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CONTROL OF KIMCHI FERMENTATION BY LACTIC ACID BACTERIA

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Introduction

Kimchi is a fermented vegetable food in Korea. It has been traditionally served as side dishes at meals along with cooked rice and other dishes. Many types of Kimchi are available according to raw materials, processing methods, season and locality (1). In 2000, an amount of Kimchi consumed in Korea is estimated to be 1,557,000 M/T per year, of which 467,000 M/T (30%) is produced commercially. The market of commercial Kimchi production is about $500 million and has been increasing by 15-20% yearly since 1988.

Kimchi is fermented naturally by many microorganisms from various raw materials. Kim et al. (2-4) isolated 150 anaerobes, 50 aerobes, and 2 yeasts from Kimchi. Among 200 bacteria isolated, important microorganisms in Kimchi fermentation were *Lactobacillus plantarum, L. Brevis, Streptococcus faecalis, Leuconostoc mesenteroides,* and *Pediococcus pentosaceus.* Most strains of *Lactobacillus* have been found in Kimchi. Some strains of the aerobic bacteria such as *Achromobacter, Flavobacterium* and *Pseudomonas* were also identified (5).

Ecological changes of various microorganisms during Kimchi fermentation have been investigated to understand the Kimchi fermentation mechanism. Kim and Chun (4) reported that the growth of aerobes increased at the early stage of Kimchi fermentation and then decreased, while the growth of anaerobes continued to increase during storage of 50 days. A rapid growth of aerobes after the storage of 50 days was due to the proliferation of film-forming yeasts. Results on distribution of the isolated and identified microorganisms indicated that *Leu. mesenteroides* grew actively in the early stage of Kimchi fermentation, thereby producing lactic acid and carbon dioxide which could acidify Kimchi and created an anaerobic state to suppress the growth of aerobes. *Streptococcus* grew actively in the early stage of fermentation, whereas *Pediococcus* grew in the mid-stage, and *L. plantarum* and *L. brevis* grew in the late stage, which could affect the ripening of Kimchi. Therefore, it is clear that Kimchi fermentation is initiated and conducted by bacteria that are present in raw materials, rather than by enzymatic action of the vegetables involved (6). However, few studies to compare Kimchi fermentation characteristics of each of lactic acid bacteria have been carried out because there were no ways to make it grow under the similar conditions as Kimchi.

Kimchi tastes best when properly fermented (7-10). At the optimum time of fermentation, the pH and the titratable acidity of Kimchi were 4.2 and 0.6-0.8% (as lactic acid), respectively (9). Sugars in raw materials used for Kimchi preparation are converted to lactic acid as well as other acids by lactic acid bacteria during fermentation, and these acids are responsible for the unique taste of Kimchi. Important organic acids detected from Kimchi are lactic, malic, citric, tartaric, succinic, acetic, oxalic and fumaric acid (11). However, after the optimum time of fermentation, production of excessive acids and breakdown of pectic substances occur, and off-flavors are developed due to the growth of aerobes. These changes in Kimchi are significantly affected by salt concentration and fermentation temperature.

In this study, control of Kimchi fermentation by the lactic acid bacteria selected as Kimchi starter cultures were investigated in the pasteurized fresh Kimchi (Kimchi media) as well as the fresh Kimchi in order to produce kimchi with constant quality.

Materials and Methods

1. Raw materials

Baechu (Chinese cabbage; *Brassica campestris* L. spp. *pekinensis* (Lour) Olsson), moo (radish; *Raphanus sativus* L.), and other raw materials such as garlic, ginger, green onion and hot pepper powder were purchased at a grocery store. Bay salts used in salting process and partially refined salts (with purity greater than 88%) used in seasoning mixture process for adjusting the final salt content were purchased at the same store. Water used in all processes of Kimchi preparation was tap water.

2. The lactic acid bacteria

Eight strains of the lactic acid bacteria which were selected as the Kimchi starter strains in the previous study(12) and were used in this study were *Lactobacillus plantarum* KFRI 813, *Lactobacillus plantarum* S-1, *Leuconostoc mesenteroides* subsp. *mesenteroides* KFRI 819, *Leuconostoc mesenteroides* subsp. *dextranicum* KFRI 184, *Lactobacillus maltaromicus* KFRI 235, *Lactobacillus sake* KFRI 816, *Lactobacillus brevis* KFRI 805, *and Pediococcus pentosaceus* S-5.

3. Media and chemicals

MRS agar and broth (Difco Laboratories, U.S.A.) were used for counting and culturing lactic acid bacteria, respectively. Petrifilm plates (3M, U.S.A.) were used for counting other microorganisms. All chemicals used in these experiments were reagent grade.

4. Preparation of baechu Kimchi and moo Kimchi

Baechu and moo kimchi types were prepared as in the previous study (12). Baechu and moo trimmed were cut into halves or quarters lengthwise and small slices, respectively. The cabbage sections and radish slices were subjected to a salting process which combined both wet and dry salting methods. They was soaked in the brine solution until softened, rinsed three times with running tap water and then drained gravitationally. The brined cabbage sections were cut crosswise into smaller slices.

Meanwhile, the Kimchi seasoning mixture which contained 3.1g of green onion, 2.3g of red pepper powder, 1.5g of garlic, 0.4g of ginger root and 2.9g of tap water was prepared and then was mixed with 100g of brined cabbage or radish. The final salt contents of the Kimchi types were adjusted to 2.5%(w/w).

5. Packing and pasteurization of baechu Kimchi and moo Kimchi

Each of the two Kimchi types was packed under vacuum (38 torr) with a vacuum packaging machine (Turbovac, type SB 260, Netherlands) in 300g unit. The packing film used in this experiment was composed of nylon (15μm) and polyethylene film (85μm) (13). The packed units of the Kimchi types were pasteurized in the hot water bath under different conditions (at 70℃ for 20 min in baechu Kimchi and at 65℃ for 20 min in moo Kimchi). The pasteurized Kimchi types were stored at 4℃ and used as Kimchi media for growth of lactic acid bacteria.

6. Cultivation of lactic acid bacteria in Kimchi media and fresh Kimchi

Seed cultures of the lactic acid bacteria were activated in MRS broth at 30℃ for 24 hrs. The cells were harvested by centrifugation, washed twice with sterilized saline solution (0.85%), and resuspended in the same solution. The cell suspension was used as 0.1 - 0.5%(v/w) of inoculum for cultivation of each of lactic acid bacteria in the pasteurized Kimchi media and the fresh Kimchi at 4 or 10℃.

7. Counting of microorganisms (13)

Growth of lactic acid bacteria was measured as colony-forming units(CFU) per m*l* of Kimchi liquid by using the culture pouring method with MRS agar medium. Growth of other microorganisms was measured by using the Petrifilm plating method. One m*l* of Kimchi liquid was taken aseptically as soon as each packing unit of Kimchi types was opened, and was serially diluted in 0.85% of sterile saline solution. One m*l* of the diluted Kimchi liquid was poured onto the MRS agar medium and incubated at 37℃ for 72 hr. Colonies grown on the plates were counted using a Quebec colony counter.

8. Chemical analyses

The remaining portion of each Kimchi type was blended (Kwangjin blender, Model KJ201, Korea) and filtered through multi-layered gauze. The filtrate was used for measurement of pH, titratable acidity(TA), salinity and reducing sugar content. The pH was measured with a pH meter (Orion, Model SA520, Japan). The TA was measured by titrating 10 m*l* of the filtrate with 0.1N NaOH to pH 8.3 (14), and then the TA was converted to lactic acid content(%; w/w). The salinity of the filtrate was measured by the Mohr method (15), and the reducing sugar content was measured by Miller's dinitrosalicylic acid (DNS) method (16) using glucose as a standard. For analysis of organic acids, the Kimchi liquid was diluted 25 times with the 3rd distilled water, sonicated for 10 min, and filtered through Millipore filter(0.45㎛). Organic acids were analyzed with HPLC(JASCO, Japan) using acetic, citric, fumaric, lactic, malic, malonic, oxalic, propionic, succinic and tartaric acid as standards.

Results and Discussion

1. Growth of the selected lactic acid bacteria in the baechu and moo kimchi

Fermentation characteristics of the selected lactic acid bacteria were compared in the baechu and moo kimchi media as well as in the fresh baechu and moo kimchi with regard to pH, total acidity, reducing sugars, microbial changes and organic acid composition. Among them, only *L. plantarum* KFRI 813 could grow actively in both the baechu kimchi and moo kimchi media. The other strains of lactic acid bacteria could grow actively in the baechu kimchi media, but not in the moo kimchi media.

The controlled kimchi fermentation using lactic acid bacteria as microbial starters were also compared with natural kimchi fermentation.

From the above results, *Leuconostoc mesenteroides* subsp. *mesenteroides* KFRI 819, *L. plantarum* KFRI 813 and *L. maltaromicus* KFRI 235 were selected as microbial starter strains for making kimchi.

2. Enumeration of the lactic acid bacteria in the mixed cluture system

Types of lactic acid fermentation of the selectec lactic acid bacteria were determined in HHD (Hetero-fermentative and homo-fermentative differential) media.

And, BL and BPB media was selected as the selective media for enumeration of each strain of the lactic acid bacteria differentially in the mixed culture. Colony colors and forms shown on those media were observed and were considered to be important factors to differentiate them.

3. Fermentation of kimchi inoculated with single and mixed culture of the selected lactic acid bacteria

Fermentation characteristics of thc selected microbial starter strains were investigated in the fresh baechu kimchi inoculated with 0.5% of the single and mixed culture during fermentation at 4 and 10℃. Sensory evaluation of the kimchi samples was investigated with regard to the fermentation phases of kimchi. Kimchi samples inoculated with the mixed culture showed better quality than those inoculated with the single culture.

4. Preparation of the lactic acid bacterial starters in the cryoprotectants

For production of commercial kimchi products inculated with microbial starters, those lactic acid bacteria should be prepared to be a commercial product with high viability by using various cryoprotectants such as skim milk, lactose, sucrose and gelatin. Garlic paste which is used as one of subingredients for making Kimchi was also selected as a good cryoprotectant. During storage at -70℃ for about 150 days, each strain of the selected lactic acid bacteria showed good viability in 10% solution of the cryoprotectants.

5. Fermentation of kimchi inoculated with the lactic acid bacterial starters in the cryoprotectants

The microbial starters of the selected lactic acid bacteria were prepared by using 10% garlic paste or 10% skim milk as cryoprotectants. Fermentation characteristics of the microbial starter were investigated in the fresh baechu kimchi inoculated with 0.5% of the single and mixed culture during fermentation at 4 and 10℃. Sensory evaluation of the kimchi samples was investigated with regard to the fermentation phases of kimchi. Kimchi samples inoculated with the mixed culture showed better quality than those inoculated with the single culture. Also, kimchi samples inoculated with the microbial starters in 10% garlic paste showed better quality than those in 10% skim milk.

Conclusion

It was shown in this study that Kimchi fermentation could be controlled by using lactic acid bacteria as the microbial starters for making kimchi. However there are various problems to solve for production of Kimchi products with constant quality by using microbial starters. One of those problems is reduction of the initial microbial loads in the raw materials to significant levels. Unfortunately, most of raw materials used for making kimchi are vegetables labile to heat. At present, any other methods do not show the remarkable reduction of the initial microbial loads in raw materials. Therefore, some researches should be done in the future to develop technology to reduce the initial microbial loads in the raw materials, to improve the microbial starter strains, to produce of kimchi with a constant quality and to establish the quality contol system.

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