 g K_2HPO_4, 0.04 g KH_2PO_4, 0.01 g $MgSO_4 \cdot 7H_2O$, 0.01 g $CaCl_2 \cdot 2H_20$, 2.0 g $NaHCO_3$, 0.5 g L-cysteine·HCl, and 0.5 g bile salt. Media were adjusted to pH 5.5 with 0.5 M acetic acid. The fermentation medium was of a composition similar to that of the basal media with the exception that glucose was replaced by sago RS (at concentrations varying from 1.25−7.50 g/L), FOS (at 5.00 g/L), or corn starch (at 5.00 g/L).

Sago RS_3. Preparation of sago RS_3 was done as described previously (*18*), whereby 200 g of native sago starch was suspended in 1 L of sodium acetate buffer, pH 5.0, in a 2 L conical flask. Pullulanase enzyme (16% v/w, equivalent to 64 PUN/g starch) was added, and the starch suspension was incubated for 24 h at 60 °C with agitation at 150 rpm in an orbital incubator−shaker (Certomart*SII*, B. Braun Biotech International, Melsungen, Germany) to debranch the amylopectin portion before heating (80 °C, 10 min) to deactivate the enzyme. The starch suspension was then heated to 121 °C for 90 min and cooled to room temperature before refrigeration (4 °C, 16 h). This was followed by heating (95 °C, 72 h) and cooling (4 °C, 24 h) before freeze-drying and grinding the pellets to a powder with a particle size of 250 μm. RS content was determined and calculated (*19*) as follows:

$$Percentage\ of\ RS = \frac{mg\ glucose \times dilution\ factor \times 0.9 \times 100}{Sample\ weight\ (mg, dry\ basis)}$$

Fermentations. Prior to fermentation experiments, the bacterial isolates were precultured in 5 mL of sterile growth medium and incubated at 37 °C for 24 h. An inoculum of 10^5 cells was transferred into 20 mL of fermentation medium containing sterile test carbohydrates. Both precultures and fermentations were incubated in an anaerobic atmosphere system consisting of 85% N_2, 10% H_2, and 5% CO_2 at 37 °C with no pH control or agitation. After 24 h of fermentation, tubes were taken out for the following analyses: viable count, total residual carbohydrate in medium, and total short chain fatty acids (SCFA) and butyric acid. In addition, to confirm that negligible growth occurred from the use of extraneous carbon sources present in the base medium, each type of bacterium was also grown in a control fermentation containing the base medium with no added carbon source.

Analysis. *Measurement of Growth*. Enumerations of *Lactobacillus* spp. and *Bifidobacterium* were carried out by serially diluting the samples with sterile 0.1% (w/v) peptone water before pour plating the solutions onto MRS agar supplemented with 0.05% (w/v) of filter-sterilized L-cysteine·HCl. Agar plates were placed into anaerobic jars that contained GasPak Envelopes (Becton Dickinson, Cockeysville, Maryland, U.S.A.) for 36 h at 37 °C. After incubation, single colonies were counted and reported as the total *Lactobacillus* spp. and *Bifidobacterium* counts.

Determination of pH. The pH was determined by a pH meter with a glass electrode (DELTA 320, Shanghai, China).

Carbohydrate Analysis. Total residual carbohydrate in the media was determined by the phenol-sulfuric acid assay (*20*).

Total SCFA, Acetic, Propionic, and Butyric Acids. Free fatty acids were quantified using a gas chromatograph (*21*) on a Shimadzu gas chromatography unit (GC-17 AF, Kyoto, Japan) equipped with a flame ionization detector, fitted with a bonded−phase (polyethylene glycol BP-21), fused-silica capillary column (0.25 mm i.d. × 30 m; 0.25 μm film thickness, SGE, Australia). Organic fatty acid standards, acetic, propionic, and butyric acids, were obtained from Sigma Aldrich, Steinheim, Germany. Total SCFA was determined by the summation of these three free fatty acid concentrations.

Statistical Analysis. Triplicate fermentation experiments were conducted in duplicate tubes, and all analyses were performed in duplicates. The data were subjected to one-way analysis of variance (ANOVA), and the significance of the difference between means was determined by the Duncan test, where $p < 0.05$ was considered statistically significant. The Statistical Package for Social Science, version 11.5 (SPSS Inc., Illinois, U.S.A.) was used for the analysis.

RESULTS AND DISCUSSION

In this study, commercial Raftilose FOS, Hi-maize 1043, and Hi-maize 240 were used as substrates for comparison to sago RS_3 as there are numerous studies pertaining to the prebiotic effects of FOS (*22*−*25*) and Hi-maize (*26*). Type III RS was produced in this research from *Metroxylon sagu* starch with a 12.2% (w/w) RS content. Using the same determination method, the RS content in two commercial starches, Hi-maize 1043 and Hi-maize 240, were found to be 32.4% and 28.1%, respectively. Hi-maize, commercially available since 1993, was developed from a corn hybrid containing 80% amylose (*27*). Although Hi-maize contained higher RS contents than sago RS, the corn starches were actually categorized as type II RS. Therefore, a preferential growth comparison can also be made between RS_3 and RS_2 in addition to studying the ability of strains to grow on sago RS.

The abilities of selected *Lactobacillus* spp. and *Bifidobacterium* to grow on indigestible carbohydrates are displayed in **Table 1**. The bacterial growth levels on sago RS were also significantly higher than on the commercial carbohydrates. Sago RS_3 (5 mg/mL) was the best growth substrate as compared to commercial carbohydrates for *L. acidophilus* FTCC 0291, *L. bulgaricus* FTCC 0411, and *L. casei* FTCC 0442, as exhibited by having the highest counts after 24 h of fermentation, reaching 6.41, 7.20, and 8.11 $\log_{10}$ cfu/mL, respectively. As substrate concentration is an important factor in determining the formation of end products and viability of the probiotic bacteria in fermentation (*28*, *29*), the influence of different concentrations of sago RS on total viable count of probiotics, substrate consumption, changes in the pH of media were determined in this study. As the concentration of sago RS increased, the total viable count and carbohydrate consumption increased with a significant reduction of pH in media when fermented by *L. acidophilus* FTCC 0291, *L. bulgaricus* FTCC 0411, and *L. casei* FTCC 0442. Fermentations were carried out at 37 °C for 24 h as breakdown of substrates will be significantly overestimated for fermentation conducted more than 24 h (*30*). Moreover, many reports (*23*, *31*) had shown that the extent of fermentation remained stable after 24 h with a maximum SCFA production observed before 24 h of fermentation. In all cases, the

Table 1. Effect of Non-Digestible Carbohydrates on Probiotic Growth, Carbohydrate Consumption, and Changes in the pH of Media[a]

	sago RS (mg/mL)							
probiotic	1.25	2.50	5.00	6.25	7.50	Hi-maize 240	Hi-maize 1043	FOS
				Total Viable Count (log cfu/mL)				
L. acidophilus FTCC 0291	6.1 ± 0.1 bc	6.2 ± 0.2 cd	6.4 ± 0.2 d	6.7 ± 0.1 e	6.8 ± 0.1 e	6.2 ± 0.2 cd	5.9 ± 0.2 ab	5.77 ± 0.08 a
L. bulgaricus FTCC 0411	6.6 ± 0.0 b	6.9 ± 0.0 c	7.2 ± 0.1 d	7.7 ± 0.0 e	7.9 ± 0.1 f	6.6 ± 0.2 b	6.5 ± 0.2 b	4.56 ± 0.04 a
L. casei FTCC 0442	7.9 ± 0.1 cd	8.0 ± 0.1 de	8.1 ± 0.1 e	8.6 ± 0.1 f	8.8 ± 0.0 g	7.8 ± 0.1 bc	7.6 ± 0.0 a	7.69 ± 0.10 ab
B. bifidum BB12	5.0 ± 0.1 b	5.3 ± 0.0 c	5.0 ± 0.1 b	5.0 ± 0.1 b	5.1 ± 0.1 b	6.0 ± 0.1 d	5.9 ± 0.1 d	4.51 ± 0.09 a
				Total Carbohydrate Consumption (%)				
L. acidophilus FTCC 0291	0.26 ± 0.10 a	1.63 ± 0.06 b	3.68 ± 0.04 e	5.30 ± 0.35 f	6.75 ± 0.15 g	2.21 ± 0.16 c	2.05 ± 0.16 c	2.76 ± 0.14 d
L. bulgaricus FTCC 0411	2.00 ± 0.05 c	3.50 ± 0.20 d	4.60 ± 0.03 e	4.71 ± 0.01 ef	4.76 ± 0.00 f	1.64 ± 0.04 b	1.50 ± 0.10 b	0.20 ± 0.05 a
L. casei FTCC 0442	2.21 ± 0.11 a	7.49 ± 0.16 c	8.84 ± 0.18 d	10.63 ± 0.06 e	14.33 ± 0.06 f	2.50 ± 0.01 a	3.97 ± 0.25 b	2.05 ± 0.10 a
B. bifidum BB12	1.32 ± 0.11 a	1.21 ± 0.11 a	2.12 ± 0.08 b	3.25 ± 0.01 c	6.63 ± 0.10 d	2.20 ± 0.20 b	2.50 ± 0.01 b	7.21 ± 0.02 e
				pH				
L. acidophilus FTCC 0291	6.01 ± 0.04 e	5.87 ± 0.01 d	5.68 ± 0.03 c	5.59 ± 0.01 b	5.53 ± 0.00 a	5.97 ± 0.03 e	6.01 ± 0.04 e	6.20 ± 0.02 f
L. bulgaricus FTCC 0411	6.09 ± 0.04 e	5.89 ± 0.02 c	5.87 ± 0.01 c	5.78 ± 0.01 b	5.69 ± 0.01 a	6.02 ± 0.01 d	6.05 ± 0.04 de	6.29 ± 0.02 f
L. casei FTCC 0442	5.85 ± 0.05 d	5.67 ± 0.02 c	5.64 ± 0.15 bc	5.54 ± 0.02 b	5.40 ± 0.04 a	6.11 ± 0.01 e	6.06 ± 0.03 e	5.91 ± 0.01 d
B. bifidum BB12	6.30 ± 0.04 d	6.25 ± 0.03 bcd	6.22 ± 0.01 bc	6.18 ± 0.07 ab	6.12 ± 0.06 a	6.28 ± 0.01 cd	6.27 ± 0.01 cd	6.25 ± 0.03 bcd

[a] Results are expressed as means ± standard deviation; values are the means of duplicate analysis from three separate runs ($N = 3$). Means in the same row followed by different lower case letters are significantly different ($P < 0.05$). Fermentation media contained 5 mg/mL individual corn starch or FOS.

Table 2. Molar Concentration of Total Short Chain Fatty Acid (SCFA) and Molar Percentage of Acetic (AA), Propionic (PA), and Butyric Acid (BA) Production by Probiotics Fermented in Different Substrates[a]

		sago RS	Hi-maize 240	Hi-maize 1043	FOS
L. acidophilus FTCC 0291	SCFA	7.89 ± 0.13 A	9.52 ± 0.35 B	8.98 ± 0.48 B	12.56 ± 0.64 C
	AA	83.22 ± 1.07 c	79.00 ± 0.42 b	78.05 ± 1.88 ab	75.07 ± 3.01 a
	PA	13.75 ± 1.63 a	15.02 ± 1.12 ab	16.95 ± 1.51 b	16.97 ± 1.10 b
	BA	3.02 ± 0.55 a	5.97 ± 1.54 bc	4.98 ± 0.38 ab	7.95 ± 1.91 c
L. bulgaricus FTCC 0411	SCFA	6.51 ± 0.28 B	7.98 ± 0.42 D	7.25 ± 0.30 C	2.57 ± 0.11 A
	AA	89.55 ± 1.87 b	78.72 ± 2.47 a	75.36 ± 0.42 a	88.38 ± 2.78 b
	PA	7.88 ± 1.07 a	14.81 ± 1.71 b	20.07 ± 0.43 c	6.37 ± 2.61 a
	BA	2.56 ± 0.80 a	6.46 ± 0.77 c	4.56 ± 0.00 b	5.24 ± 0.39 b
L. casei FTCC 0442	SCFA	8.97 ± 0.05 A	10.52 ± 0.57 B	11.73 ± 0.68 C	18.65 ± 0.35 D
	AA	85.00 ± 0.13 c	80.61 ± 3.14 b	78.52 ± 0.9 b	74.20 ± 0.41 a
	PA	10.98 ± 0.14 a	11.68 ± 1.95 ab	14.23 ± 0.60 c	13.49 ± 0.69 bc
	BA	4.01 ± 0.28 a	7.71 ± 1.20 b	7.25 ± 0.28 b	12.30 ± 0.26 c
B. bifidum BB12	SCFA	9.25 ± 0.48 A	10.98 ± 0.35 B	12.25 ± 1.15 C	20.56 ± 0.17 D
	AA	80.60 ± 0.59 c	73.06 ± 3.95 b	75.03 ± 0.64 b	65.25 ± 0.47 a
	PA	13.63 ± 0.31 a	17.97 ± 1.95 b	14.98 ± 0.24 a	20.36 ± 0.89 c
	BA	5.73 ± 0.89 a	8.96 ± 2.00 b	10.98 ± 0.39 b	14.40 ± 1.35 c

[a] Results are expressed as means ± standard deviation; values are the means of duplicate analysis from three separate runs, $N = 3$. Means in the same row followed by different upper (SCFA) or lower case (AA, PA, and BA) letters are significantly different ($P < 0.05$). Fermentation media contained 5 mg/mL individual carbohydrate.

fermentation of sago RS by *L. casei* 0442 gave the highest total viable count, total carbohydrate consumption, and the lowest in final pH.

Previous in vivo and in vitro studies have shown that FOS is a very effective substrate for fermentation in the large intestine. FOS is considered a good prebiotic because diets containing FOS decrease fecal pH and increase total volatile fatty acids and the concentration of lactobacilli (*32*, *33*). However, it is interesting to note that FOS, a commercial prebiotic, gave the lowest cell count for *L. acidophilus* FTCC 0291, *L. bulgaricus* FTCC 0411, and *B. bifidum* BB12. This suggests that FOS was not a suitable substrate for these probiotic strains. It was demonstrated that *L. bulgaricus* strains, although ordinarily used for yogurt manufacture, and *B. bifidum* were FOS nonfermenters (*24*). Nevertheless, it is well known that sugar metabolism by lactic acid bacteria is species and even strain dependent (*34*).

FOS is an example of the inulin-type fructans, which are linear D-fructose polymers linked by $\beta(2-1)$-glycosidic bonds, often with a terminal glucose moiety that is linked by an $\beta(1-2)$-glycosidic bond, as in sucrose. The degree of polymerization (DP) of FOS varies between 2 and 10. The $\beta(2-1)$ linkage of this fructan prevents its digestion in the upper part of the human gastrointestinal tract and is responsible for its reduced caloric value and dietary fiber-like effects (*35*). The inability of the four studied strains to metabolize FOS indicated that they produced lower levels of the essential enzymes β-1,2-glycosidase and fructofuranosidases to hydrolyze the bonds. These *Lactobacillus* strains also preferred RS_3 to RS_2 as growth substrates as indicated by the lower growth observed in media with Hi-maizes. However, as with *B. bifidum* BB12, a descending order of growth was seen, as follows: Hi-maize 204 ≥ Hi-maize 1024 > Sago RS_3 > FOS.

The percentage of carbohydrate consumption by the probiotic strains is shown in **Table 1**. Generally, all the carbohydrates were utilized to some extent, from 0.20% to 14.33%, with the highest consumption by *L. casei* FTCC 0442. Sago RS_3 (5 mg/mL) was consumed to a significantly greater extent than the other substrates during fermentation with *L. acidophilus* FTCC 0291 (3.68%), *L. bulgaricus* FTCC 0411 (4.60%), and *L. casei* FTCC 0442 (8.84%). The percentage of consumption was low in other substrates, ranging from 3.97% to 0.20%, especially in the case of FOS by *L. bulgaricus* FTCC 0411, in which only about 0.20% of the substrate was consumed. This trend of low substrate consumption was correlated with the observation of low growth in the probiotic bacteria.

Sago RS_3 had also shown a significant pH reduction in the fermentation medium (**Table 1**). As a lowering of pH is preferable in fermentation, the low pH in the gastrointestinal tract may cause the binding of potentially toxic NH_4^+, which in bound form is nondiffusible and thus has the positive effect of lowering blood ammonia level. In addition, the acidic condition also has a detrimental effect on pH-sensitive pathogenic species such as *E. coli* and *Salmonella* found in the gut (*36*).

The SCFA such as butyrate and propionate are able to prevent the synthesis of cholesterol and fatty acids, respectively, in the liver (*37*). It was shown that the production of free fatty acids were strain and substrate-dependent. It was demonstrated that *Lactobacillus* sp. produced organic acids such as lactic, acetic, formic, and butyric acids when fermented in MRS broth (*38*) and sourdough (*39*). The total and individual SCFA produced during fermentation of nondigestible carbohydrates are presented in **Table 2**. Hi-Maize 240 and Hi-Maize 1043 produced significantly higher total SCFA than sago RS when fermented by *L. acidophilus* FTCC 0291, *L. bulgaricus* FTCC 0411, *L. casei* FTCC 0442, and *B. bifidum* BB12. This was likely because the amount of RS in both Hi-Maize 240 and Hi-Maize 1043 was higher than that in sago RS; higher amounts of RS contributed to the higher production of SCFA. Overall, the production of total SCFA from Hi-Maize 240, Hi-Maize 1043, and sago RS was lower compared to the production of total SCFA from FOS by the probiotic bacteria, except for *L. bulgaricus* FTCC 0411. FOS was found to produce the highest concentrations of total SCFA when fermented by *B. bifidum* BB12, followed by *L. casei* FTCC 0442 and *L. acidophilus* FTCC 0291, at amounts of 20.56 mM, 18.65 mM, and 12.56 mM, respectively. In general, all the cultures produced the highest molar percentage of acetic acid as compared to propionic and butyric acids. Sago RS was found to produce the highest molar percentage of acetic acid (81−90%) by all of the strains.

Total SCFA is a group of fatty acids such as acetic, propionic, lactic, butyric, valeric, iso-valeric, iso-butyric, etc. (*23*); thus, the choice of free fatty acids analyzed will greatly contribute to the level of total SCFA. It was shown that acetic acid, propionic acid, and butyric acid had accounted for more than 90% of SCFA present in the colonic fermentation of RS (*23*).

However, the main acidic fermentation products of *Lactobacilli* and *Bifidobacterium* were acetic, lactic, and formic acids (*40*). Nevertheless, total SCFA concentration alone cannot be used to judge the potential of sago RS. The fermentation of sago RS_3 was found to be clearly different from those of FOS, Hi-maize 1043, and Hi-maize 240. Generally, sago RS at 5 mg/ml had shown that it was the most preferred substrate on the basis of viable counts, substrate consumption, and reduction in the pH of media; thus, its potential as a growth substrate of probiotics warrants further investigation to fully understand its metabolism by probiotics.

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