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Prebiotic properties of germinated riceberry rice (*Oryza sativa* L.) fermented with *Pleurotus ostreatus*

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ABSTRACT: In this study, we examined the prebiotic properties of ungerminated riceberry rice (R), germinated rice (GR), fermented rice with the oyster mushroom *Pleurotus ostreatus* mycelium (FR), and α-amylase. The resulting residues, along with inulin (I) and β-glucan (BGC) as a control, were then added to a basal culture medium containing probiotic bacteria: *Lactobacillus acidophilus* (LA), *Lactobacillus casei* subsp. *rhamnosus* (LR), and *Streptococcus lactis* (SL). Our study revealed that the BGC content in the FR sample was 1.5 g per 100 g of the dried sample. This is approximately 4.6 times higher than that found in both the R and GR samples. Additionally, the prebiotic index of FR for LA was 9.75 ± 0.39, significantly higher than that of I, recorded at 1.47 ± 0.48. Moreover, the prebiotic activity scores of FR and BGC against LA were 4.86 ± 0.10 and 7.14 ± 0.56, respectively, which are approximately 5 times greater than that of I (-0.45 ± 1.32). For LR, the prebiotic indices were 2.13 ± 0.60, 2.47 ± 0.17, and 4.00 ± 0.34 for FR, BGC, and I, respectively, with no significant difference between FR and BGC. However, SL showed less effectiveness for all samples. Interestingly, the prebiotic activity scores of LR and SL were less effective compared to the growth of non-probiotic bacteria, *Escherichia coli*. These findings suggest that fermented rice exhibits potential prebiotic properties, indicating its potential for the development of various functional food products.

KEYWORDS: germinated riceberry rice, *Pleurotus ostreatus*, *in vitro* digestion, β-glucan, prebiotic

INTRODUCTION

Prebiotics are non-digestible compounds that promote the growth of probiotic microorganisms. These are commonly beneficial bacteria that reside in the gastrointestinal (GI) tract such as bifidobacteria and lactobacilli. Prebiotics can resist digestive enzymes in the upper GI tract such as α-amylase and polysaccharide hydrolases in the oral cavity and small intestine but can be used as a substrate in the lower GI tract by probiotic bacteria. Examples of oligosaccharides that have shown prebiotic properties include galactooligosaccharides (GOS), fructooligo-saccharides (FOS), xylooligosaccharides (XOS), I, and BGC. BGC is mostly found in the cell walls of plants, cereal grains, algae, and mushrooms. It has been reported to exhibit various biological activities, including anticancer, anti-inflammatory, immuno-stimulatory, and sugar level regulating properties [1–3]. Furthermore, BGCs have demonstrated potential as prebiotics, capable of restoring probiotics in the gastrointestinal tract [4]. The relationship between prebiotics and probiotics can be used to promote host health and well-being. Probiotics are microorganisms that are beneficial for hosts and comprise part of the gut microbiota in the gut ecosystem [5]. Gut microbiotas are linked to health and disease states. Balanced microbiota has been associated with reduced stress and anxiety [6] and reduced risk of colon cancer [7]. However, gut microbiota can be managed and regulated by consuming prebiotics, which have the potential to improve overall health and well-being [8].

Rice is a staple food for more than half of the world's population. Furthermore, brown and colored rice are becoming increasingly popular due to their higher antioxidant and vitamin content [9]. Riceberry rice is a purple-black grain with a high nutritional and protein content. It possesses potential antioxidant, anti-inflammatory, anticancer, antihyperlipidemic, and hypoglycemic activities [10]. Riceberry rice nutritional and prebiotic properties can be improved by germination or microorganism-based fermentation [11]. Particularly, germination is a crucial process in cereal grains that significantly increases nutrient content. This includes increased protein, amino acid, sugar and vitamin content, and bioactive compounds such as the total amount of phenolics, γ-oryzanol, antioxidants, and γ-aminobutyric acid (GABA) [12].

Pleurotus ostreatus, also known as the oyster mushroom, is rich in polysaccharides, including BGC. This mushroom is consumed globally either directly or as a key ingredient in plant-based protein products. Beyond its nutritional value, *P. ostreatus* has been the subject of numerous studies due to its potential medicinal properties. These include anti-inflammatory, antioxidant, and anticancer effects [13]. Moreover, the

fermentation with *P. ostreatus* mycelium can increase the BGC content in germinated riceberry rice, thus making it a promising subject for further research in the field of functional foods and nutraceuticals [11]. The aim of this study was to investigate the nutrient content and prebiotic properties of germinated riceberry rice fermented with *P. ostreatus* mycelium. To assess the prebiotic score and probiotic index, *in vitro* enzymatic digestion of commercial BGC, riceberry rice, germinated riceberry rice, and fermented germinated riceberry rice was conducted using pepsin and α-amylase. The digestive residues were subsequently supplemented to 3 probiotic bacteria: LA, LR, and SL to evaluate the prebiotic potential.

MATERIALS AND METHODS

Materials

Riceberry rice (*Oryza sativa* L.), sourced from a farmer group in Chiang Rai, Thailand, and *P. ostreatus* spawn, obtained from a local mushroom farm in Chiang Rai, Thailand, were used for this study. The mushroom spawn was incubated at 25 °C in complete darkness with the atmospheric humidity adjusted to 75–80% for 10 days. This allowed the mushroom mycelium to fully cover the spawn surface. Then, the mushroom spawn was aseptically cut into pieces of 5 × 5 mm using a sterile blade. These pieces were placed on potato dextrose agar and incubated at 25 °C in darkness for an additional 15 days. The prepared spawn served as the starter for the *P. ostreatus* mycelium (M). Laboratory-grade chicory inulin and enzymes, including pepsin and α-amylase, were obtained from Sigma-Aldrich Pte. Ltd., Singapore. Food-grade BGC was from Pronova Laboratories (Thailand) Co., Ltd. All necessary cultivation media and supplements for the study were purchased from Hi Media Laboratories, LLC, India.

Sample preparation for measuring prebiotic activities

In this study, R was germinated by modifying a previous protocol from Soodpakdee et al [11]. In brief, R was rinsed and soaked with sterile distilled water in plastic baskets covered by a colander for 12 h. Water was poured out, and rice was soaked again. These steps were repeated for 3 cycles (12 h each in total of 36 h). After that, the germination was stopped by incubating the rice at 60 °C for 24 h. The GR was kept in a desiccator at room temperature prior to further experiments.

The preparation of M followed the protocol outlined by Zhu et al [14]. To summarize, the edge of the mycelium, cultured for 15 days, was cut into small pieces with a diameter of 5 mm using a sterile cork borer. These pieces were then aseptically transferred to 300 ml of potato dextrose broth (PDB). The broth was incubated at 25 °C in darkness for 8 days on a reciprocal shaker operating at 125 rpm. This process produced the seed inoculum for the solid-state fermentation of GR, utilizing a 20% (v/w) mycelium inoculum. The fermentation process was carried out for 3 days at 25 °C in darkness under static conditions, and then the FR were dried in a hot air oven, which was set at 60 °C for 24 h. All rice samples (R, GR, FR) were ground and sieved through an ASTM (American Standard Test Sieve Series) stainless-steel mesh sieve No. 60 corresponding to a sieve opening of 250 µm. The samples were stored in an amber zip-lock bag and placed in a desiccator at room temperature prior to further experiments.

β-glucan assay

The β-glucan assay kit (Mixed Linkage) (Megazyme, Ltd., Bray, Ireland) was used to determine content of 1,3/1-6-β-D-glucans in riceberry rice and mycelium. The assay method was according to the standard protocol stated by the manufacturer's protocol (Megazyme, Ltd.).

In vitro intestinal enzymatic digestion using pepsin and α-amylase

This study focused on the digestion of carbohydrates and proteins in the stomach and small intestine with pepsin and α-amylase serving as representative enzymes. The *in vitro* digestion protocol was adapted from Cleary et al [15]. Briefly, 2 g of R, GR, FR, and BGC samples were soaked in 50 ml of phosphate buffer (pH 6.9), and the pH was adjusted to 1.5 with 1 N HCl and digested with pepsin (115 U/g substrate) at 37 °C for 30 min. Next, the pH was adjusted to 6.9 with 1 N NaOH and digested with α-amylase (110 U/g substrate) at 37 °C for 3 h. The digested crude was harvested by centrifugation at 5,000 rpm for 10 min and dried at 60 °C for 24 h, then stored until use.

Bacterial strains

Three probiotic bacteria, LA (strain TISTR 2365), SL (strain TISTR 457), and LR, were used in this study. Additionally, *Escherichia coli* (strain TISTR 527) was employed as a representative pathogenic strain in the intestine. Probiotic bacteria were inoculated in de Man Rogosa Sharpe (MRS) broth medium following the method of Gulhane et al [16], while *E. coli* was maintained on nutrient broth (NB). All bacterial strains were stored at −80 °C before the experiment.

Growth stimulation of probiotic bacteria

Bacterial suspensions were prepared at a concentration equivalent to 0.05 OD_{600}. To stimulate the growth of probiotic bacteria, 1% (v/v) of bacteria was inoculated in the basal media containing 0.1% peptone, 0.1% yeast extract, and 1% (w/v) of either lactose (L, control), I, or digested samples of R, GR, FR, and BGC. The bacterial suspensions were then incubated at 37 °C with reciprocal shaking at 200 rpm for 24 h. Each

treatment was sampled every 4 h for 48 h to determine growth based on the number of colony-forming units (CFUs).

The evaluation of prebiotic potential using Prebiotic index and prebiotic activity score

According to Figueroa-Gonzalez et al [17], the potential of a prebiotic can be assessed by the Prebiotic index, which is the ratio of the growth of probiotic bacteria after supplementation with a sample to the growth after supplementation with lactose (control carbohydrate). A higher Prebiotic index indicates that the sample has a positive effect on prebiotic growth, while a lower than 1 Prebiotic index indicates that the sample has a less effective or negative effect on prebiotic growth. The Prebiotic index was calculated using the following Eq. (1).

$$\text{Prebiotic index} = \frac{\text{CFU of probiotic in sample}}{\text{CFU of probiotic in control carbohydrate}} \quad (1)$$

Additionally, the prebiotic activity score measures the difference between the growth of probiotic and *E. coli*, which is a pathogenic bacterium in the GI tract. Ideally, the growth of *E. coli* on prebiotics should be lower than that of probiotic on lactose. The prebiotic activity score was calculated using Eq. (2).

$$\text{Prebiotic activity score} = \frac{(\text{Log P}_t - \text{Log P}_0)_{\text{prebioticcarbohydrate}}}{(\text{Log P}_t - \text{Log P}_0)_{\text{lactose}}} - \frac{(\text{Log E}_t - \text{Log E}_0)_{\text{prebioticcarbohydrate}}}{(\text{Log E}_t - \text{Log E}_0)_{\text{lactose}}} \quad (2)$$

Where: Log P is the log growth (CFU/ml) of probiotic bacteria at 24 h (P_t) and 0 h (P_0); Log E is the log growth (CFU/ml) of *E. coli* at 24 h (E_t) and 0 h (E_0); t is the time point at 12 and 24 h.

Thin layer chromatography (TLC) analysis

The oligosaccharide pattern was determined using the TLC analysis method modified from Apirak-sakorn et al [18]. The digested crude samples (5 mg), including R, GR, and FR, were dissolved in 1 ml of deionized water and incubated at 60 °C for 12 h. The samples were then centrifuged at 12,000 rpm for 5 min, after which the supernatant was collected and stored at 4 °C until further use. In this experiment, glucose (G), fructose (F), sucrose (S), FOS, XOS, and MOS were used as standard sugars. A 3 µl aliquot of samples G, F, and S, and a 5 µl aliquot of samples FOS, XOS, MOS, and digested samples were loaded onto a TLC plate (Kiesel gel 60, Merck, Darmstadt, Germany). The mobile phase was prepared using a solvent mixture composed of butanal, ethanol, and deionized water in a ratio of 5:3:2, respectively. The TLC plate was developed in a glass chamber for 40 min, immediately removed from the tank, and dried with a hot air drier. The oligosaccharide spots were developed using 5% v/v sulfuric acid in ethanol, and the TLC plate was then dried in a hot air oven (Memmert/une-500, Büchenbach, Germany) at 150 °C for 5 min.

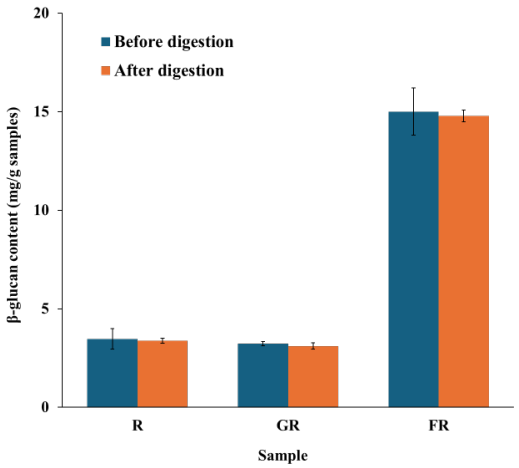

Fig. 1 The BGC content (w/w) in samples both before and after digestion: R, ungerminated riceberry rice; GR, germinated riceberry rice; and FR, fermented germinated riceberry rice. The presence of different superscript letters (a and b) signifies significant differences at $p < 0.05$.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics Program Version 23 (SPSS, Inc., Chicago, IL, USA) using ANOVA, followed by the Duncan multiple comparison test. $p < 0.05$ denoted statistical significance. All experiments were done in triplicates, and results are expressed as mean values with standard deviations.

RESULTS AND DISCUSSION

Determination of BGC content

The results showed that the BGC content, initially measured at 0.35 ± 0.05 g/100 g in R and 0.32 ± 0.01 g/100 g in GR, increased to 1.50 g/100 g in FR, and it was not degraded after intestinal enzymatic digestion as depicted in Fig. 1.

From this result, it appears that the germination process does not directly influence BGC production. Instead, it alters the content of metabolites, proteins, and carbohydrates, which are involved in energy and lipid production [19, 20]. The BGC in the FR sample is primarily derived from the M. This fungus produces enzymes such as laccase, endo-xylanase, endoglucanase, and β-glucosidase, which are involved in the degradation of polysaccharides in GR [21]. As the fungal mycelium digests and absorbs the nutrients present in GR, we observed an increase in the biomass of the mycelium, which in turn leads to an increase in the

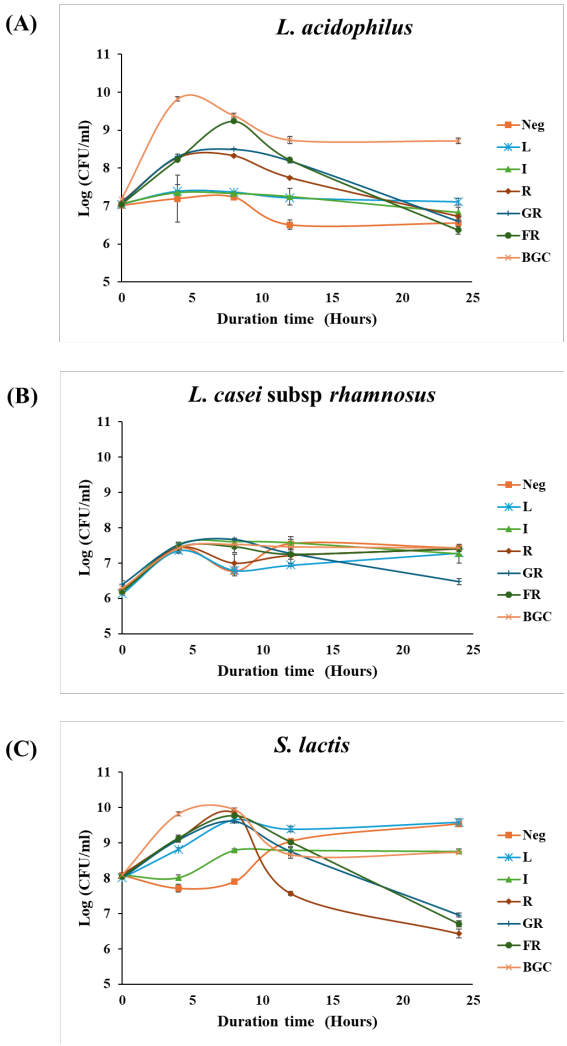

Fig. 2 Growth of *L. acidophilus* (A), *L. casei* subsp. *rhamnosus* (B), and *S. lactis* (C). Neg, Negative control; L, Lactose; I, Inulin; R, Raw riceberry rice; GR, Germinated riceberry rice; FR, Fermented riceberry rice; and BGC, commercial β-glucan.

content of BGC [11, 22].

Probiotic growth stimulation

To stimulate the human digestive system, pepsin and α-amylase digested samples of R, GR, FR, and BGC were used. These samples were each added separately to basal media at 1% (w/v) and grown with LA, LR, and SL. Inulin (a commercial prebiotic) and lactose were used as controls. It was observed that LA exhibited growth in all the digested samples, including BGC (9.82 ± 0.06 log CFU/ml), with cell growth accelerating rapidly and peaking at 4 h. Cell growth in FR reached its peak at 8 h with a count of 8.50 ± 0.02 log CFU/ml. All digested samples prolonged the cell growth of LA for up to 12 h (Fig. 2). These results suggested that this basic medium and digested samples might not be suitable for LR. The growth curves for almost all samples indicate that all media can accelerate cell growth for up to 4 h with log CFU/ml values of 7.44 ± 0.07, 7.36 ± 0.05, 7.44 ± 0.07, and 7.53 ± 0.04 for Neg, L, R, GR, and FR, respectively. However, exceptions are observed in the case of I and BGC, which show the highest cell growth at 12 and 8 h, respectively, with log CFU/ml values of 7.67 ± 0.04 and 7.48 ± 0.04, respectively. The results of LA demonstrate the potential for prolonged cell growth up to 24 h in all samples, except for the GR sample where cell growth begins to decline after 8 h. As for SL, the growth curve indicates that all samples, including L, I, R, GR, FR, and BGC, reach their peak cell growth (9.66 ± 0.08, 8.79 ± 0.04, 9.84 ± 0.08, 9.62 ± 0.05, 9.77 ± 0.03, and 9.94 ± 0.05 log CFU/ml, respectively) at the 8 h mark, after which growth of all samples starts to decline.

Prebiotic index

The prebiotic index of all samples was tested on all probiotic bacteria at 12 and 24 h. Prebiotic index is the comparative growth between samples and the control carbohydrate (lactose). If the prebiotic index is < 1, the prebiotic is not as effective [17]. The results demonstrated that BGC had the highest prebiotic index on the growth of LA, while R, GR, and FR had a higher prebiotic index than I at 12 h (Table 1). Moreover, I had the highest prebiotic index on the growth of LR, while R had the lowest prebiotic index. There was no significant difference between GR, FR, and BGC. Nevertheless, the prebiotic index of the samples grown with SL was low as shown by the lesser growth of this bacterium.

The results supported previous reports on the prebiotic activity of I on the growth of *Bifidobacterium* and *Lactobacillus* [23]. The results of GR and FR showed that L was more effective at 12 h on LA and LR. The sample that was used to supplement the experiment was digested, hence the remaining polysaccharide or fiber should be a non-digestible polysaccharide and might be fermented by probiotics (i.e., breaking down the remaining fiber into food) [24]. The remaining crude R content should contain high fiber [25]. The results show a significantly increased prebiotic index after germination (GR) and fermentation with *P. ostreatus* mycelium (FR) on the growth of probiotic bacteria LA and LR.

However, all samples had the lowest prebiotic index on the growth of SL, suggesting that this probiotic bacterium may require other polysaccharides that could have been removed by the *in vitro* digestion with pepsin and α-amylase. All samples had a positive effect on LA only at 12 h, while within 24 h only BGC had an effect. Regarding LR, all samples had a positive effect

Table 1 Prebiotic index at 12 and 24 h after cultured in the basal media supplement with 1% digested crude samples.

Bacterial strain	Time	Prebiotic index					
	(h)	I	Neg	R	GR	FR	BGC
LA	12	1.47 ± 0.48^{c}	0.02 ± 0.05^{c}	3.31 ± 0.17^{c}	9.27 ± 1.19^{b}	9.75 ± 0.39^{b}	33.14 ± 6.82^{a}
	24	0.54 ± 0.15^{b}	0.28 ± 0.04^{b}	0.41 ± 0.08^{b}	0.31 ± 0.10^{b}	0.18 ± 0.05^{b}	40.36 ± 6.59^{a}
LR	12	4.00 ± 0.34^{a}	3.12 ± 0.98^{ab}	1.42 ± 0.34^{e}	1.64 ± 0.33^{cd}	2.13 ± 0.60^{cd}	2.47 ± 0.17^{bc}
	24	1.32 ± 0.05^{a}	1.37 ± 0.26^{a}	1.29 ± 0.19^{a}	0.16 ± 0.03^{b}	1.32 ± 0.04^{a}	1.39 ± 0.30^{a}
SL	12	0.25 ± 0.04^{b}	0.45 ± 0.04^{a}	0.02 ± 0.00^{c}	0.24 ± 0.07^{b}	0.43 ± 0.01^{a}	0.19 ± 0.05^{b}
	24	0.15 ± 0.03^{b}	0.89 ± 0.13^{a}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.14 ± 0.01^{b}

LA, *L. acidophilus*; LR, *L. casei* subsp. *rhamnosus*; SL, *S. lactis*; I, inulin; Neg, no supplement; R, raw riceberry rice; GR, germinated riceberry rice; FR, fermented riceberry rice; and BGC, commercial β-glucan. Each bacterial strain is analyzed independently from other strains. The different superscript letters (a–d) indicate significant differences ($p < 0.05$).

on growth at 12 and 24 h, with the exception of the GR sample, which showed a reduced prebiotic index at 24 h.

Prebiotic activity score

The prebiotic activity score measures the ability of a prebiotic to stimulate the growth of probiotics relative to other colonic microorganisms, such as *E. coli*, in this study. A higher score indicates a more effective prebiotic activity. The results showed that BGC had a positive score, while I was the least effective among all the prebiotics tested. On the other hand, R, GR, and FR samples enhanced the growth of only LA, and their scores were higher than that of I at 12 h ($p < 0.05$), as shown in Table 2. However, these samples were less effective than BGC when compared to the growth of *E. coli* especially after 24 h. This could be because *E. coli* has the ability to adapt and produce enzymes that can break down the prebiotics from oligosaccharides into suitable nutrients [26].

Based on prebiotic index and prebiotic activity score, R, GR, and FR have high effectiveness when compared to I for LA at 12 h. However, these samples have the least effectiveness on LR and SL. These results agree with those of Sookpakdee et al [11] in terms of the GR and FR.

Thin layer chromatography (TLC) analysis

The oligosaccharide patterns of all rice sample residues, both before and after enzymatic digestion, were analyzed using TLC (Fig. 3). The TLC patterns of R, GR, and FR were similar to those of the oligosaccharide pattern presented in MOS but with a higher sucrose content. The TLC analysis revealed that the sucrose content in the GR sample was higher than that in the R sample. This could be explained by the fact that during the germination process, the enzymes in the seed digested the carbohydrates into sucrose to support the growth of the plant embryo [27, 28]. Interestingly, the sucrose content in the FR sample was lower than that of R and GR, but the oligosaccharides of trimers to pentamers of maltose were higher. Although the pattern of oligosaccharides presented in MOS was similar to that of XOS, this study found that the digestion of polysaccharides by α-amylase resulted in short-chain polysaccharides consisting of 3–4 subunits of maltose sugar rather than xylose sugar [22, 29]. After digestion, sucrose was not detected in any of the rice sample residues. However, the maltotriose and maltotetraose were more abundant than in the non-digested sample. This suggests that carbohydrates undergo enzymatic digestion, resulting in oligosaccharides, which could act as a prebiotic substrate for probiotic bacteria. This could explain why we observed a high prebiotic index in these digested rice samples, especially in FR, which promoted the growth of LA at 8 h better than R and GR. This could be due to the oligosaccharides and BGC contents in the FR sample (Fig. 3).

The results of the prebiotic activity and TLC analysis suggest that the non-digestible oligosaccharides in rice samples could enhance the growth of probiotic bacteria. Furthermore, BGC also plays a pivotal role in promoting growth and acting as a prebiotic for probiotic bacteria, especially *Lactobacillus* sp. Our results indicate that the BGC content in all rice samples remained unchanged before and after enzymatic digestion. This finding aligns with our previous study, which reported less effective prebiotic properties in non-digested R and GR samples on LA [11]. In this study, we observed that the digested R and GR samples could enhance the prebiotic properties towards probiotic bacteria. This could be attributed to the oligosaccharides produced after the enzymatic digestion of carbohydrates in the samples, as supported by the TLC pattern. Moreover, the prebiotic activity of FR could be derived from both BGC and oligosaccharides after enzymatic digestion. The growth of LA was highest at 4 h when treated with BGC, while FR promoted growth at 6 h and 10 h in R and GR (Fig. 2). This could be explained by the non-digestible polysaccharide in riceberry rice acting as a prebiotic for the growth

Table 2 Prebiotic activity score at 12 and 24 h after cultured in the basal media supplement with 1% digested crude samples and control.

Bacterial strain	Time	Prebiotic activity score					
	(h)	I	Neg	R	GR	FR	BGC
LA	12	-0.45 ± 1.32^{e}	-4.08 ± 0.73^{d}	1.47 ± 0.14^{c}	4.45 ± 0.34^{b}	4.86 ± 0.10^{b}	7.14 ± 0.56^{a}
	24	-4.35 ± 2.03^{b}	-8.13 ± 1.13^{c}	-7.59 ± 1.28^{c}	-9.29 ± 2.22^{c}	-12.30 ± 1.89^{e}	24.05 ± 1.20^{a}
LR	12	-7.72 ± 0.53^{d}	-5.89 ± 1.82^{cd}	-2.37 ± 1.41^{ab}	-0.47 ± 1.26^{a}	-4.80 ± 1.78^{bc}	-4.59 ± 0.43^{bc}
	24	0.50 ± 0.11^{a}	0.64 ± 0.59^{a}	0.37 ± 0.45^{b}	-7.67 ± 0.56^{c}	0.05 ± 0.08^{ab}	0.38 ± 0.59^{a}
SL	12	-0.25 ± 0.05^{a}	0.15 ± 0.04^{a}	-2.65 ± 0.08^{d}	-2.31 ± 0.03^{c}	-2.31 ± 0.05^{c}	-0.11 ± 0.01^{b}
	24	-1.05 ± 0.05^{a}	-0.36 ± 0.02^{a}	-2.79 ± 0.04^{d}	-1.67 ± 0.09^{c}	-1.43 ± 0.01^{b}	1.80 ± 0.08^{a}

LA, *L. acidophilus*; LR, *L. casei* subsp. *rhamnosus*; SL, *S. lactis*; I, inulin; Neg, no supplement; R, raw riceberry rice; GR, germinated riceberry rice; FR, fermented riceberry rice; and BGC, commercial β-glucan. Each bacterial strain is analyzed independently from other strains. The different superscript letters (a–e) indicate significant differences ($p < 0.05$).

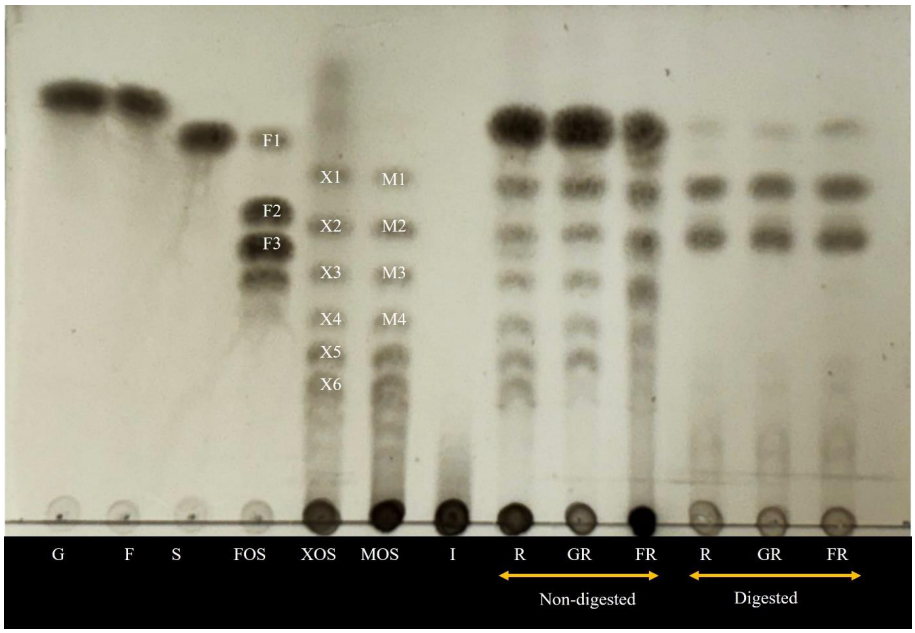

Fig. 3 The TLC analysis. G, F, and S represent glucose, fructose, and sucrose, respectively. FOS, XOS, MOS, and I stand for fructooligosaccharide, xylooligosaccharide, maltooligosaccharide, and inulin, respectively. R, GR, and FR denote riceberry rice, germinated rice, and fermented riceberry rice, respectively. F1, F2, and F3 represent kestose, nystose, and fructo-syfuranosylstose, respectively. X1 through X6 represent xylose, xylobiose, xylotriose, xylotrihalose, xylopentose, and xylohexose, respectively. M1 through M4 represent maltotriose, maltotetraose, maltopentaose, and Maltohexaose, respectively.

of probiotic bacteria [30]. Humans are incapable of producing β-amylase and β-glucosidase, enzymes necessary for the digestion of these oligosaccharides. As a result, the remaining oligosaccharides are utilized by gut microbes, which can produce β-amylase, β-glucosidase, and pullulanase. These enzymes can catalyze the breakdown of oligosaccharides or short-chain BGCs [31]. Furthermore, LA produces several enzymes, including lactase, amylase, phytases, esterase, and pullulanase [32]. LR is commonly used in milk fermentation, and SL primarily produces enzymes such as lipase, esterase, α-glucosidase, β-glucosidase, and α-fucosidase [33, 34]. The production of β-amylase and pullulanase in LA can catalyze the remaining oligosaccharides after digestion. This could explain why different probiotic strains respond differently to the prebiotic supplement.

CONCLUSION

The process of germination and fermentation with mushroom mycelium can enhance the prebiotic potential of riceberry rice by increasing its BGC content.

Following *in vitro* enzymatic digestion with pepsin and α-amylase, the digested crude samples were digested into oligosaccharides and found to stimulate the growth of probiotic bacteria, namely *L. acidophilus* and *L. casei* subsp. *rhamnosus*, more effectively than lactose (except for *S. lactis*). The prebiotic activity score was used to compare the growth of pathogenic bacteria in the GI tract (*E. coli*) with the growth of LA induced by the R, GR, and FR samples. These samples were found to enhance the growth of LA more than I, but less than BGC. To support prebiotics that can promote the growth of LR and SL, further bioinformatics studies should be conducted to explore the enzymatic pathway of these probiotic bacteria. This would be valuable for the development of prebiotic products in synbiotic product development.

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