Korean Society for Biotechnology and Bioengineering Journal 30(1): 33-37 (2015)
http://dx.doi.org/10.7841/ksbbj.2015.30.1.33 ISSN 1225-7117 / eISSN 2288-8268

연구논문

탄소원으로서 입국을 이용한 유산균 발효

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Lactic Acid Fermentation with Rice Koji as a Carbon Source

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Received: 27 December 2014 / Accepted: 25 February 2015

Abstract: Recently, several health benefits of rice wine, makgeolli, were known due to the interest on the traditional Korean liquor and the researches on the rice wine are increasing. Organic acids produced during the process of rice wine fermentation play important roles in the taste and flavor. In this study, we have examined the optimal conditions for lactic acid production in rice koji as a carbon source. Skim milk was also used as a supplementary ingredient for the optimization of lactic acid fermentation. Bacterial growth of *Lactobacillus sakei* was monitored under this condition. The pH, acidity of the culture and the ethanol tolerance of this bacterium were also tested. Through these experiments, we were able to optimize the growth condition of lactic acid bacteria by the addition of skim milk. This was also able to affect the change of pH, acidity, sugar concentration and alcohol tolerance, which might contribute to the improvement of the quality of rice wine. The optimal condition for the growth was 2 days with 10% (w/v) of skim milk concentration. With these results, it was confirmed that rice koji was an effective carbon source for the growth of lactic acid bacteria.

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Keywords: Rice koji, Carbon source, Lactic acid, *Lactobacillus sakei*

1. INTRODUCTION

Rice wine is traditional “just fitter” liquor in Korea [1]. Recently, rice wine have receiving attention as a dietary health supplements food known to be beneficial for blood circulation, constipation, and anti-cancer effect [2,3]. Therefore, rice wine is becoming a popular alcoholic beverage to elder and youth in domestic as well as in overseas [2,3]. Main ingredient of rice wine is the cereal, with yeast as a leavening agent [4]. As well as the yeast, lactic acid bacteria affect the taste of the rice wine during the fermentation process [5]. The fermentation process of rice wine is generally composed of sequential growth of mold, yeast and lactic acid bacteria. Among them, lactic acid bacteria play important roles in quality of rice wine. The lactic acid bacteria are lowering the pH of the culture, which prevents contamination of bacteria in early stage of the fermentation process [6]. These lactic acid bacteria also give significant impacts on the flavor of the rice wine, and eliminate the fatigue substances in the body to promote metabolism [7].

Usually, about 0.8% of lactic acid was contained in the rice wine [7]. A group of lactic acid bacteria might increase the fermentation efficiency of yeast. However, the optimal condition for lactic acid bacteria in rice wine fermentation was not determined yet.

On the other hand, rice koji refers to a pure culture which

was inoculated artificially with a station mold (i.e., *Aspergillus*) in the steam-cooked rice. Rice koji is used as a starter culture of *Aspergillus* strain, which plays an important role during the fermentation of rice wine. Therefore, we have examined the optimal conditions for lactic acid fermentation in rice koji as a carbon source. Skim milk was also used as a supplementary ingredient for the optimization of lactic acid fermentation. This process will also be used as a supplementary data for the production of traditional fermented drinks with rice and probiotics food contained lactic acid bacteria.

2. MATERIALS AND METHOD

2.1. Culture of *Lactobacillus sakei*

Lactobacilli MRS broth was used for the cultivation of *Lactobacillus sake*i (KCCM 40264). An aliquot of *L. sakei* was inoculated in 15 mL of *Lactobacilli* MRS broth and incubated for 24 h at 30°C. This seed culture was then transferred into rice koji containing 1%, 10%, 50%, or 100% of skim milk and incubation at 30°C for 48 h. Bacterial cell growth was measured by streak plated method. Serially diluted bacterial cultures were plated onto *Lactobacilli* MRS agar and incubated at 30°C for 24 h. The total cell number was obtained by multiplying the number of colonies formed to the dilution folds.

Sugar concentration of the medium was measured with the saccharometer (PAL-1, Atago Co., Fukui, Japan) and by oBx.

2.2. Assays of acidity and pH measurement

Acid production of the bacteria was monitored by measuring the pH. After centrifugation of the culture broth, the pH of the culture was measured with a pH meter (EcoMet P25, Istek Co., Seoul, Korea). A clear supernatant was also used for the assays of acidity. The amount of 0.1 N NaOH was measured to change the color of supernatant containing an aliquot of 1% (w/v) phenolphthalein. Lactic acid content was showed by adequate amount of NaOH added to the supernatant.

2.3. Determination of ethanol tolerance

To determine the ethanol tolerance, the lactic acid bacteria was cultivated in the broth containing 0%, 5%, 10%, or 15% of ethanol and incubated at 30°C for 2 days. The bacterial cell growth was measured with the spectrophotometer (U-2800, Hitachi Co., Ltd., Tokyo, Japan) at 660 nm.

2.4. Statistical analysis

All tests and analyses were repeated at least three times. The results are expressed as means±SD. A one way analysis of variance (ANOVA) and Duncan test were used for multiple comparisons using the SPSS program (version 16.0 for windows, SPSS Inc.). Values were considered to differ significantly if the *P* value was less than 0.05.

3. RESULTS

3.1. Bacteria growth in skim milk-enhanced rice koji

To enhance the growth of lactic acid bacteria skim milk was added to rice koji. The bacterial growth was monitored with a series of concentrations of skim milk. After 2 days incubation at 30°C, it was confirmed that the addition of skim milk effectively enhanced the bacterial growth (Fig. 1). When zero to 1% (w/v) of skim milk was added the bacterial growth was significantly retarded. With 5% (w/v) or 10% (w/v), it showed comparably high growth rate and increased cell number after incubation for 48 h. However, the bacterial cell numbers showed sudden decrease after 2 days. Therefore, it was confirmed that the optimal condition for the growth was 2 days with 10% (w/v) of skim milk concentration.

During the growth of lactic acid bacteria, the sugar concentration in rice koji was also monitored (Fig. 2). As the lactic acid bacteria grew sugar concentration in rice koji was increased. This result might support that lactic acid bacteria in rice koji might have positive effect in further alcohol fermentation.

3.2. Acid production and pH of the culture

Acid production and the pH is one of the important factors in the quality of rice wine. Lowering the pH not only enhances the refreshing flavor of the rice wine but also prevents the conta-

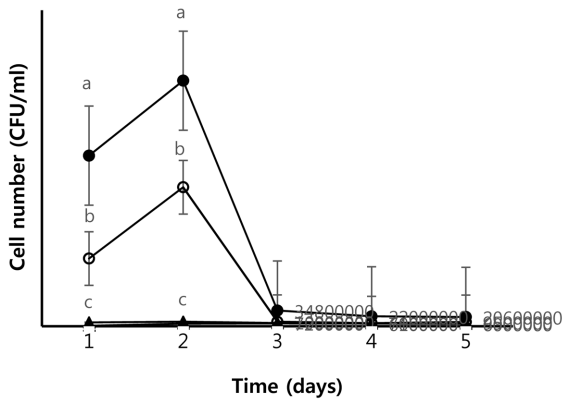

Fig. 1. Bacterial growth of *L. sakei* in rice koji with the supplement of skim milk. Various concentrations of skim milk were added to rice koji. Bacterial cell growth was described by CFU/mL. Error bars represent the standard deviations. Difference letters superscripts in the same incubation time indicate significant difference ($p \leq 0.05$). Symbols: △, 0% of skim milk; ▲, 1%; ○, 5%; ●, 10%.

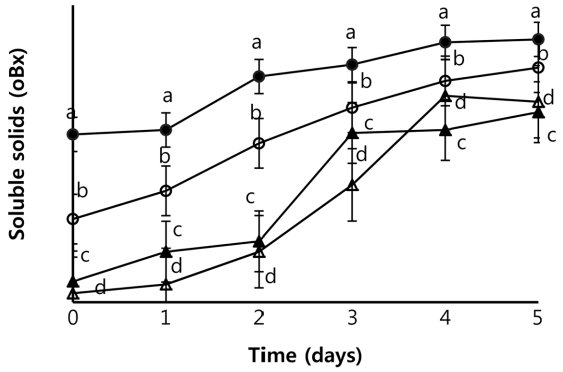

Fig. 2. Changes of sugar concentration in rice koji during the cultivation of *L. sakei*. *L. sakei* was inoculated and cultured on rice koji supplemented with various concentrations of skim milk. Error bars represent the standard deviations. Difference letters superscripts in the same incubation time indicate significant difference ($p \leq 0.05$). Symbols: △, 0% of skim milk; ▲, 1%; ○, 5%; ●, 10%.

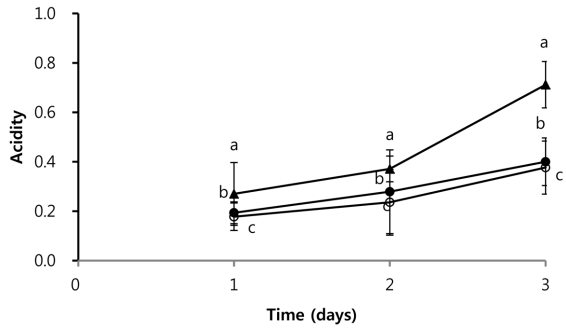

Fig. 4. Acidity change of *L. sakei* culture. The amount of 0.1 N NaOH was measured to change the color of supernatant containing an aliquot of 1% phenolphthalein. Error bars represent the standard deviations. Difference letters superscripts in the same incubation time indicate significant difference ($p \leq 0.05$). Symbols: ○, 1% of skim milk; ●, 5%.▲, 10%.

mination of unnecessary bacteria [7]. However, excessive acid production, on the other hand, might result a strong sour flavor that impairs the taste of rice wine. The pH of the culture showed the lowest value when no skim milk was added (Fig. 3). However, it is said that the optimal pH of the rice wine is between 4 and 5. As depicted in Fig. 3, these pH ranges were obtained when 5% (w/v) or 10% (w/v) of skim milk was added. However, the pH of culture decreased below 4 after incubation for 24 h.

The decrease of pH in the culture is mainly due to the production of acids by the lactic acid bacteria. Therefore, the amount of acids produced during the culture was monitored. Over the incubation periods, acidity of the culture was gradually increased till 3 days. However, the acid production was not proportional to the skim milk concentration. The acidity showed the highest value with 1% of skim milk at 3 days (Fig. 4).

3.3. Ethanol tolerance

Acid production is important because it might have influence to the flavor of rice wine. However, it is also important that the lactic acid bacteria might also be present during the alcohol fermentation step. Therefore, alcohol tolerance was monitored in the presence of 10% (w/v) skim milk.

The bacterial growth was slowly retarded with the increase of the ethanol concentration (Fig. 5). There was no significant difference of bacterial growth between 5% (v/v) and 10% (v/v)

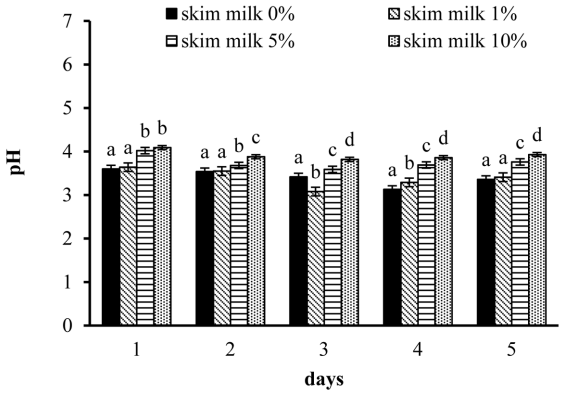

Fig. 3. Changes of pH in rice koji during the cultivation of *L. sakei*. *L. sakei* was inoculated and cultured on rice koji supplemented with various concentrations of skim milk. Error bars represent the standard deviations. Difference letters superscripts in the same incubation time indicate significant difference ($p \leq 0.05$).

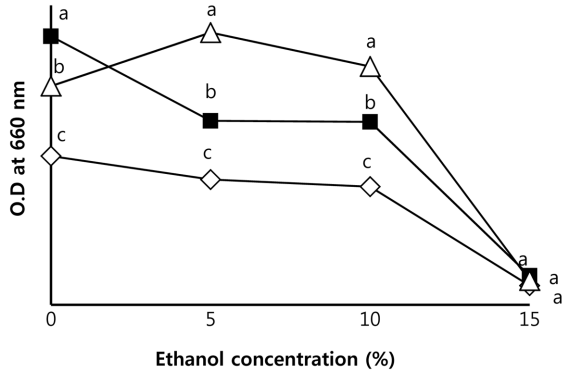

Fig. 5. Determination of ethanol tolerance. *L. sakei* was cultivated in the broth containing 0%, 5%, 10%, and 15% of ethanol, respectively and incubated at 30°C for 2 days. The bacterial cell growth was measured with the spectrophotometer at 660 nm. Error bars represent the standard deviations. Difference letters superscripts in the same ethanol concentrations indicate significant difference ($p \leq 0.05$). Symbols: ◇, 24 h; , 48 h; △, 72 h.

of alcohol concentrations. There was no remarkable growth of lactic acid bacteria at 15% (v/v) of ethanol.

4. DISCUSSION

Rice wine is made by simultaneous process of saccharification and alcohol fermentation [8]. Nuruk is a leavening agent of rice wine[2]. Several microorganisms are related in the fermentation process of rice wine such as yeasts, molds and lactic acid bacteria. Among them lactic acid bacteria play an important role in the taste and flavor of rice wine [5]. Moreover, lactic acid bacteria in the alcohol fermentation process is lowering the pH of the culture and prevent its contamination from bacteria in the early process of fermentation [6]. It also has been reported that lactic acid in rice wine might be able to eliminate the fatigue substances in the body to promote metabolism [7]. Lactic acid bacteria are having a variety of configurations of nutritional requirements such as amino acids, peptides, vitamins and several mineral components depending on the species [9]. There were several reports about the facilitated the growth of lactic acid bacteria with the addition of vegetable extracts [10,11]. In this paper, we want to promote the growth of lactic acid bacteria supplemented with skim milk instead of vegetable extracts.

Skim milk contains a large amount of non-fat-solids in it, which might be able to accelerate the growth of lactic acid bacteria[12]. In this report, we have find out that higher concentration of skim milk was able to promote the growth of lactic acid bacteria (Fig. 1). More precisely, the highest growth of the lactic acid bacteria was shown in 10% (w/v) of skim milk. The addition of skim milk also influenced the acidity, sugar concentration and ethanol tolerance of the bacteria.

On the other hand, even the bacterial growth was not significantly detected with 0% or 1% of skim milk, sugar concentrations were slowly increased as the incubation time increased (Fig. 2). This might be the release of sugar from the rice koji. These results imply that the supplementation of skim milk might improve the taste and flavor of rice wine through the promotion of the growth of lactic acid bacteria.

The change of pH and acidity in the rice wine is important. It is well known that the change of pH might deteriorate the fermentation process of rice wine. Therefore, it is an important indicator for alcohol fermentation [13]. The change of pH is due to a series of organic acids produced during the fermentation process (Fig. 3) [14,15]. In this experiment the pH change came from the growth of the lactic acid bacteria. Therefore it might be the result of lactic acid production by the bacteria. However, the pH of the culture went a little higher with the higher the concentration of the skim milk. This result was opposite to the previous research results [14,15]. It might be necessary for further study. The pH was initially decreased till 72 h and slightly increased after 72 h regardless of the concentration of skim milk. However it was maintained the proper pH of less than pH 4.5. This result is similar to the previous research reports of Kim et al. [16], and Lee et al. [17].

The total acid of the rice wine is an index factor that indicates the content of the acid substances such as organic acids, carbon dioxide and acidic amino acids, produced during the fermentation process. It is well known that these acids affect the taste of the rice wine [18]. In this study, we measured the total acidity of the culture with the addition of 10% (w/v) of skim milk (Fig. 4). Total acidity of the culture was gradually increased throughout the culture time. In general, with comparably low acid content makes rough taste and higher content of acid makes sour taste [13].

Sugars used as substrates for microbial fermentation are important in the production of ethanol and might influence on the creation of scent and sweetness [19,20]. So, sugar concentration is also an important factor of the texture of the rice wine. The change of sugar concentration was also examined in this study with the addition of skim milk. Soluble solid concentration was increased with a higher concentration of skim milk. This result implies that the addition of skim milk might enhance the saccharification of rice koji. This result showed skim milk was able to affect the increase of sugar concentration. As the incubation time flows the sugar concentration might be decreased since sugars were used as a substrate of microbes [21,22].

Finally, alcohol concentration is also one of the most important elements in the fermentation of all alcohol drinks and that affects the quality of the rice wine [23,24]. Generally, during the fermentation process of rice wine with traditional yeasts, the growth of lactic acid bacteria is rapidly decreased with the increase of alcohol concentration because lactic acid bacteria are difficult to grow in more than 5% (v/v) of alcohol [25,26]. In this study, the number of lactic acid bacteria also decreased rapidly with 10% (v/v) of alcohol concentration (Fig. 5). However, *L. sakei* used in this study was able to survive even at 15% (v/v) of alcohol concentration (data not shown). This result showed that alcohol tolerance of *L. sakei* was enough to alcohol fermentation up to the concentration of 15% (v/v) of ethanol.

5. CONCLUSION

Based on these results, we were able to optimize the growth conditions of lactic acid bacteria by the addition of skim milk.

The optimal condition for the growth was 2 days with 10% (w/v) of skim milk concentration. This was also able to affect the change of pH, acidity, sugar concentration and alcohol tolerance, which might contribute to the improvement of the quality of rice wine. With these results, it was confirmed that rice koji was an effective carbon source for the growth of lactic acid bacteria.

ACKNOWLEDGEMENTS

This research was financially supported by Chonnam National University, 2012.

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