Selective Fermentation of *Lactobacillus* and *Streptococcus* *In Vitro*: Effects of Chinese Fermented Glutinous Rice on the Growth Promotion of Potential Probiotics

Wenliang Ma,1 Xiao Ni,1 Ying Guo,1 Yu Zhang,1 Chaojie Zhu,1 Yinpeng Li,1 Chi Shen,1 Biao Yuan2 and Xiao Xu1,3

1School of Life Science, Shaoxing University, Shaoxing, Zhejiang 312000, China
2Department of Food Quality and Safety, National R&D Center for Chinese Herbal Medicine Processing, College of Engineering, China Pharmaceutical University, Nanjing, Jiangsu 211198, China
3National Engineering Laboratory for Cereal Fermentation Technology, Jiangnan University, Wuxi, Jiangsu 214122, China

Correspondence should be addressed to Biao Yuan; yuanbiao@cpu.edu.cn and Xiao Xu; xiaolexu@126.com

Received 30 July 2021; Revised 15 October 2021; Accepted 12 November 2021; Published 23 December 2021

A functional Chinese fermented glutinous rice has been developed with the supplementation of Fu brick tea (CRW-FBT). In this study, we aimed to evaluate its effect on the growth of potential probiotic strains in the *Lactobacillus*, *Streptococcus*, and *Weissella* genus, compared with traditional Chinese fermented glutinous rice (CRW). LQ_h growth profiles of lactic acid bacteria were analyzed based on fermentations *in vitro*, and the optical densities were recorded at 600 nm during the whole fermentation. Growth curve, maximum OD 600 nm, and growth rate were measured and compared among samples with different ratios of CRW-FBT and CRW addition. Through the multiple analysis of growth parameters, we found that all the tested strains obtained better growth results when CRW-FBT was supplemented to the media, compared with the CRW and basic media. The bacterial growth was promoted by exhibiting the shortened lag time, prolonged logarithmic phase and stationary phase, and increased growth rate and cell density, as well as the better performance after 24 h and 48 h fermentation. Besides, short-chain fatty acids and organic acids in CRW-FBT were founded. Our work demonstrated the positive effect of Fu brick tea supplemented in the CRW and illustrated its beneficial role in the food fermentation industry for the purpose of microorganism enrichment and the improvement of microbial metabolism.

1. Introduction

In recent years, prebiotics have been received increasing attention as ‘a substrate that is selectively utilized by host microorganisms conferring a health benefit’ [1]. The consumption of food-based prebiotics is one of the practicable approaches to modulate the composition and structure of human intestinal microbiota. These prebiotics reach the large intestine and were selectively metabolized by certain intestinal bacterial species, such as lactobacilli and galactococcus [2]. In general, the prebiotics could promote short-chain fatty acids, resulting in various health benefits [3]. Nowadays, numerous new functional foods emerged endlessly aiming at improving host health, while novel beneficial food supplements achieve rapid development in possession of stimulating the growth of probiotics and leading to intestinal microflora homeostasis.

Polyphenols have been found to have the capabilities of influencing cell growth of microorganisms and their metabolisms. Zhou et al. showed the addition of gallic and protocatechuic acids significantly reduced the concentration of ethyl carbamate during Chinese glutinous rice wine fermentation [4]. Chen et al. reported the finding that one type of polyphenols in oolong tea influenced the protein expression and meliorated alcohol-induced intoxication of *Saccharomyces cerevisiae* [5]. Figueiredo et al. presented the
finding that the phenolic aldehydes, coniferaldehyde, 3,4-dihydroxybenzaldehyde, p-hydroxybenzaldehyde, and sinapaldehyde significantly affected the growth of Oenococcus oeni, while flavonoids showed slight differences such as myricitin and the flavan-3-ols studied (catechin and epicatechin) [6, 7]. Thus, polyphenols played crucial roles in food fermentation and were used to contribute to the population adjustment of microorganism, leading to the enhanced fermentation efficiency and food quality.

Fermented foods and beverages have been developed for nearly 14,000 years as irreplaceable staples and drinks of human diets. In recent years, fermented foods and beverages has been increasingly popular for their functional properties. Lactic acid bacteria and their metabolites in fermented foods, such as exopolysaccharide, organic acids, galactooligosaccharides, and phenolic acids, have many positive effects on human health [8]. Hence, it needed to establish relationships among lactic acid bacteria in fermented foods, their metabolites, and gut health. Chinese fermented glutinous rice wine (CRW), as one of the oldest alcoholic beverages, was brewed dating as far as 5000 BCE in China and recently is consumed widely in the world. CRW usually appears in daily diet as a drinking or seasoning matter during cooking. CRW was a primary source of polyphenols intake in eastern diets, just like beer in western diets. Phenolic acids, flavonoids, tannins, and flavan-3-ols were biotransformed by microorganisms and released from materials during the fermentation. Furthermore, CRW had many pharmacological benefits and value as its various nutritional compounds including polysaccharides, peptides, and amino acids [9, 10]. CRW was also used to supplement as one of foodstuffs during the food fermentation process with an effect to promote microbial growth, ameliorate flavor, and participate in substance metabolism. A certain type of Chinese glutinous rice wine in the region of Shaoxing city (Zhejiang province, China) called “xiangxue” was made by mixing with fermented glutinous rice wine during the primary fermentation. The flavor of xiangxue was special and this processing technique was popular, but few studies focus on the effect of fermented glutinous rice wine supplementation, especially on microbial growth and their metabolism. It is also difficult to ascertain the biological effects on microbial metabolism and the promotion of the special species growth.

Chinese glutinous rice wine has been reported to contain ten individual phenolic compounds such as syringic acid, rutin, (−)-epicatechin, (+)-catechin, gallic acid, and vanillic acid [11]. Xu et al. studied the promoted fermentation efficiency exhibited by Chinese fermented glutinous rice when supplementation with Fu brick tea (CRW-FBT) in the primary fermentation, because of an increased concentration of polyphenols leading to an increased population of yeasts and enzyme activities [8, 12]. Thus, it is rational that Fu brick tea as a supplement of polyphenols was utilized into fermentation process to modulate microbial community. In this study, Chinese fermented glutinous rice with Fu brick tea was pressed to collect the low-alcohol juice, and its ability of supporting the growth of potential intestinal probiotics and lactic acid bacteria was analyzed. A series of substrate utilization examinations were carried out, by pure cultures of strains, such as Streptococcus thermophilus, Lactobacillus plantarum, Lactobacillus rhamnosus, and other 8 species. The dynamic changes of microorganism in various fermentation stages were displayed based on the growth curves, maximum microorganism population, growth rates, and lag parameters. We compared the microbial growth rate when growing at original media with those growing at which carbohydrate was partially replaced with CRW-FBT or CRW. We aimed to obtain information about the growth profiles of potential probiotics under different conditions based on the model analysis, along with the influence CRW-FBT and CRW may have on the fermentation ability of lactic acid bacteria during fermentation process.

2. Method

2.1. Materials and Reagents. Glutinous rice (Oryza sativa var.) was bought from a local market. Fu brick tea (Camellia sinensis L.) was obtained from a tea factory in Yiyang city, Hunan province, China. The starter this study used was commercial starter (Angel Yeast Co., Yichang, Hubei Province, China). Media components were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). MRS (Man-Rogosa and Sharp) was utilized as a basic medium for the growth of Lactobacillus and Streptococcus strains, enriched with carbohydrates, nitrogen sources, mineral elements, and 0.1% (v/v) Tween 80. Besides, 2% (w/v) of the experimental fraction was supplemented into basic media. The standards were as follows: oxalic acid, tartaric acid, succinic acid, pyruvic acid, malic acid, lactic acid, citric acid, formic acid, acetic acid, propionic acid, and butyric acid were obtained from Aladdin Chemical Reagent Co. (Shanghai, China). LC-grade methanol was from Merck (Darmstadt, Germany). All the other reagents were of analytical grade.

All strains in this study were isolated from traditional fermented foods in our lab. Lactobacillus helveticus MB2-1 and Streptococcus thermophilus MB5-1 were isolated from Sayram ropy yogurt in Xinjiang [13]. Weissella hellenica D1501 was obtained from Dongzhu fermented meat [7]. L. plantarum 70810 and L. rhamnosus LS-8 were isolated from Chinese pickle [14]. L. delbrueckii subsp. bulgaricus 1-4 was obtained from fermented dzho milk. L. plantarum B1-6 was isolated from a type of fermented cereal beverage in Xinjiang province of China called Kirgiz boza. L. plantarum 17-1 was obtained from Zhalaba, a special type of fermented vegetable in Guizhou province of China.

2.2. Preparation of Chinese Fermented Glutinous Rice with Fu Brick Tea. Chinese fermented glutinous rice was fermented with/without Fu brick tea (CRW-FBT/CRW) according to the method of our previous study [12]. In brief, Fu brick tea extract was performed by extracting with water at 100°C for 5 min in a ratio of 2:100 (g/mL). Glutinous rice was soaked and steamed for 30 min firstly and then mixed with starters and Fu brick tea extracts at a ratio of 2%, followed by incubating at 30°C. The liquor was collected after 4-day
fermentation by pressing and filtered with 0.45 μm membrane for the following analysis.

2.3. Microorganisms and Culture Media. Bacterial strains in our work belong to *Lactobacillus* and *Lactococcus* genera. The strains were cultured in MRS broth and incubated at 37°C in a 5% CO₂ atmosphere under anaerobic conditions (Thermo Forma 5111, Thermo Fisher Scientific Co., USA). The CRW-FBT extracts were supplemented into basic media into different ratios of 1%, 2%, 5%, and 10% as the experiment groups. The CRW extracts were prepared as a control group that was supplemented at ratio of 1%. The blank group was MRS basic media. The whole lactic acid bacteria were cultured and revived twice before the measurement of bacterial growth.

2.4. Measurement of Organic Acids in the Fermented Samples. The organic acids were detected according to a HPLC analysis. All CRW-FBT and CRW samples were prepared by centrifuging at 10000 × g for 10 min at 4°C (Allegra64R, Beckman Coulter, Inc., Brea, USA). The supernatant was collected and filtrated with 0.22 μm membrane and then injected into a HPLC system (Agilent Technologies, Wilmington, DE, USA) equipped with a XSelect HSS T3 analysis column (4.6 mm × 250 mm, 5 mm, Agilent) and a diode-array detector. The injection volume was 20 μL. Each sample was eluted at a flow rate of 0.7 mL/min for 20 min by mobile phases (96% 0.01 M (NH₄)₂HPO₄ and 4% methanol). The column temperature was maintained at 30°C. Lactic acid and other 9 organic acids were monitored at the wavelength of 215 nm. The concentrations of identified compounds in samples were quantified due to standards.

2.5. Evaluation of Bacterial Growth. The microbial strains were cultured in MRS for 36 h, and then the cells were harvested by centrifuging at 8000 × g for 10 min at 4°C. After washing twice with 0.85% NaCl solution, the cells were used as seed cultures of each strain and were diluted 1:100 into media for the assays described below. The media used in this study included basic media, MRS plus CRW-FBT and MRS plus CRW. According to the method of Ahn et al., bacterial growth was performed in 300 μL wells of sterile 100-well honeycomb microplates by repeating three replicates [15]. Each strain was inoculated into 300 μL fresh medium in individual wells and grown at 37°C for 50 h. To monitor growth patterns, the optical densities at 600nm (OD₆₀₀nm) were recorded at 20 min intervals using a BioScreen C automated growth curve analysis system (Oy Growth Curves AB Ltd., Helsinki, Finland).

2.6. Statistical Analysis. All the results in the present study were presented as means ± standard deviation. One-way analysis of variance (ANOVA) and *t*-tests were implemented to consider the significant differences (*p* < 0.05), using SPSS version 24.0 (SPSS Inc., Chicago, IL, USA). In order to analyze the microplate assays comprehensively, the representative results were expressed as maximum growth rate (max, h⁻¹) and lag time (lag, h) calculated by fitting the curves to a growth model using Microsoft Excel [14].

3. Results and Discussion

To understand the effects of Chinese fermented glutinous rice fermented with Fu brick tea (CRW-FBT) and Chinese fermented glutinous rice (CRW) on the growth of lactic acid bacteria, the selected strains of the genera *Lactobacillus* and *Streptococcus* were used due to their widespread utilization in fermented foods [16]. The growth curves of representative strains grown on supplementation of CRW-FBT at ratios of 1%, 2%, 5%, and 10%, as well as 1% CRW, are depicted in Figure 1. The growth curves showed the growth phases of bacteria, covering the lag phase, logarithmic phase, and stationary phase. All the strains did not meet their decline phases during the 50 h incubation, and thus the overall stationary phases were not exhibited in order to compare differences of other three growth phases evidently.

As shown in Figure 1(a) and Figure 1(b), *L. plantarum* 17-1 and *L. plantarum* 70810 reached their stationary phases earlier compared with other 6 bacteria. Similarly, the needed time that *L. delbrueckii* subsp. *bulgaricus* 1-4 reached the stationary phase was a little shorter than that of the other groups. The OD₆₀₀nm values still preserved a slight raising tendency during its stationary phase, especially in the supplementation groups of 10% CRW-FBT and 5% CRW-FBT (shown in Figure 1(f)). Moreover, the OD₆₀₀nm values in these two experiment groups at different growth periods displayed higher results than those in the control group. 2% CRW-FBT, 1% CRW-FBT, and 1% CRW groups. These results illustrated that the growth and reproduction of lactic acid bacteria were promoted by the supplementation of high content of CRW-FBT. This phenomenon appeared significantly in the growth of *L. plantarum* B1-6, in which a longer lag phase and logarithmic phase were needed, while reaching stationary phase cost about another 5 h compared with experiment groups (Figure 1(c)). Interestingly, the densities of *L. rhamnosus* LS-8 bacteria in 10% CRW-FBT group at any time were higher than those in 5% CRW-FBT, followed by those in 2% CRW-FBT and 1% CRW-FBT, while the much lower were exhibited in the control group.

To analyze the characteristics of the proliferation under the effect of nutrient supplements, the growth rates were calculated (Figure 2). Usually, in the lag phase, the growth rates increase laggingly and then increase fleetly to the maximum when reaching the logarithmic phase. There were plenty of differences displayed by the growth profiles of *L. helveticus* MB2-1 in different media (Figure 2(e)). Obviously, the CRW actuated a promotion of microbial growth, leading to shortening lag phase period and costing less time for the maximum growth rates. The influences of CRW-FBT caught better results, in which the least time of the lag phase was spent by the selected strains culturing in the media with the supplementation of 10% CRW-FBT, as well as costing the least time to reach the maximum growth rate. The trends of growth rates of 5% CRW-FBT, 2% CRW-FBT, and 1% CRW-FBT were semblable and spent a little more time staying on lag phase and reaching the maximum growth rates than the 10% CRW-FBT group. As for
Figure 1: Continued.
W. hellenica D1501 (Figure 2(d)) and L. delbrueckii subsp. bulgaricus 1-4 (Figure 2(f)), these phenomena could be noted, but the influences were insignificant compared with L. helveticus MB2-1. Furthermore, it was inconsistent in all strains that a high concentration of CRW-FBT had a high growth rate. For example, the least time was spent by W. hellenica D1501 with the supplementation of 1% CRW-FBT running to the maximum growth rates, followed by 2% CRW-FBT and 5% CRW-FBT. LQ_his may be because some microorganisms were sensitive to higher concentration of acids and polyphenols in the supplementation, whereas a synchronous trends were displayed in the growth rate of L. plantarum 17-1 grown in all samples (Figure 2(a)). To better describe the effect of all samples on the growth of L. plantarum 17-1, we analyzed another two parameters, the maximum growth rate ($\mu_{\text{max}}$) and lag time (lag), which were used to compare the substrates preferences of microorganism [17].

As shown in Table 1, through fitting the curves to a sigmoid growth model, the fastest growth rate ($\mu_{\text{max}}$) of L. plantarum 17-1 was 0.69 h$^{-1}$ with the supplementation of 1% CRW-FBT and 1% CRW, followed by the control group, and other high concentrations of CRW-FBT. However, in
Figure 2: Continued.
the case of *W. hellenica* D1501 and *S. thermophilus* MB5-1, the highest $\mu_{\text{max}}$ was 0.40 h$^{-1}$ and 0.49 h$^{-1}$, endowed by both 5% CRW-FBT and 10% CRW-FBT. As for these two strains, the higher $\mu_{\text{max}}$ parameters emerged in the high concentrations of CRW-FBT. LQ$\_\text{hat}$ illustrated the finding that less than 5% of the addition proportion would be enough to perform an optimal role to promote the bacterial growth in the culture of these species. Similarly, the fastest growth rate endowed by 5% CRW-FBT occurred with *L. plantarum* 70810, *L. plantarum* B1-6, *L. helveticus* MB2-1, and *L. delbrueckii* subsp. *bulgaricus* 1-4 reaching values of 0.49 h$^{-1}$, 0.44 h$^{-1}$, 0.36 h$^{-1}$, and 0.47 h$^{-1}$, followed by 0.48 h$^{-1}$ of 10% CRW-FBT, 0.41 h$^{-1}$ of 2% CRW-FBT, 0.34 h$^{-1}$ of 2% CRW-FBT, and 0.45 of h$^{-1}$ 10% CRW-FBT, respectively.

In general, a higher $\mu_{\text{max}}$ implied that growth of the strains keeps a higher rate, while indirectly reflecting a stronger capability of all samples. But possessing the fastest growth rate could not be a necessary and sufficient condition for the strongest bioactivity; therefore, the lag time (lag) was used to present the profiles of microbial lag phases. The
fermentation of basic medium by *L. helveticus* MB2-1 with a long lag time (6.31 h) and the lowest $\mu_{\text{max}}$ values (0.24 h$^{-1}$) was characterized as being much slower than fermentation in the sample-supplemented medium, with 4.57 h of lag time by 5% CRW-FBT and 5.31 h of lag time by 1% CRW. However, the positive effect of CRW-FBT and CRW would not be achieved during the fermentation of other lactic acid bacteria, such as *W. hellenica* D1501, in which control group (1.90 h) with the shortest lag time indicated that supplementing experimental samples could prolong the lag phase of bacterial growth. In addition, as for *L. plantarum*, different subspecies exhibited different bioactivities. We found that the lag times of *L. plantarum* 17-1 and *L. plantarum* 70810 were prolonged, while the lag times of *L. plantarum* B1-6 were shortened by various constituents of samples. Tang et al. also reported their distinct performance when different subspecies of *L. plantarum* grow on the exopolysaccharide fractions [18]. In fact, the shortening lag time was considered as a way to accelerate the reproduction of microorganisms, as well as to push the process of microbial metabolism and substrate utilization [19].

In terms of the comprehensive comparison among samples, Figure 3 shows the time of lag phases and logarithmic phases and reaching the maximum OD$^{600\text{nm}}$ (OD$^{\text{max}}$) during the stationary phases. The addition of CRW-FBT and CRW observed with *L. rhamnosus* LS-8 resulted in the significant longer time of logarithmic phases, which were prolonged about ten hours compared with the control MRS medium. A longer time was spent by *L. delbrueckii* subsp. *bulgaricus* 1-4 for obtaining OD$^{\text{max}}$ when it was grown in the supplementation of 10% CRW-FBT and 5% CRW-FBT. That was also the reason why significant higher OD$^{\text{max}}$ values were endowed with the high concentrations of CRW-FBT compared to other media. During the prolonged stationary phase, the growth rates were positive till reaching the OD$^{\text{max}}$ value, and further leading to the delayed decline phase. It would be a beneficial result to food industry, particularly to a long-time fermentation process. In the cases of three *L. plantarum* samples, different effects were made by adding the experimental samples. *L. plantarum* 17-1 spent a longer time reaching the OD$^{\text{max}}$ value in 10% CRW-FBT and 5% CRW-FBT supplemented media, while the OD$^{\text{max}}$ values were obtained quickly after the end of logarithmic phase when grown in other media. *L. plantarum* 70810 had a shorter time to reach OD$^{\text{max}}$, but a longer time of logarithmic phase, as a reason of obtaining the highest OD$^{\text{max}}$ in the shortest time. *L. plantarum* B1-6 were the least affected by the samples, exhibiting a little shorter lag time and longer stationary time in the high concentrations of CRW-FBT. Furthermore, the *L. helveticus* MB2-1 was influenced with the shorter time in the three periods we detected, indicating that CRW-FBT promoted the bacterial proliferation. Thus, 2% CRW-FBT could be utilized to shorten the production time and improve the fermentation efficiency.

With respect to mirroring the growth performance through microbial densities during the fermentation, the maximum OD$^{600\text{nm}}$ (OD$^{\text{max}}$) was recorded (Figure 4), and the OD$^{600\text{nm}}$ values at 24 h and 48 h fermentation were summarized (Table 2). Obviously, the highest OD$^{\text{max}}$ was obtained by supplementing experimental samples. *S. thermophilus* MB5-1 and *L. helveticus* MB2-1 cultured in 10% CRW-FBT fraction possessed the highest OD$^{\text{max}}$ which were much higher than those in 1% CRW and basic media. Meanwhile, these two strains obtained the higher OD$^{600\text{nm}}$ at both 24 h and 48 h in the media supplemented with CRW-FBT, whose effect was in a dose-dependent manner. Similarly, three *L. plantarum* strains reached better significant growth results when 10% CRW-FBT was added to MRS broth than with a low concentration of CRW-FBT and CRW. In the case of *L. plantarum* 17-1, the highest OD$^{\text{max}}$ (2.17) was observed in 10% CRW-FBT fermentation, which was much higher than 1.97 of OD$^{\text{max}}$ in 1% CRW-FBT, 1.94 of OD$^{\text{max}}$ in 1% CRW and 1.88 of OD$^{\text{max}}$ in basic MRS media. Thereby, 10% CRW-FBT addition had a positive effect on obtaining a better bacterial growth behavior during a long-time fermentation, especially after 24 h fermentation. Otherwise, the growth performances of *L. rhamnosus* LS-8 and *W. helenica* D1501, by the supplementation of 5% CRW-FBT, reached 2.19 and 2.21 of OD$^{600\text{nm}}$ after 24 h fermentation, as well as 2.21 and 2.18 of OD$^{600\text{nm}}$ after 48 h fermentation, which were a little higher compared with those grown in other media. Meanwhile, at the supplementation concentration of 10%, these two strains, coupled with *L. delbrueckii* subsp. *bulgaricus* 1-4, gave a similar performance in the OD$^{600\text{nm}}$ values at 24 h and 48 h, as well as OD$^{\text{max}}$.

All the data demonstrated that CRW-FBT and CRW fractions stimulated the growth of the tested lactic acid bacteria in the typical food fermentation environment. The occurrence of components in CRW-FBT and CRW seems to influence the $\mu_{\text{max}}$ values, lag time, and maximum OD$^{600\text{nm}}$.

---

**Table 1: The lag time (lag, h) and the fastest growth rate ($\mu_{\text{max}}$, h$^{-1}$) of representative selected strains during 50 h fermentation with the supplementation of CRW-FBT and CRW.**

<table>
<thead>
<tr>
<th></th>
<th>lag</th>
<th>17-1</th>
<th>70810</th>
<th>B1-6</th>
<th>D1501</th>
<th>MB2-1</th>
<th>1-4</th>
<th>MB5-1</th>
<th>LS-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% CRW-FBT</td>
<td>0.67</td>
<td>0.62</td>
<td>3.89</td>
<td>0.48</td>
<td>4.61</td>
<td>0.40</td>
<td>4.73</td>
<td>0.40</td>
<td>5.73</td>
</tr>
<tr>
<td>5% CRW-FBT</td>
<td>2.64</td>
<td>0.64</td>
<td>3.81</td>
<td>0.49</td>
<td>4.54</td>
<td>0.44</td>
<td>4.84</td>
<td>0.40</td>
<td>4.67</td>
</tr>
<tr>
<td>2% CRW-FBT</td>
<td>2.28</td>
<td>0.64</td>
<td>3.73</td>
<td>0.47</td>
<td>4.60</td>
<td>0.41</td>
<td>4.76</td>
<td>0.43</td>
<td>5.37</td>
</tr>
<tr>
<td>1% CRW-FBT</td>
<td>2.23</td>
<td>0.69</td>
<td>3.56</td>
<td>0.45</td>
<td>4.68</td>
<td>0.38</td>
<td>4.63</td>
<td>0.40</td>
<td>5.40</td>
</tr>
<tr>
<td>1% CRW</td>
<td>2.20</td>
<td>0.69</td>
<td>3.64</td>
<td>0.45</td>
<td>4.70</td>
<td>0.38</td>
<td>4.42</td>
<td>0.39</td>
<td>5.31</td>
</tr>
<tr>
<td>Control</td>
<td>1.90</td>
<td>0.67</td>
<td>3.31</td>
<td>0.42</td>
<td>4.82</td>
<td>0.37</td>
<td>4.15</td>
<td>0.38</td>
<td>6.31</td>
</tr>
</tbody>
</table>

Figure 3: Continued.
Figure 3: The time of lag phase and logarithmic phase and to reach OD\textsubscript{max} during stationary phase of selected fermentation of representative strains with the supplementation of CRW-FBT and CRW. L. plantarum 17-1 (a), L. plantarum 70810 (b), L. plantarum B1-6 (c), Weissella helenica D1501 (d), Lactobacillus helveticus MB2-1 (e), L. delbrueckii subsp. bulgaricus 1-4 (f), Streptococcus thermophilus MB5-1 (g), and L. rhamnosus LS-8 (h).

Figure 4: Continued.
values of the individual strains, such as the polysaccharides, polyphenols, and short-chain fatty acids (SCFAs). In the survival of the model probiotic *L. plantarum*, Ramos et al. reported that the presence of the exopolysaccharides β-glucans enhanced the beneficial properties of probiotic bacteria [20]. In addition, Gomez et al. evaluated the low-molecular-weight pectic oligosaccharides according to the selective fermentation by potential probiotic *Lactobacillus* and *Bifidobacterium* strains, and the fermentation capability of probiotic bacteria was modified [17]. LQ_he stimulatory effects on the growth and malolactic activity of lactic acid bacteria were exhibited by fermentation with low concentrations of gallic acid [21]. We also found CRW-FBT contained a low concentration of gallic acid [12]. That was one of reasons why the better growth parameters were observed in the selective fermentation when supplementing with CRW-FBT.

The metabolism of lactic acid bacteria produces the short-chain fatty acids (SCFAs), which have been confirmed to contain several healthy benefits, especially to the improvement of colonic health, like reducing the growth of potential pathogenic bacteria and ameliorating probiotics [22, 23]. As shown in Table 3, the contents of acetic acid were the highest (0.095 ± 0.03 g/L), followed by the propionic acid (0.138 ± 0.007 g/L) in CRW-FBT, and 0.015 ± 0.004 g/L and 0.033 ± 0.008 g/L, respectively in CRW. Acetic acid

![Figure 4: The maximum OD<sub>600</sub> (OD<sub>max</sub>) of representative selected strains during 50 h fermentation with the supplementation of CRW-FBT and CRW. *L. plantarum* 17-1 (a), *L. plantarum* 70810 (b), *L. plantarum* B1-6 (c), *Weissella hellenica* D1501 (d), *Lactobacillus helveticus* MB2-1 (e), *L. delbrueckii* subsp. *bulgaricus* 1-4 (f), *Streptococcus thermophilus* MB5-1 (g), and *L. rhamnosus* LS-8 (h).]

**Table 2:** The OD<sub>600</sub> values of representative selected strains after 24 h and 48 h fermentation with the supplementation of CRW-FBT and CRW.

<table>
<thead>
<tr>
<th></th>
<th>17-1</th>
<th>70810</th>
<th>B1-6</th>
<th>D1501</th>
<th>MB2-1</th>
<th>1-4</th>
<th>MB5-1</th>
<th>LS-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% CRW-FBT</td>
<td>2.09</td>
<td>2.16</td>
<td>2.27</td>
<td>2.26</td>
<td>2.30</td>
<td>2.21</td>
<td>2.19</td>
<td>2.13</td>
</tr>
<tr>
<td>5% CRW-FBT</td>
<td>2.02</td>
<td>2.14</td>
<td>2.25</td>
<td>2.23</td>
<td>2.27</td>
<td>2.19</td>
<td>2.18</td>
<td>2.10</td>
</tr>
<tr>
<td>2% CRW-FBT</td>
<td>1.89</td>
<td>2.05</td>
<td>2.17</td>
<td>2.20</td>
<td>2.24</td>
<td>2.16</td>
<td>2.15</td>
<td>2.10</td>
</tr>
<tr>
<td>1% CRW-FBT</td>
<td>1.84</td>
<td>1.97</td>
<td>2.11</td>
<td>2.18</td>
<td>2.16</td>
<td>2.14</td>
<td>2.06</td>
<td>2.12</td>
</tr>
<tr>
<td>1% CRW</td>
<td>1.78</td>
<td>1.85</td>
<td>2.09</td>
<td>2.16</td>
<td>2.06</td>
<td>2.05</td>
<td>2.03</td>
<td>2.07</td>
</tr>
<tr>
<td>Control</td>
<td>1.74</td>
<td>1.85</td>
<td>2.04</td>
<td>2.08</td>
<td>2.04</td>
<td>2.08</td>
<td>2.00</td>
<td>2.06</td>
</tr>
</tbody>
</table>

usually could serve as a source of energy for human body tissues, such as brain, heart, and peripheral tissues [24]. Propionic acid has various beneficial effects and was reported to affect the cell metabolism and immune system, thus reducing hepatic and serum cholesterol content in vivo [25]. Butyrate can regulate the precursors of M2 macrophages and T cells, regulate oxidative stress, and contribute to intestinal homeostasis [22]. The content of butyric acid in CRW-FBT was 0.011 ± 0.001 g/L, which was higher than in CRW significantly with 0.006 ± 0.001 g/L. Campos et al. also reported that special strains could produce butyric acid during food fermentation [26]. The total content of organic acids (in Table 3) except for SCFAs in CRW-FBT (1.706 ± 0.051 g/L) was a little lower than CRW (2.157 ± 0.056 g/L), covering the significant differences of lactic acid contents and constituent proportion. Malic acid and pyruvic acid were not found in CRW, while they were quantified by 0.006 ± 0.001 g/L and 0.006 ± 0.001 g/L in CRW-FBT. Malic acid is one of the major organic compounds which contribute to the flavor and taste of wine, juices, and other drinks [27]. Malolactic fermentation was usually studied in red wine fermentation because of its benefits of increasing flowery aroma and adjusting the balance of saccharinity and acidity [28]. In our work, the significant differences were also present in the contents of tartaric acid (0.082 ± 0.002 g/L in CRW-FBT and 0.034 ± 0.008 g/L in CRW).

4. Conclusion

This study evaluated the potential beneficial role of fermented glutinous rice with Fu brick tea (CRW-FBT) by establishing a pure culture system. Eight strains used in traditional fermented foods were selected for bacterial fermentation in vitro. Our results suggested CRW-FBT may have a great potential as supplementation to shorten lag time, raise the growth rate, and prolong the proliferation period, leading to a higher cell density. A high concentration of CRW-FBT was considered to possess the bioactive capability of stimulating the growth of probiotic strains related to the human intestinal health. In sum, these results could provide a novel perspective for fermented glutinous rice development in food fermentation industry.

Data Availability

The raw data used to support the findings of this study are currently under embargo while the research findings are commercialized. Requests for data, 6 months after publication of this article, will be considered by the corresponding author.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Authors’ Contributions

Xiao Xu conceived the project idea and obtained fundings. Xiao Xu and Biao Yuan were assigned the project and wrote this article. Wenliang Ma and Xiao Ni finished all the researches. Chi Shen, Ying Guo, and Biao Yuan revised the paper. Other authors were participants in the method optimization and review. All authors approved the final manuscript draft.

Acknowledgments

Financial support from the Natural Science Foundation of Zhejiang Province, China (LQ21C200003), Natural Science Foundation of Shaoxing University (2019LG1012), and Scientific Research Start-Up Fund of Shaoxing University (20195015) was acknowledged.

References

[1] G. R. Gibson, R. Hutkins, M. E. Sanders et al., “The International scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of...


