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Microbial Population Dynamics and Temperature Changes during Fermentation of Kimjang Kimchi

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A distinct subset of lactic acid bacteria that are greatly influenced by temperature play an important role during kimchi fermentation. However, microbial population dynamics and temperature control during kimjang kimchi fermentation, which is traditionally fermented underground, are not known. Here we show that *Lactobacillus sakei* predominates in kimjang kimchi, perhaps due to suitable fermentation (5~9°C) and storage (-2°C) temperatures. The temperature of this kimchi gradually decreased to 3.2°C during the first 20 days of fermentation (-0.3°C/day) and then was stably maintained around 1.6°C, indicating that this simple approach is very efficient both for fermentation and storage. These findings provide important information towards the development of temperature controlling systems for kimchi fermentation.

***Keywords*:** kimchi, kimjang kimchi, Leuconostoc, *Lactobacillus sakei*, population dynamics

Kimchi is a fermented cabbage product containing a host of different spices such as hot red pepper and garlic (Cheigh and Park, 1994). Temperature plays an important role during kimchi fermentation and storage. We previously reported that the population dynamics of lactic acid bacteria in kimchi is greatly influenced by temperature and can greatly influence the organoleptic and storage properties of the products (Cho *et al*., 2006). Recently in Korea, it is typical that homemade or commercially prepared kimchi products are stored in kimchi refrigerators, which provide dynamic temperature control for expediting fermentation and efficiently reducing microbial growth by rapid shifts to subzero temperatures. However, the optimal parameters for both fermentation and storage in these refrigerators have not been determined and require further investigation.

To provide information about better temperature control during kimchi production we investigated the temperature changes that occur during traditional fermentation of kimjang kimchi. Kimjang kimchi (approximately 200 Chinese cabbages) was prepared in early December and was fermented in a traditional ceramic pot (60 cm in diameter, 90 cm in height; Fig. 1C) that was buried underground during the winter months from December through February. We examined both the sample temperatures and the microbial population dynamics. Rapid microbial growth occurred in the temperature range of 4.8～10.0°C, a point at which most lactic acid bacteria grow well. The maximum lactic acid bacterial cell density was recorded at day 10, when temperature at the depth of about 10 cm from the top of kimchi sample was 4.8°C and at the bottom of the sample was 6.8°C (Fig. 1C), indicating a significant temperature gradient throughout the sample height (Fig. 1A). The rapid decline in total lactic acid bacterial population observed after day 10 might be due to the high cell density and a somewhat high acidity, but not due to limited food sources (Fig. 2A). The temperature of the kimchi sample gradually decreased after day 10 and then reached stability at approximately 1.6°C around day 40, perhaps stabilizing the growth of the microbial community. However, it is important to note that this low temperature remained stable despite the severe fluctuation in temperature within the soil immediately above the ceramic pot (Fig. 1A).

We next analyzed microbial population dynamics by sampling about 500 g of kimchi at given time points and identified the range of species via 16S rRNA analysis (Cho *et al*., 2006). The maximum cell density was achieved at day 10 and our analysis identified *Lactobacillus sakei* as the major species and *Leuconostoc citreum*, *Leuconostoc gasicomitatum*, and *Leuconostoc lactis* as minor constituents of the bacterial population (Fig. 1B). Given that these four species are commonly found in early phases of kimchi fermentation (Choi *et al*., 2003; Cho *et al*., 2006), they likely compete for similar environmental niches. However, all the *Leuconostoc* species (with the exception of *L. gasicomitatum*) disappeared gradually when the pH dropped below 4.7. However, *Lactobacillus sakei* maintained its dominance throughout the later stages of fermentation, indicating that unlike most *Leuconostoc* species *Lactobacillus sakei* is notably acid-tolerant. Although the total microbial population decreased rapidly between day 10 and 13, the species *Weissella koreensis* showed

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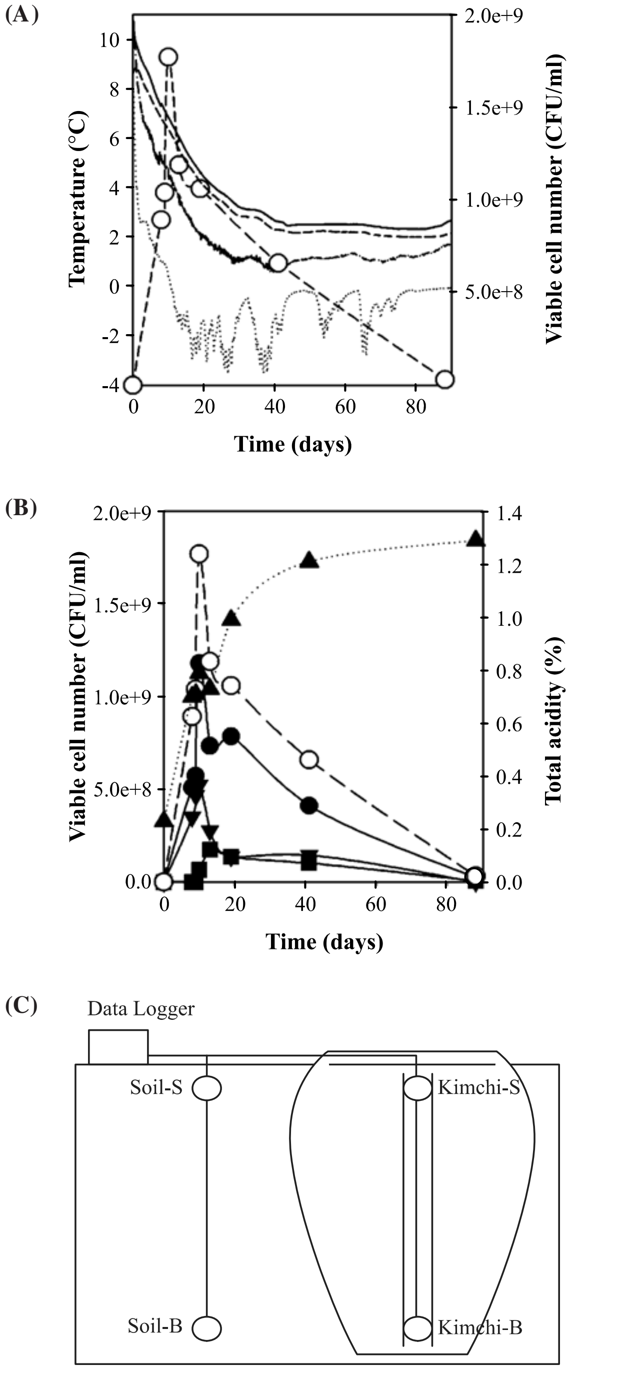

Fig. 1. Temperature profile during kimjang kimchi fermentation. (A) Temperatures at four different locations, namely surface kimchi (dash-dot line), bottom kimchi (solid line), surface soil (dotted line), and deep soil (medium dash line) were measured every 30 min for 88 days using a Data Logger DL2 (Delta-T Devices Ltd, UK). Total viable cell number (○), (B) Microbial population dynamics in kimjang kimchi. *Lactobacillus sakei* (●) outcompetes *Leuconostoc* species (▼) as well as *Weissella koreensis* (■). The *Leuconostoc* species identified from the kimchi sample include *Leuconostoc citreum, L. gasicomitatum*, and *L. lactis*. Total viable cell number (○); total acidity (▲). (C) A diagram of the kimchi-fermenting kimjang dok, which was buried underground and the location of the four temperature probes. Kimchi-S, surface kimchi; Kimchi-B, bottom kimchi; Soil-S, surface soil; Soil-B, deep soil.

a slight population increase during this time period then decreased as the pH approached 4.7, perhaps due to growth competition by *Lactobacillus sakei* (Fig. 1B). *Weissella koreensis* cannot compete with the aforementioned four species in the presence of sucrose, because *W. koreensis* has limited potential to utilize sucrose and in addition, sucrose is a better sugar to utilize than fructose or glucose for *Leuconostoc* species and perhaps for *Lactobacillus sakei* (Table 1), suggesting rapid growth of these species by consuming sucrose during the earliest phage of fermentation (Dols *et al*., 1997; Cho *et al*., 2006). Overall, *Lactobacillus sakei* dominated the microbial population throughout kimchi fermentation up to day 88.

Microbial population dynamics is influenced by factors such as metabolites and waste products. To evaluate these conditions, we next analyzed the sugar content within the kimchi sample. The concentration of glucose within the kimchi sample was higher than that of fructose and sucrose

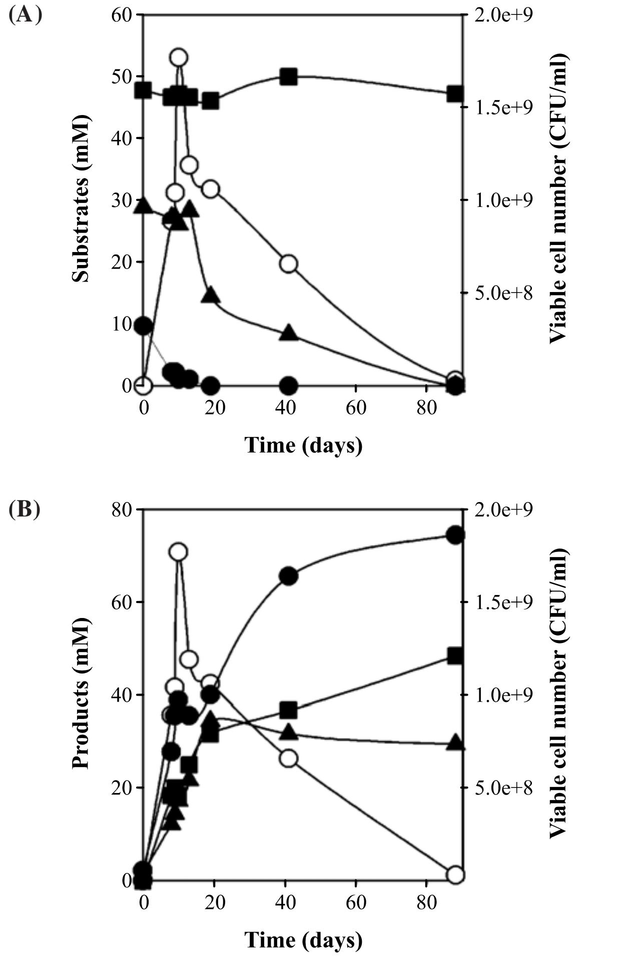

Fig. 2. Time course of sugar consumption and fermentation products in kimjang kimchi fermentation. The fermentation substrates and products of kimchi samples were analyzed via high performance liquid chromatography (HPLC) as previously described (Cho *et al*., 2006). (A) Sucrose (●); glucose (■); fructose (▲); total viable cell numbers (○). (B) Lactate (●); acetate (■); mannitol (▲); total viable cell number (○).

Table 1. Fermentation profile of lactic acid bacterial strains isolated from kimchi

Bacteria	Consumed sugars (mM)			Products (mM)			
	Glucose[a]			Lactate	Acetate	Ethanol	Mannitol
L. citreum	84			73	27	22	0
L. gasicomitatum	72			41	18	17	0
Lb. sakei	50			53	20	0	0
W. koreensis	69			41	20	15	0
	Sucrose[b]						
L. citreum	58			41	32	22	46
L. gasicomitatum	58			23	20	0	29
Lb. sakei	23			58	17	0	0
W. koreensis	18			17	7	0	0
	Glucose[c]	Fructose[c]					
L. citreum	47	48		50	33	35	49
L. gasicomitatum	24	43		31	28	0	36
Lb. sakei	21	7		46	13	0	0
W. koreensis	33	33		38	23	30	17
	Glucose[d]	Fructose[d]	Sucrose[d]				
L. citreum	28	28	15	40	27	13	40
L. gasicomitatum	12	17	15	19	18	0	47
Lb. sakei	18	16	15	58	23	0	1
W. koreensis	28	28	3	32	15	37	8

Bacterial cultures were conducted at 15°C for 3 days. Data are average of two independent experiments.
[a] a glucose (111 mM) medium, [b] a sucrose (58 mM) medium, [c] a glucose (56 mM)-fructose (56 mM) mixture, [d] a glucose (28 mM)-fructose (28 mM)-sucrose (15 mM) mixture. *L.*, *Leuconostoc*; *Lb.*, *Lactobacillus*; *W.*, *Weissella*

(Fig. 2A). The low concentration of sucrose (10 mM), which is a common additive to kimchi, was completely depleted within 10 days of fermentation, indicating fast consumption of sucrose even in the presence of higher concentrations of glucose. This non-diauxic growth, characterized by consuming both glucose and other sugars such as sucrose or fructose simultaneously, has also been observed in *Leuconostoc mesenteroides* and *Leuconostoc oenos* populations (Salou *et al*., 1994; Dols *et al*., 1997). The constitutive expression of phosphoenolpyruvate-phosphotransferase systems for sugars such as glucose, fructose and sucrose was also confirmed in *Streptococcus mutans* (Ajdic and Pham, 2007). Concentrations of fructose and glucose in our kimchi sample appear to decrease slightly when sucrose was being consumed during the first 10 days. At day 10, the accumulated lactic acid was around 39 mM. Given homo and heterolactic fermentation by these lactic acid bacteria, the total concentration of lactic acid could not be accounted for as produced solely from sucrose. Furthermore, about 20 mM acetate and 20 mM mannitol were also produced during this period, suggesting simultaneous consumption of other sugars. Indeed, the concentration of fructose decreased rapidly after day 13, at which point the total microbial population decreased gradually (Fig. 2A). However, the exact determination of the amount of glucose or fructose consumed during this period was not possible because these sugars are released steadily from cabbage throughout fermentation. Subsequently, the level of glucose showed little fluctuation throughout fermentation.

We were able to detect lactate, acetate, and mannitol as fermentation products. The amount of lactate (39 mM) was higher than that of acetate (18 mM) at day 10 when the cell density was at its maximum (Fig. 2B). However, inconsistent with our previous report (Cho *et al*., 2006), we could not detect ethanol, suggesting some amount of homolactic fermentation. To clarify this non-production of ethanol during fermentation, we examined both sugar usage patterns and fermentation products when we cultured strains isolated from kimchi samples. Single strain from each species was tested. Strains were cultured without aeration at 15°C for 3 days in modified MRS media (10 ml) containing glucose (111 mM), sucrose (58 mM), a glucose (56 mM)-fructose (56 mM) mixture, or a glucose (28 mM)-fructose (28 mM)-sucrose (15 mM) mixture. *Lactobacillus sakei* consumed glucose and produced lactate and acetate, but not ethanol, indicating both homo and heterolactic fermentation. In addition, ethanol was not produced when this strain was fermented on sucrose, a glucose-fructose mixture, or a sucrose-glucose-fructose mixture, consistent with our observations from traditional kimjang kimchi fermentation (Table 1). Similarly, *Leuconostoc gasicomitatum*, a distinct minor population present during kimchi fermentation, also did not produce ethanol under the culture conditions tested except in the presence of glucose. In contrast, *Weissella koreensis* and the other strains tested (*Leuconostoc citreum*, *L. lactis*, *L. mesenteroides*, and *Lactobacillus plantarum*) produced ethanol under all culture conditions tested (data not shown). Therefore, these results indicate that of the major microbial populations of kimjang kimchi those that do not produce ethanol during fermentation are *Lactobacillus sakei* and *Leuconostoc gasicomitatum* as determined in these experiments (Fig. 1B).

Total acidity, contributed mainly by lactic acid and acetic acid, was 1% at day 19 and gradually increased to 1.3% up to day 88, suggesting that the organoleptic qualities of kimchi are best during this period (Fig. 1B). Consumption of sucrose and fructose usually leads to the production of mannitol under anaerobic conditions (Dols *et al*., 1997). The production rate of mannitol was almost the same as that of acetate during the early phase of fermentation (Fig. 2B), indicating that fructose acts as an electron acceptor so that more ATP is produced (Dols *et al*., 1997). Mannitol is also believed to be an important product during kimchi fermentation because it is half as sweet as sucrose, but is not effectively metabolized by humans (Grobben *et al*., 2001).

It is not surprising that *Lactobacillus sakei* was determined to be a dominant species in our kimchi samples because it is strongly resistant to high salt concentrations and also expresses a sucrose transporter and sucrose phosphorylase that are both required for sucrose metabolism (Chaillou *et al*., 2005). Dominance of this bacterial species is possible because of the ideal fermentation temperatures (1.6~9.0°C; Fig. 1A) and the large initial inoculum size (63% at day 0; Fig. 1B). Previously we demonstrated that *Lactobacillus sakei* is a dominant microbe in kimchi at 10°C, while only a minor portion of the population at 15°C or -1°C, indicating that temperature greatly influences its growth (Choi *et al*., 2003; Cho *et al*., 2006). Similarly, *Leuconostoc citreum* can be a dominant microbe in kimchi at 15°C or when it is added to kimchi samples as a starter culture (Choi *et al*., 2003). Furthermore, *Weissella koreensis* can be a dominant species in kimchi at -1°C (Cho *et al*., 2006). Therefore, several factors such as temperature and sugar sources can influence the major microbial populations of kimchi.

In summary, our results indicate that *Lactobacillus sakei* can be a dominant species in kimjang kimchi fermented within the temperature range of 1.6~9.0°C. We speculate that proper control of the gradual temperature changes we observed during kimjang kimchi fermentation could have significant value to the food industry for controlling kimchi quality and establishing starter culture systems.

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