Comparison of the Biochemical Activities of Commercial Yogurts and *Lactobacillus acidophilus*-containing Yogurt

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시판용 요구르트와 *Lactobacillus acidophilus* 요구르트의 생화학적 활성의 비교

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Lactic acid-producing bacteria such as *Lactobacillus* spp. function to ferment carbohydrates and produce ATP. Such *Lactobacillus* spp. are used for the production of commercial yogurts. *Lactobacillus* spp. are beneficial to the intestinal tract, and *Lactobacillus acidophilus*-containing yogurts have received considerable attention because of their preventive effects against early-stage cancer of the large intestine. In this study, lactic acid-producing bacteria were cultured from three different groups: commercial solid yogurt (for eating), commercial liquid yogurt (for drinking), and *Lactobacillus acidophilus*-containing yogurt. We first determined the optimum culture conditions for *Lactobacillus* spp. and then analyzed turbidity and pH in order to compare the growth abilities and lactic acid-production capacities among the groups. Finally, high-performance liquid chromatography was used to measure the lactic acid content in the culture supernatants, and the antibacterial activities against *Staphylococcus aureus* and *Escherichia coli* were compared among the three groups. The optimum culture conditions for *Lactobacillus* spp. were MRS medium at 25°C, for 24 h. The highest turbidity was found in *L. acidophilus*-containing yogurt, followed by liquid yogurt and solid yogurt. Similarly, the highest lactic acid production ability was found in *L. acidophilus*-containing yogurt, followed by liquid yogurt and solid yogurt. Culture supernatants from the three groups did not show any antibacterial activity towards *S. aureus*; however, supernatants derived from *L. acidophilus*-containing yogurt resulted in a 1.8 mm inhibitory zone against *E. coli* in a paper disk diffusion test. These results revealed the high level of lactic acid-production capacity and antibacterial activity in *L. acidophilus*-containing yogurt.

Keywords: *Lactobacillus acidophilus*, Yogurt, Lactic acid, Antibacterial activity

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Introduction

Lactic acid-producing bacteria such as *Lactobacillus* spp., which ferment carbohydrates and produce ATP (Kandler,

1983) are known for their considerable benefits to the health of the human intestine. Indeed, probiotic substances produced by *Lactobacillus* spp. are used in the food and pharmaceutical industries for manufacturing products that maintain

the stability of the normal intestinal flora. These probiotic substances have various effects on the physiology and health of individuals. For example, these probiotics have been shown to protect against cancer, decrease cholesterol levels in the blood, promote the growth of beneficial bacteria, and improve the absorption of lactose (Robinson *et al.*, 1984; Kim *et al.*, 1993; Andersson *et al.*, 2001; Ranadhheera *et al.*, 2010; Ooi and Liong, 2010). Additionally, *Lactobacillus acidophilus* has been shown to prevent the development of cancer of the large intestine (Wollowski *et al.*, 2001). Thus, *Lactobacillus* spp. have a range of biological effects that are beneficial to health.

In the food industry, *Lactobacillus* spp. are used extensively for producing cheese, bean paste, fermented milk, kimchi, soy sauce, yogurt, and yogurt-based products (Sugiyama, 1984; Lücke, 1996; Leory and Vuyst, 2004; Lee *et al.*, 2011). Yogurt is classified into solid and liquid types. Solid-type yogurt was first introduced in Korea in 1981. Since the early 1970s, after the sale of liquid-type yogurt containing 3% milk solids produced by Japan, the consumption of yogurt has increased dramatically every year. Moreover, consumption of solid yogurt is gradually increasing in consumers with a high standard of living.

Staphylococcus aureus is a gram-positive bacterium isolated from the nasal cavities of healthy adult and from sites of infection; this bacterium is associated with hospital and community-acquired infections (Kim, 2012; Han and Kim, 2014). Some strains of *S. aureus* are resistant to antimicrobial agents (Kim, 2012; Han and Kim, 2014). *Escherichia coli* is a part of the normal flora of the human intestine; however, this bacterium can occasionally cause opportunistic infection and exhibit antimicrobial resistance (Lee and Choi, 2007; Kim *et al.*, 2012; Sung *et al.*, 2013).

Although many commercial yogurts have been used to promote health, the content and activities of lactic acid bacteria in solid and liquid yogurts have not been fully elucidated. Therefore, in this study, we aimed to compare the lactic acid-producing capacities and antibacterial activities of commercial solid and liquid yogurts and a homemade yogurt containing *L. acidophilus*. We expect that our results will provide important insights into the production of more

effective probiotic yogurts.

Materials and Methods

1. Materials

Solid and liquid-type yogurts from three different companies purchased from local supermarkets and a single strain of *L. acidophilus* were included in this study. All materials were analyzed within 24 h after storage at 10°C in a refrigerator.

2. Culture and preprocessing

One milliliter of each sample type (solid yogurt, liquid yogurt and *L. acidophilus*-containing yogurt) was cultured in 200 mL MRS broth with Tween 80 (MB Cell, Los Angeles, CA, USA) for 24 h in an incubator (J-2000, JISICO, Seoul, Korea) at 25°C and centrifuged three times at 3000 rpm for 1 min. The turbidity of each sample was adjusted to 0.5 McFarland units by spectrophotometry (MAP LAB PLUS, BSI, Arezzo, Italy) at 630 nm. The cultures for the three groups were maintained in incubators at 37°C or 25°C and divided into subgroups on the basis of incubation time (24, 48, or 72 h) in MRS broth with Tween 80.

3. pH analysis

pH was determined for each sample more than three times with a pH meter (HM-25R, Analyticon, Springfield, NJ, USA) at 25°C. Buffers at pH 4.01 and pH 6.86 were used for calibration.

4. Turbidity test

Commercial yogurts from three different companies (a, b, and c; 100 mL each) and a strain of *L. acidophilus* were cultured in MRS broth with Tween 80 for 24 h at 25°C. The absorbance was then measured at 630 nm by spectrophotometry. Sterile MRS broth with Tween 80 was used for blank samples.

5. High-performance liquid chromatography (HPLC) analysis

Lactic acid was used as the index component. Culture supernatants were obtained from 24 h cultures in MRS broth

with Tween 80 by centrifugation at 3000 rpm for 10 min.

The HPLC system (LC-20AD, Shimadzu, Nakagyo, Kyoto, Japan) comprised a pump, auto sampler, RS column compartment, UV-VIS detector, photo-diode array detector, high-pressure switching valve, and column (Shiseido, Minato-ku, Tokyo, Japan) maintained at 30°C. The mobile phase was 0.1% phosphoric acid: acetonitrile (9:1). The wavelength was measured at 254 nm at a flow rate of 1.0 mL/min. The injection volume was 10 µL.

6. Antibacterial activity

Sensitivity tests were conducted to investigate the antibacterial effects of the solid-type, liquid-type, and *L. acidophilus*-containing yogurts against two strains of bacteria commonly associated with food poisoning, namely *S. aureus* (ATCC 25923), and *E. coli* (ATCC 25922). Nutrient broth (BD, Franklin Lakes, NJ, USA) was used for culturing both *S. aureus* and *E. coli*. Mueller-Hinton agar (BD) was used for the sensitivity tests. Frozen samples of *S. aureus* and *E. coli* were cultured for 24 h in TSB (BD). Next, each strain was mixed in 0.85% saline and the mixture was adjusted to a turbidity of 0.5 McFarland units (spectrophotometer, 630 nm). Twenty microliters of each yogurt culture supernatant obtained from 24 h cultures in MRS broth with Tween 80 was dispensed onto paper disks for the disk diffusion assay.

7. Statistical analysis

All experiments were repeated at least three times. The results are presented as the mean ± standard deviation (SD). All experimental data were analyzed using the SPSS program (SPSS 18.0, SPSS Institute, Chicago, IL, USA). One-way analysis of variance (ANOVA) with Duncan's multiple range tests was used to examine the differences between groups. Differences with *p* values of less than <0.05 were considered statistically significant.

Results

1. Turbidity test

Increased turbidity suggests the growth of bacteria. Liquid yogurt was found to have a significantly higher absorbance

than solid yogurt, while *L. acidophilus*-containing yogurt had a significantly higher absorbance than liquid yogurt (Fig. 1). These data implied that *L. acidophilus*-containing yogurt had the highest growth ability among the three groups.

2. pH meter analysis

pH is indicative of acid-producing activity. The lowest pH was in the *L. acidophilus*-containing yogurt followed by liquid yogurt and solid yogurt (Fig. 2). Thus, the *L. acidophilus*-containing yogurt had the highest lactic acid production, while solid yogurt had the lowest. However, the pH values of the liquid yogurt and *L. acidophilus*-containing yogurt were not significantly different.

3. HPLC analysis

HPLC analysis revealed that lactic acid production was in the order of *L. acidophilus*-containing yogurt > liquid yogurt

Fig. 1. Comparison of absorbances in commercial solid and liquid yogurts and *L. acidophilus*-containing yogurt. (A) Comparison of the absorbances of commercial yogurts (a, b, and c indicate commercial yogurts from three different companies). (B) Comparison of the mean absorbances of solid and liquid yogurts in (A) and *L. acidophilus*-containing yogurt. Values with different superscripts were significantly different at *p*<0.05.

> solid yogurt (Fig. 3).

4. Antibacterial activity

Culture supernatants from the commercial yogurts and *L. acidophilus*-containing yogurt did not show any antibacterial

activity against *S. aureus* (data not shown). However, *L. acidophilus*-derived supernatants showed an inhibitory zone of 1.8 mm in response to *E. coli* in a paper disk diffusion test (Fig. 4).

Discussion

In this study, culture turbidity and pH were measured to compare the growth ability and lactic acid production among solid-type, liquid-type, and *L. acidophilus*-containing yogurt. *L. acidophilus*-containing yogurt had the highest turbidity and lowest pH. Additionally, *L. acidophilus*-containing yogurt exhibited the highest lactic acid production, followed by

Fig. 2. Comparison of the pH of commercial solid and liquid yogurts and *L. acidophilus*-containing yogurt. (A) Comparison of pH values of commercial yogurts (a, b, and c indicate commercial yogurts from three different companies). (B) Comparison of mean pH values of solid and liquid yogurts in (A) and *L. acidophilus*-containing yogurt. Values with different superscripts were significantly different at $p < 0.05$.

Fig. 3. HPLC chromatograms of commercial yogurts and *L. acidophilus*-containing yogurt. (A) Liquid yogurt (area: 1887126), (B) *L. acidophilus*-containing yogurt at 25°C (area: 3455638), (C) Solid yogurt at 25°C (area: 161772).

Fig. 4. *E. coli* inhibition zones induced by *L. acidophilus*-containing yogurt and commercial yogurt. (A) *E. coli* inhibition by *L. acidophilus*-containing yogurt (1.8 mm), (B) *E. coli* inhibition by commercial yogurt.

liquid yogurt and solid yogurt. *L. acidophilus*-containing yogurt also showed the highest antibacterial activity. Thus, our data provide important insights into the production of more effective probiotic yogurts.

Lactic acid is a weak-organic acid and plays a role in diverse biochemical processes (Ibrahim *et al.*, 2008). Ibrahim *et al.* (2008) reported that lactic acid inhibits the growth of pathogens such as *Salmonella* and *E. coli* and Alakomi *et al.* (2000) showed that lactic acid exerts antimicrobial activity through inducing the permeabilization of gram-negative bacteria and lowering of the pH.

S. aureus and *E. coli* can cause hospital and community-acquired infections or opportunistic infection. Moreover, some strains show resistance to antimicrobial agents (Lee and Choi, 2007; Kim, 2012; Kim *et al.*, 2012; Sung *et al.*, 2013; Han and Kim, 2014). In our study, *L. acidophilus*-containing yogurt showed antibacterial ability against *E. coli* but, not against *S. aureus*. High lactic acid production was also observed for this sample. These data indicated that *L. acidophilus* may be a source of antibacterial substances.

In summary, *L. acidophilus*-containing yogurt had high lactic acid production and antimicrobial activity. Thus, further studies are warranted to ascertain whether commercial food products produced using *L. acidophilus* may also affect other substances within the human gastrointestinal tract, including gastric acids, bile salts, or various enzymes.

요 약

Lactobacillus spp. 같은 유산균은 탄수화물을 발효하여 ATP를 생성하며, 시판되는 요구르트 생산에 이용되고 있다. *Lactobacillus* spp.는 장관계 건강에 유익한 균으로, 특히 *Lactobacillus acidophilus*를 함유하는 요구르트는 대장암 초기단계를 예방하는 효과가 있는 것으로 나타나 관심이 모아지고 있다. 본 연구에서는 고형 요구르트와 액상 요구르트, 그리고 *Lactobacillus acidophilus* 세 그룹의 유산균을 배양하였다. 먼저 *Lactobacillus* spp.의 최적의 배양조건을 조사하였고 세 그룹의 성장능과 젖산생성을 비교하기 위해 혼탁도와 pH를 측정하였다. 배양 상층액의 젖산을 비교하기 위해 HPLC를 시행하였으며 *Staphylococcus aureus*와 *Escherichia coli*에 대한 항균력을 측정하였다. *Lactobacillus* spp.의 최적의 배양조건은 25°C의 온도로 MRS배지에서 24시간 배양이었다. 혼탁도와 젖산 생성은 *L. acidophilus*가 가장 높았고

액상 요구르트, 고형 요구르트 순으로 나타났다. Paper disk법을 이용한 항균력 시험에서는 *S. aureus*에 대해서는 세 그룹 모두 항균력이 없었으며 *E. coli*에 대해서는 *L. acidophilus*에서 1.8 mm의 억제대를 보였다. 이 연구결과로 *L. acidophilus*는 높은 젖산 생성 능력과 항균력을 가지고 있음을 확인하였다.

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Conflict of interest: None

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