Change of Lactobacillus in Cabbage and Radish Caused by the Change of Soil: Quality Hindrance of Kimchi and the Solution to It

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***Abstract*—The existence of lactobacillus on the surface of cabbage and radish allows these vegetables to be made into kimchi (Korea's most famous vegetable side dish). But as soil temperatures rise, the lactobacillus cultured in radish and cabbage loses its vitality, precluding it not only from being engrafted alive onto leaves and stems of radish and cabbage, but also from helping the growth of soil bacteria. This suggests that temperature change of soil caused by climate change may damage the growth of lactobacillus on radish and cabbage. Moreover, for lactobacillus strains that dropped in vitality after being exposed to temperature change, changes in the bacteria's pH and acidity levels reveal that the degree of vitality in lactobacillus decreases considerably during cabbage and radish's fermentation process to become kimchi. As a solution for this problem, based on studies indicating that propolis enhances the vitality of lactobacillus, this study injected lycopene (the anti-oxidative component of tomatoes) and fragments of lactobacillus into samples of growing cabbage and radish. The results reveal higher levels of vitality in lactobacillus strains that were exposed to changes in temperature.**

***Index Terms*—kimchi, lactobacillus, lycopene, propolis**

I. Introduction

Kimchi, produced by the fermentation of lactobacillus, is an important food for Korean dietary life. The first step to make kimchi is salting cabbage and radish in high concentrated salt water to remove moisture of those vegetables using infiltration. In this process and treatment with red pepper powder, while most bacteria die out, lactobacillus survives, ripens kimchi, and produces its unique taste. Throughout the prior experiment, it was confirmed that lactobacillus exists on the surface of radish and cabbage (Prior Experiment 1) [1]. By the next prior experiment, it was checked that this lactobacillus proliferates also in the soil (Prior Experiment 2) [2]. Furthermore, as a result of injecting lactobacillus cultured in radish and cabbage and planting radish and cabbage in sterilized soil, lactobacillus injected into the soil was detected in stems and leaves of radish and cabbage, where lactobacillus grows inside (Prior Experiment 3) [3]. Throughout these three experiments, it was confirmed that lactobacillus existing in cabbage and radish comes from the soil. In case of lactobacillus separated and cultured in lactic beverage, the survival rate was greatly decreased compared the rate of lactobacillus in cabbage and radish. As the number of lactobacillus found in radish and cabbage was decreased as well (Prior Experiment 4), it was checked that only lactobacillus separated from radish and cabbage moves from the soil to radish and cabbage [4]. The fact that different environmental changes occurs the soil change can be checked from Climate Change Confrontational Strategy of Jeonbuk Agricultural Technology Administration, (http://www.jbares.go.kr), and as soil change accompanies the change of microorganism existing in soil, there will be a change of growth and propagation of lactobacillus in the soil [5]. This change will cause the change of lactobacillus moving into radish and cabbage, thus it is expected to influence the fermentation of kimchi. From the reference research, it was hypothesized that this change will have negative effect on lactobacillus existing in the soil [6], [7]. Therefore, the research was designed in order to seek the way to solve the problem on kimchi's quality deterioration caused by the impact of climate and environmental change on lactobacillus moving into cabbage.

Prior Experiment 1) Production of MRS Media and Confirmation of the Existence of Lactobacillus in Radish and Cabbage using the Media

1. 72g of MRS media powder was mixed with 1L of sterilized distilled water in 2L Erlenmeyer flask.
2. The mouth of the flask was sealed with aluminum foil, then the mixture was sterilized in 121 ℃, 2 atm, for 15 minutes.
3. After the flask was taken out and cooled down in a room temperature, it was moved into a clean bench and was poured in petri dishes. It was refrigerated in 4 ℃ before the use in order to prevent the media from other bacteria.
4. MRS broth media without agarose was produced with the same method, but after the sterilization, the mixture was moved into 50mL conical tube, and was refrigerated in 4 ℃.
5. The radish was peeled off, and the peel was cut into pieces. After the peel was moved into a clean bench, it was put into 15mL conical tube containing 5mL of MRS

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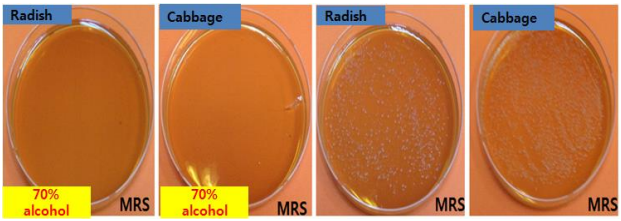
broth media. 5 μℓ of MRS broth media containing the peel was collected with a micropipette, and after the media was injected into MRS media, it was smeared with a spreader and was cultured. The experiment was done with the cabbage as well.

6. After the peel of radish and cabbage were cut into pieces, they were put into 50mL conical tube containing 70% of alcohol. They were stored in for 30 minutes and 5> was done. Using GasPak ™EZAnaerobeContainerSystem, anaerobic culture was done in 30 ℃.

Result



As a result of the experiment, the proliferation of lactobacillus in radish and cabbage with alcohol treatment was not detected, while that in radish and cabbage without alcohol treatment was detected. (MRS media is a selective media that lactobacillus can only grow, thus only lactobacillus could be cultured. When petri dish containing culturing microorganism was put into GasPak ™EZAnaerobeContainerSystem and sealed, CO2 occurs and anaerobic condition can be provided. Lactobacillus is cultured in anaerobic condition, and the best culturing temperature of lactobacillus is 28-30 ℃.)

Prior Experiment 2) Proliferation of Lactobacillus, Existing in Radish and Cabbage, *in Soil*

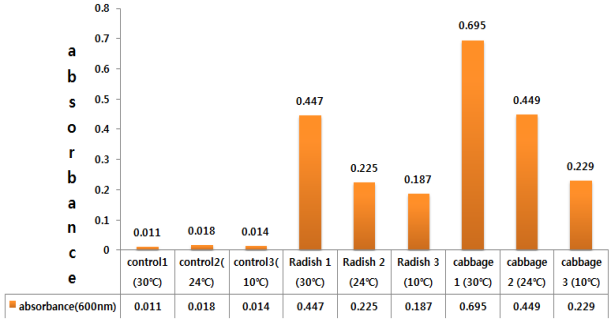
1. Garden soil was put into a beaker, and the beaker was sealed with aluminum foil. It was put into high temperature and pressure sterilizer and was sterilized in 121 ℃, 2 atm, for 15 minutes.

2. Lactobacillus colonies cultured in MRS media from Prior Experiment 1 was collected using a platinum loop. The colonies were injected into MRS broth media, and anaerobic culture was done in 30 ℃.

3. 20g of sterilized soil was put into a petri dish, and 10 μℓ of lactobacillus that was cultured in 30 ℃ anaerobic condition was injected into the soil. The soil was stored in a constant temperature of 30 ℃ for 1 week.
4. 0.1g of soil in step 3 was collected and was put into 1.5mL micro tube with 1mL of MRS Broth media. Centrifugation was done to separate MRS broth media containing lactobacillus of soil.

4. 5 μℓ of the MRS broth media containing lactobacillus was injected into 15mL conical tube containing 3mL of MRS broth media. After the media was cultured in a constant temperature of 30,2410 ℃ for 12 hours, the absorbance was measured in 660nm with UV-SPECTROPHOTOMETER to count the number of lactobacillus culture in soil. The temperature of the soil that lactobacillus culture was finished was measured.

Result



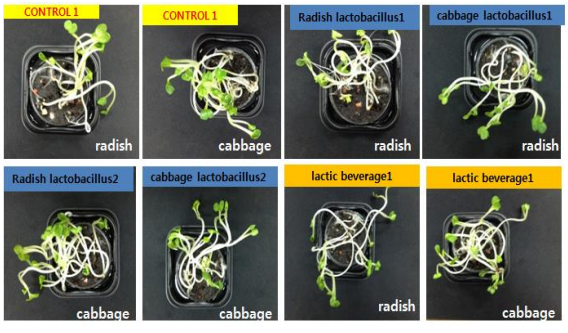
As a result of measuring the absorbance, lactobacillus growing in radish and cabbage showed the highest growth rate when it was cultured in 30 ℃, and the growth was impeded when the temperature decreased to 24, 10 ℃.

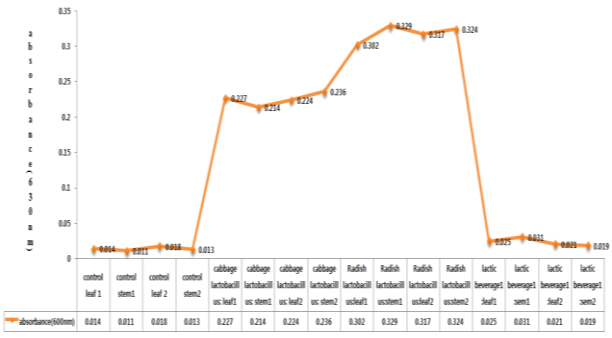
When the core temperature of the soil was measured, the soil cultured in 30 ℃ for a week showed the temperature of 28-24 ℃, the soil cultured in 24 ℃ showed of 20 ℃, and the soil cultured in 10 ℃ showed 8-7 ℃; therefore, the temperature in soil was 2-4 ℃ lower than the culturing temperature.

Prior Experiment 3, 4) Distribution of Lactobacillus in Stems of Radish and Cabbage Leaves Grown in the Soil

1. After soil containing lactobacillus was cultured following the process of Prior Experiment 2, seeds of cabbage and radish were planted and put into sterilized sealed plastic box. The seeds were grown for a week in a room temperature, then stems and leaves of radish and cabbage were collected and put into 15mL conical tube containing 1mL of MRS broth. After they were cultured in 30 ℃ for 12 hours, the lactobacillus on leaves and stems were checked by measuring the absorbance using UV_SPECTROPHOTOMETER. Lactobacillus from lactic beverage was cultured in MRS media, and the experiment was repeated.

Result





In case of lactobacillus cultured in radish, cabbage, and lactic beverage, no significant influence on the growth of cabbage and radish even the lactobacillus was injected into the soil. However, lactobacillus was not detected in leaves and stems of radish and cabbage grown in the soil containing lactobacillus separated cultured in lactic beverage and in the soil not containing lactobacillus. This result shows that only lactobacillus separated from radish and cabbage can exist in leaves of cabbage and radish.

II. Materials and Methods

A. Materials

Radish, cabbage, sterilized distilled water, lactic beverage, radish seeds, cabbage seeds, gardening soil, clean bench, constant temperature incubator, UV-Spectrophotometer, bacteria culture media, thermometer, centrifuge, tubes, sodium chloride, etc.

B. Methods

Experiment 1) Change of Vitality of Lactobacillus in Radish and Cabbage According to Changes of Soil Temperature

1. Gardening soil was sterilized in 121 ℃ and 2 atm using high temperature and pressure sterilizer, so that all microorganism living in the soil can be removed. After the sterilization, the soil was refrigerated until the use in order to impede the proliferation of microorganism.

2. 5g of sterilized soil was put into small petri dish, and 100 μℓ of MRS media that lactobacillus in radish and cabbage leaves was cultured in was injected using micro pipette. All process was done in a clean bench. In order to provide moisture in soil, 1mL of sterilized distilled water was provided into the soil.

3. After the petri dish containing the soil that lactobacillus was injected into was stored in 28 ℃ for 3 days, 0.1g of soil was collected and stored in 4 ℃, and this soil was stored in 4, 28, 40 ℃ for three days. When a week of storing was finished, the temperature of soil was measured with electronic thermometer to observe the temperature change of the soil.

4. 0.1g of soil, which lactobacillus was injected into, stored for a week in conditions of 4, 28, and 40 ℃ measured with electronic scale and put into 1.5mL micro tube. After 1mL of sterilized distilled water was added, the soil and the water was mixed by shaking the tube. The mixture was put into a centrifuge and the centrifugation was done with a speed of 6000 rotations per minute to separate the aqueous solution containing soil bacteria from the soil.

5. After 5 μℓ of aqueous solution containing soil bacteria separated from 4 was injected into MRS media, the soil bacteria was cultured by smearing with a spreader. The culture was done in a constant temperature of 28 ℃ incubator for 12 hours.

6. After 5 μℓ of aqueous solution containing soil bacteria separated from 4 was injected into MRS media, the spinner culture was done in 28 ℃ for 12 hours. The absorbance was measured with UV-SPECTROPHOTOMETER to observe the proliferation of lactobacillus.

Experiment 2) Proliferation of Lactobacillus Existing in Cabbage and Radish that Experienced the Change in Vitality According to Different Temperature Changes of Soil and Change of Lactobacillus Distribution of Radish and Cabbage Grown in Soil Containing Lactobacillus in Radish and Cabbage

1. Lactobacillus in radish and cabbage treated with different temperatures from Experiment 1) was injected into the petri dish containing 5g of gardening soil (Different types of soil microorganism exist, as it is a soil that was not sterilized). After the soil was stored in room temperature for 1 week, seeds of radish and cabbage were planted.

Experiment 3) About the Relationship between Soil Bacteria and Lactobacillus

1. 0.1g of the soil from Experiment 2) was measured with electronic scale and was added into 1.5mL micro tube. After 1mL of sterilized distilled water was added as well, the micro-tube was shaken to mix the water and the soil. The mixture was put into a centrifuge and the centrifugation was done with a speed of 6000 rotations per minute to separate the aqueous solution containing soil bacteria from the soil.

2. 5 μℓ of aqueous solution containing soil bacteria separated from 1 was injected into NA media, and it was smeared with spreader in order to culture. The culture was done in a constant temperature of 28 ℃ incubator for 12 hours.

3. 5 μℓ of aqueous solution containing soil bacteria separated from 1 was injected into liquid NB media, and spinner culture was done in 28 ℃ for 12 hours. The proliferation of soil bacteria was observed by measuring the absorbance using UV-SPECTROPHOTOMETER.

4. The colonies of soil bacteria cultured in NA media from 2 were collected by each type using sterilized platinum loop and were injected into NA media. Each type of soil bacteria whose growth was confirmed was cultured in 15mL conical tube containing 3mL of NB media so that each type can be separated.

5. 5 μℓ of the soil bacteria from 4 was injected into 3mL of NB media, and 5 μℓ of MRS media that lactobacillus from Experiment 1) and lactobacillus that had not experienced temperature treatment were cultured was injected. After it was cultured in 28 ℃ for 12 hours, the proliferation of soil bacteria was observed by measuring the absorbance with UV-SPECTROPHOTOMETER. Soil bacteria was cultured without adding lactobacillus as a control, and the results were compared.

6. The experiment was done with the experimental condition of 1>-5 in the temperature of 40 ℃, and the results were compared.

Experiment 4) Growth Change of Plants and Change of Soil in Soil Containing Lactobacillus

1. Arabidopsis, whose gene can be analyzed, was planted into the soil from Experiment 2) and was bred for 2 weeks. Through RT-PCR, the growth of arabidopsis was compared.

2. Soil component was analyzed based on process test. The total nitrogen was titrated into 0.001N H2SO4 by measuring with Kjeldahl method. Total phosphorus, TP, was measured by measuring 0.05g of sample with carbon measuring instrument (SSM-500A, Shimadzu, Japan). Total inorganic phosphate was measured using spectroscope in 440nm after applying color development of 1g of sample with 1-amino-2-naphtol-4-sulfonic acid. For extractable ammonium (NH4+), after 10g of soil sample was dried, the leachate was added in and NH4+ was extracted and filtered in constant temperature incubator, and then it was titrated 0.001 N H2SO4. Lastly, for S04-2, 2.5mL of 25% metric acid, 2mL of acetic-phosphoric acid, 0.5mL of barium sulfate suspension, and 0.2g of barium chloride were added, and it was measured and analyzed in 440nm.

Experiment 5) Production of Kimchi Using Lactobacillus in Radish and Cabbage that Experienced Vitality Change and that Did not Experienced Vitality Change and Comparison of Lactobacillus

1. Before salting the prepared cabbage, solution of lactobacillus existing in cabbage and radish diluted with sterilized distilled water was treated on the surface of the cabbage, and the cabbage was stored for about a day before producing kimchi. When kimchi was ripen, kimchi was stored in kimchi refrigerator so that is will not ripe more. The experiment was done with lactobacillus separated from lactic beverage as a control.

2. 1g of kimchi from 1 and kimchi produced generally were added into 25mL erlenmeyer flask. 100mL of sterilized distilled water was put in, and the mixture was stirred with magnet spinner.

3. Aqueous solution of erlenmeyer flask from 2 was put into a centrifuge, and the centrifugation was done with the speed of 6000 rotations per minute. After supernatant was collected, 5 $\mu\ell$ of this supernatant was injected into solid and liquid MRES media. Those media was cultured, and the absorbance was measured to analyze the proliferation of lactobacillus.

Experiment6) Quality Test for Kimchi Produced in Experiment 5) Throughout Lactobacillus Cultivation and Acidity Test

1. pH and acidity of each produced kimchi were measured. pH was measured with pH meter (M220, Corning, Tewksbury, MA, USA) in room temperature. In order to measure acidity, following the AOAC standard test (16), kimchi juice was diluted 20 times, and 0.1N NaOH was added in this sample. The number of 0.1N NaOH mL used to make the pH of this sample into 8.4 was measured. Titrated value was converged into the content of lactic acid(%), and the conversion equation was acidity(%)=[(mLof 0.1 N NaOH×normality of NaOH×0.09)/NaOH×0.09)/weight of sample(g)]×100.

Experiment 7) About the Way to Recover Lactobacillus that has Lost its Vitality by Temperature *Change*

1. Tomato juice containing lycopene, antioxidant substance, was collected, and its effect was enhanced by boiling the juice. Collected juice was contained in MRS media during the process of culturing lactobacillus separated from radish and cabbage (lactobacillus whose vitality was weakened due to growing in the soil of high temperature (40℃)). Centrifugation for this separated lactobacillus was done to remove media, and after leftover lactobacillus was decomposed using ultrasonic wave, and this decomposed lactobacillus was added into MRS media containing lycopene. This experiment was conducted based on the fact that treating propolis enhances the vitality of lactobacillus, the fact confirmed from the research 'Stability of Korean Lactobacillus about Propolis and Spice, and the Selection of Lactobacillus that has Outstanding Antibacterial Activation Ability in order to Enhance the Storage of Kimchi', and injecting fragments of lactobacillus is the application of 'probiotics,' the phenomenon that proliferation of lactobacillus can be accelerated when fragments or component of same type of lactobacillus, of Seigen Korea Inc.

2. Lactobacillus separated from radish and cabbage, whose vitality and growth ability were weakened due to the culture in soil with high temperature (40℃), was treated with antioxidant matter and lactobacillus fragments. After the lactobacillus was let grow, Experiment 2 and 6 were done to test the recovery of vitality.

Experiment 8) Growth Change in Soil and Recovery or Lactobacillus that Has Lost Vitality by Temperature Change

1. After lactobacillus that had recovered its vitality by lycopene and propolis through the experiment and lactobacillus that had not recovered its vitality were injected into soil, the growth changes were observed.

Experiment 9) Change of Engrafting Ability on Cabbage of Lactobacillus Treated *with Lycopene and Propolis*

1. After seeds of radish and cabbage were planted and grown in the soil of Experiment 8, the leaves were collected after 1 week to check the engraftment of lactobacillus.

Experiment 10) Operation of Vitalized Lactobacillus for Next Generation

1. Lactobacillus from Experiment 8 was injected into soil. Separation culture was done in order to check whether its vitality and effect were maintained for the next generation, thus whether engraftment on plants was successful. The process followed the experiments conducted before.

Experiment 11) Relationship between Change of Metabolism Material of Plants and Engraftment on Plants

1. Arabidopsis seeds were planted in soil containing lactobacillus. After the plant was grown for a week, its leaves were collected and put into liquid nitrogen. The leaves were broken into pieces and were put into micro tubes to make five samples. 500uL of solution that 99% ethanol and formic acid were mixed in a ratio of 2 to 8

was added, and the solution was treated in 80 ℃ for 20 minutes. During the treatment, the lid was opened regularly so that tubes would not burst. Ethanol was vaporized for 1 hour, and after 500uL of third distilled water was added and mixed for 20 minutes, the centrifugation was done in 14000rpm for 5 minutes. The supernatant was separated and was stored in 4 ℃. 500uL of third distilled water was added again and was treated with high temperature and pressure sterilizer for 3 hours. Lastly, on 50uL of supernatant, 450uL of α-amylase (1U) 4ul + α-glucosidase(1U) 7ul + 0.1M NaoAc (pH 4.8) was added, and the mixture was put into 37 ℃ incubator. Each enzyme was added, and the absorbance was measured in 520nm.

III. Results and Discussion

Experiment 1) Change of Vitality of Lactobacillus in Radish and Cabbage According to Changes of Soil Temperature

As a result of measuring the temperature change of 10 types of soil for 10 times, soil treated with 4 ℃ showed soil temperature average of 9.9 ℃, soil treated with 28 ℃ showed 22.3 ℃, and soil treated with 40 ℃ showed 38.3 ℃. Thus, Table I confirmed that the temperature change was different from storing temperature.

TABLE I. Measurement Value of Soil Temperature of Lactobacillus Existing in Radish and Cabbage (10 Times)

	1	2	3	4	5	6	7	8	9	10
4 ℃	10 ℃	11 ℃	9 ℃	9 ℃	11 ℃	10 ℃	10 ℃	10 ℃	8 ℃	11 ℃
28 ℃	22 ℃	24 ℃	24 ℃	22 ℃	20 ℃	21 ℃	23 ℃	24 ℃	22 ℃	21 ℃
40 ℃	38 ℃	37 ℃	39 ℃	39 ℃	38 ℃	38 ℃	38 ℃	38 ℃	39 ℃	39 ℃

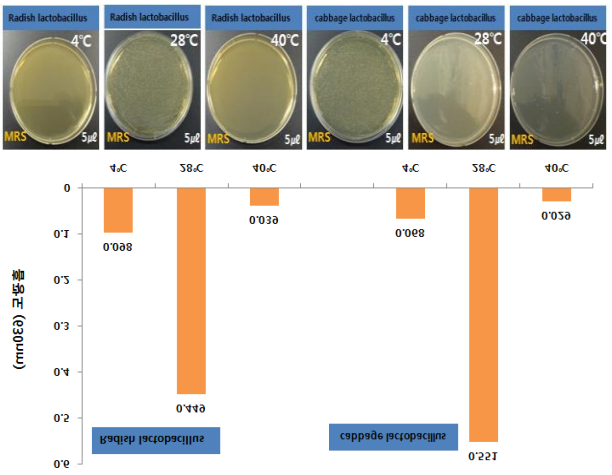


Figure 1. Injecting and culturing lactobacillus cultured in radish and cabbage that experienced temperature change (MRS media)

According to Fig. 1, when the temperature for storing soil was 28 ℃, lactobacillus collected from radish and cabbage from the soil experienced the temperature change grew normally in the soil, but lactobacillus in soil exposed to 4 and 40 ℃ could not grow well. In other words, when soil temperature does not satisfy certain condition, the growth and proliferation of lactobacillus, which exists in radish and cabbage, in soil is impeded. However, lactobacillus separated from lactic beverage was not influenced on soil temperature as much as lactobacillus separated from radish and cabbage, and lactobacillus generally cultured in a soil did not show sensitive reaction on the temperature change. Thus, it can be concluded and only lactobacillus existing in radish and cabbage reacts sensitively on the temperature change of soil. This is the result that confirms that vitality of lactobacillus existing in radish and cabbage, bacteria significant on fermentation of kimchi, is distracted by the temperature change of soil.

Experiment 2) Proliferation of Lactobacillus Existing in Cabbage and *Radish that Experienced the Change in Vitality According to Different Temperature Changes of Soil and Change of Lactobacillus Distribution of Radish and Cabbage Grown in Soil Containing Lactobacillus in Radish and Cabbage*

The experiments for Fig. 2 and Fig. 3 confirmed that the vitality and proliferation of lactobacillus separated from radish and cabbage decrease according to temperature change of the soil, and the lactobacillus exposed to the temperature change was not detected in leaves and stems of the radish and cabbage. From this result, it was hypothesized that lactobacillus would not be able to be engrafted during the process of growth of radish and cabbage, which could cause quality deterioration of kimchi.

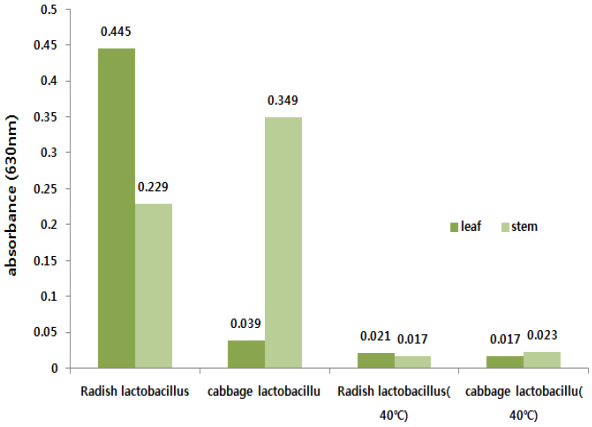


Figure 2. Change of lactobacillus engrafted on leaves and stems of plants that grew in soil containing lactobacillus of radish and cabbage whose vitality was changed by the temperature change of the soil

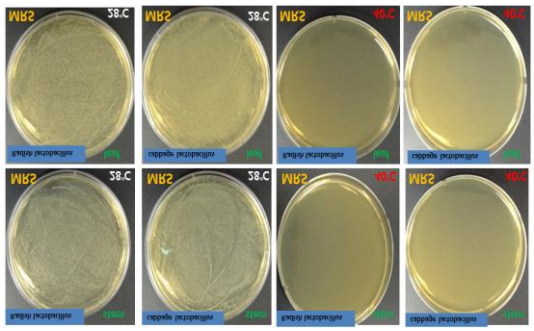


Figure 3. Culturing lactobacillus engrafted on leaves and stems of lactobacillus exposed to temperature change of soil

Experiment 3) About the Relationship between Soil Bacteria and Lactobacillus

As shown in Fig. 4, in case of lactobacillus of radish and cabbage separated from normal soil temperature (28 ℃), the culture was detected even only 5 $\mu\ell$ was injected. On the other hand, for lactobacillus of radish and cabbage separated from high temperature (40 ℃), vitality and reproductive rate decreased so greatly that at least 100 $\mu\ell$ should be injected in order to observe the growth of lactobacillus. This result can draw the inference that most lactobacillus had lost the vitality and died out by the temperature change.

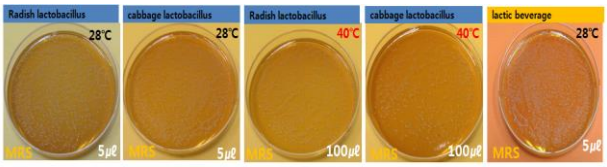


Figure 4. Growth change of lactobacillus separated from radish and cabbage in soil experiencing temperature change

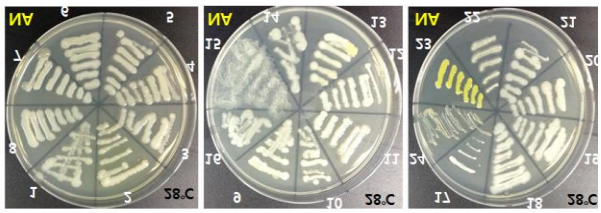


Figure 5. Separation culture of soil bacteria (24 types)

As a result of separation culture of each type of soil bacteria generally existing in soil, 24 types of soil bacteria with different shapes and growth ranges were separated. Among them, 8 types are soil bacteria separated from regular soil, other 8 types were separated from the soil contaminated by heavy metals, and the rest of them were separated from gardening soil. All of these bacteria were cultured in 28 ℃, as shown in Fig. 5.

Table II shows that when the growth temperature was 28 ℃, the growth difference of soil bacteria was not significant even when separated soil bacteria was cultured after it was mixed with lactobacillus separated from radish and cabbage grown in soil with high temperature (40 ℃) and that grown in soil with regular temperature (28 ℃). However, when the growth temperature becomes 40 ℃., while lactobacillus of radish and cabbage grown in soil temperature (28 ℃) presented similar growth rate to soil bacteria cultured in 28 ℃, the growth rate of soil bacteria mixed with lactobacillus separated from radish and cabbage grown in high temperature (40 ℃) soil decreased. This result shows that stable soil condition lets soil bacteria grow normally no matter lactobacillus in radish and cabbage exists or not, but when the soil bacteria was exposed to high temperature (40 ℃) which can suppress the growth of soil bacteria, the growth of soil bacteria can be maintained when the bacteria was mixed with separated lactobacillus.

TABLE II. RELATIONSHIP BETWEEN LACTOBACILLUS AND GROWTH OF 24 TYPES OF SOIL BACTERIA IN DIFFERENT TEMPERATURES

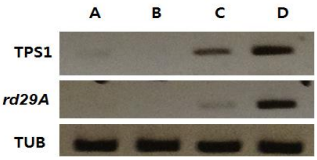
28℃	Radish lactobacillus	cabbage lactobacillus	Radish lactobacillus (40℃)	cabbage lactobacillus (40℃)	CONTROL
1	0.697	0.654	0.558	0.701	0.699
2	0.587	0.607	0.662	0.617	0.597
3	0.225	0.277	0.308	0.229	0.317
4	0.474	0.517	0.397	0.414	0.454
5	0.339	0.321	0.374	0.325	0.401
6	0.665	0.741	0.787	0.697	0.684
7	0.447	0.474	0.501	0.494	0.51
8	0.229	0.317	0.358	0.341	0.402
9	0.335	0.474	0.365	0.338	0.397
10	0.314	0.297	0.266	0.307	0.315
11	0.585	0.498	0.447	0.439	0.399
12	0.602	0.614	0.621	0.647	0.633
13	0.663	0.627	0.619	0.634	0.651
14	0.584	0.568	0.549	0.562	0.546
15	0.541	0.532	0.517	0.522	0.531
16	0.633	0.654	0.634	0.647	0.643
17	0.647	0.651	0.648	0.641	0.639
18	0.71	0.702	0.714	0.697	0.713
19	0.337	0.335	0.328	0.349	0.337
20	0.557	0.548	0.537	0.571	0.544
21	0.458	0.461	0.448	0.418	0.439
22	0.397	0.401	0.411	0.418	0.421
23	0.667	0.659	0.598	0.647	0.651
24	0.459	0.462	0.471	0.455	0.419

40℃	Radish lactobacillus	cabbage lactobacillus	Radish lactobacillus(40℃)	cabbage lactobacillus(40℃)	CONTROL
1	0.771	0.696	0.102	0.067	0.669
2	0.448	0.565	0.011	0.054	0.489
3	0.287	0.392	0.087	0.11	0.401
4	0.398	0.422	0.045	0.121	0.473
5	0.401	0.369	0.064	0.141	0.367
6	0.557	0.581	0.069	0.098	0.517
7	0.502	0.567	0.034	0.047	0.544
8	0.284	0.319	0.048	0.055	0.352
9	0.374	0.411	0.081	0.062	0.421
10	0.329	0.365	0.022	0.047	0.377
11	0.597	0.622	0.049	0.022	0.638
12	0.695	0.706	0.033	0.058	0.722
13	0.673	0.74	0.058	0.017	0.712
14	0.608	0.661	0.061	0.051	0.646
15	0.576	0.582	0.047	0.063	0.603
16	0.684	0.716	0.055	0.029	0.722
17	0.659	0.669	0.039	0.054	0.688
18	0.711	0.729	0.067	0.021	0.714
19	0.405	0.454	0.088	0.036	0.469
20	0.613	0.638	0.041	0.022	0.629
21	0.487	0.509	0.068	0.077	0.512
22	0.417	0.451	0.055	0.059	0.463
23	0.582	0.455	0.049	0.047	0.481
24	0.463	0.517	0.037	0.052	0.529

Experiment 4) Growth Change of Plants and Change of Soil Component in Soil Containing Lactobacillus

When arabidopsis is exposed to different types of stress, the vitality of TPS1 and rd29A, types of gene, increases. Based on this study, arabidopsis was planted in soil containing lactobacillus separated from radish and cabbage cultured in high temperature (40 ℃) soil, and this plant was grown for two weeks in 30 ℃, the temperature stressing arabidopsis. After this process, the appearance of TPS1 and rd29A was analyzed using RT-PCR. As shown in Fig. 6, the appearance of TPS1 and rd29A of arabidopsis did not increase in the soil containing lactobacillus of radish and cabbage cultured in the soil temperature (28 ℃), but the appearance increased when it was grown in the soil containing lactobacillus separated from radish and cabbage cultured in high temperature (40 ℃) soil. This shows that arabidopsis gained less stress when lactobacillus of radish and cabbage cultured in the soil temperature (28 ℃) was injected into the soil, while the more stress was provided to arabidopsis when lactobacillus exposed to high temperature (40 ℃) was added into the soil. The result presents that lactobacillus separated from radish and cabbage cultured in high temperature (40 ℃) soil

cannot reduce the stress of arabidopsis growing in a stressful condition. Moreover, when lactobacillus separated from radish and cabbage cultured in soil with temperature of 28 ℃ was injected into the soil, the number and types of the soil bacteria of this soil where arabidopsis was grown in 30 ℃ were relatively stabilized. In conclusion, arabidopsis was grown in stress by not receiving the nutrients that soil bacteria should provide, but when lactobacillus of radish and cabbage cultured in the soil temperature (28 ℃) was injected into the soil, the number and types of soil bacteria could be maintained, letting plants to grow normally.



A: Arabidopsis grown in soil containing lactobacillus of radish cultured in soil temperature (28 ℃)

B: Arabidopsis grown in soil containing lactobacillus of cabbage cultured in soil temperature (28 ℃)

C: Arabidopsis grown in soil containing lactobacillus of radish cultured in soil temperature (40 ℃)

D: Arabidopsis grown in soil containing lactobacillus of cabbage cultured in soil temperature (40 ℃)

Figure 6. Change of appearance of stress gene of plants after injecting lactobacillus

TABLE III. CONTENT CHANGE OF NITROGENOUS COMPOUND IN SOIL CONTAINING LACTOBACILLUS ACCORDING TO TEMPERATURE

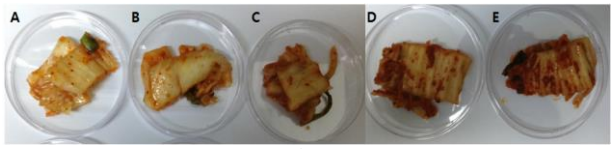
	Field station	NH_4^+ (mg/kg)	TN (g/kg)	TP (g/kg)	TPi (mg/kg)	TC (g/kg)	TOC (g/kg)	SO_4^{-2} (mg/kg)
28℃	Radish lactobacillus	3.44	1.33	0.96	342.33	1.23	12.43	0.92
	Cabbage lactobacillus	2.43	1.49	0.77	351.36	0.85	17.34	0.98
40℃	Radish lactobacillus	1.23	5.32	1.29	128.34	3.44	45.23	3.69
	Cabbage lactobacillus	0.85	6.35	2.27	116.65	2.43	62.34	4.33

TN, total nitrogen; TP, total phosphorus; TPi, total inorganic phosphorus; TC, total carbon; TOC, total organic carbon

The change of soil caused by decreased number of soil bacteria in the condition of increased temperature was observed more specifically throughout soil component analysis in Table III. The soil containing lactobacillus separated from radish and cabbage cultured in high temperature soil (40 ℃) 5 times higher compared to the soil containing lactobacillus separated from radish and cabbage cultured in TN (28 ℃); this result confirms that increase of temperature reduces the number of soil bacteria, thus it hinders the normal nitrogen circulation in normal soil. The amount of NH_4^+, the nitrogen form that plants can absorb during the process of nitrogen circulation, decreased in the soil containing lactobacillus separated from radish and cabbage cultured in high temperature soil (40 ℃). Having low NH_4^+ content despite high total amount of nitrogen can be concluded as the phenomenon caused by reduced vitality and number of soil bacteria, which displace nitrogen member into NH_4^+. Moreover, although the normal soil state should have reduced SO_4^{-2} content by the activation of anaerobic soil bacteria, the content increased in the soil containing lactobacillus separated from radish and cabbage cultured in high temperature soil (40 ℃).

Experiment 5) Production of Kimchi Using Lactobacillus in Radish and Cabbage that Experienced Vitality Change and that Did not Experienced Vitality Change and Comparison of Lactobacillus

As a result of producing kimchi with lactobacillus of radish and cabbage cultured in 28 ℃ and 40 ℃, abundant lactobacillus was detected in kimchi produced with lactobacillus cultured in normal soil temperature, but the content of lactobacillus greatly decreased when kimchi was produced with lactobacillus cultured in high temperature. This result from Fig. 7 and Fig. 8 can be analyzed into two possibilities: lactobacillus cultured in normal soil temperature proliferated during the process of making kimchi, or it helps producing other types of lactobacillus.



A: Kimchi that lactobacillus of radish cultured in soil temperature (28 ℃) was injected

B: Kimchi that lactobacillus of cabbage cultured in soil temperature (28 ℃) was injected

C: Kimchi that lactobacillus of radish cultured in soil temperature (40 ℃) was injected

D: Kimchi that lactobacillus of cabbage cultured in soil temperature (40 ℃) was injected

E: Kimchi that lactobacillus separated from lactic beverage was injected

Figure 7. Producing kimchi with lactobacillus with or without vitality change

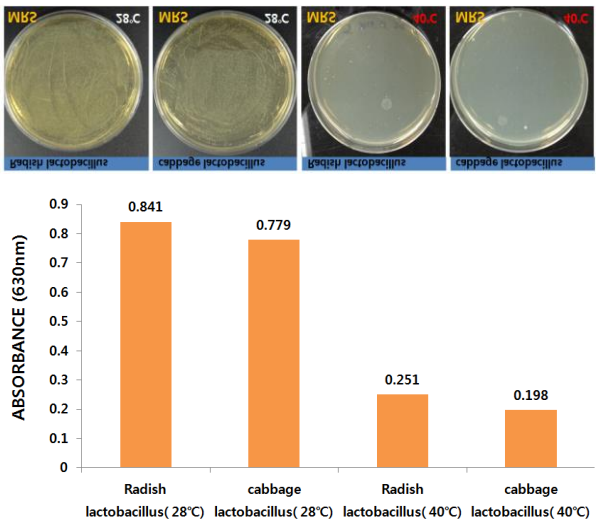
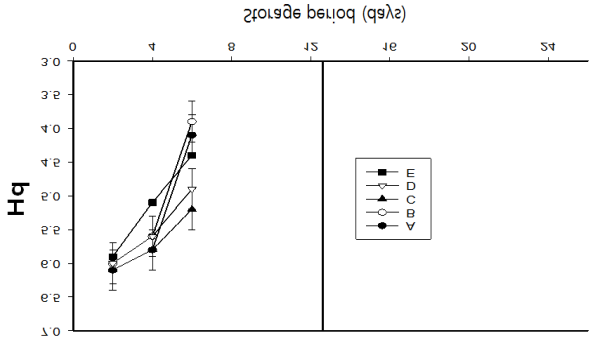


Figure 8. Change of lactobacillus of kimchi produced with Lactobacillus with or without vitality change

Experiment 6) Quality Test for Kimchi Produced in Experiment 5 Throughout Lactobacillus Cultivation and Acidity Test.

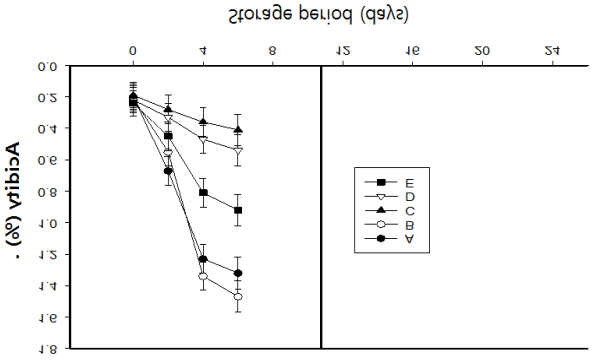
pH of Kimchi normally decreases when its fermentation began by lactobacillus. In other word, vitality of lactobacillus contained in kimchi is more active when pH of kimchi is lower. As a result of measuring pH of kimchi containing lactobacillus cultured in two different temperatures, as shown in Fig. 9, the pH of kimchi with lactobacillus cultured in normal

temperature was lower than that cultured in high temperature, and this difference got larger as time went by. This shows that the vitality of lactobacillus cultured in normal soil temperature increases during the process of fermentation of kimchi. Kimchi produced with lactobacillus separated from lactic beverage was not able to maintain its pH closer to acidic than that produced with lactobacillus cultured in normal temperature.



A: Kimchi that lactobacillus of radish cultured in soil temperature (28 ℃) was injected
B: Kimchi that lactobacillus of cabbage cultured in soil temperature (28 ℃) was injected
C: Kimchi that lactobacillus of radish cultured in soil temperature (40 ℃) was injected
D: Kimchi that lactobacillus of cabbage cultured in soil temperature (40 ℃) was injected
E: Kimchi that lactobacillus separated from lactic beverage was injected

Figure 9. Quality test of kimchi throughout pH test



A: Kimchi that lactobacillus of radish cultured in soil temperature (28 ℃) was injected
B: Kimchi that lactobacillus of cabbage cultured in soil temperature (28 ℃) was injected
C: Kimchi that lactobacillus of radish cultured in soil temperature (40 ℃) was injected
D: Kimchi that lactobacillus of cabbage cultured in soil temperature (40 ℃) was injected
E: Kimchi that lactobacillus separated from lactic beverage was injected

Figure 10. Quality test of kimchi throughout acidity test

Acidity, the unit to express the sour taste of kimchi, occurs during the process of fermentation of lactobacillus. When acidity of kimchi produced with lactobacillus cultured in two different temperatures was measured and compared, the acidity of kimchi with lactobacillus cultured in normal soil temperature was higher, as seen in Fig. 10. This shows that the quality of kimchi can be improved if lactobacillus of radish and cabbage cultivated in normal soil temperature (28 ℃) is added.

Experiment 7) About the Way to Recover Lactobacillus that has Lost its Vitality by Temperature Change

After lycopene, antioxidant component, of tomato and fragment of lactobacillus were cultured in the soil with high temperature (40 ℃), these were applied in lactobacillus separated from radish and cabbage that had lost their vitality and growth ability. As a result, the vitality of lactobacillus was recovered, as shown in Fig. 11. Thus, the rate of engraftment on leaves and stems of radish and cabbage grew.

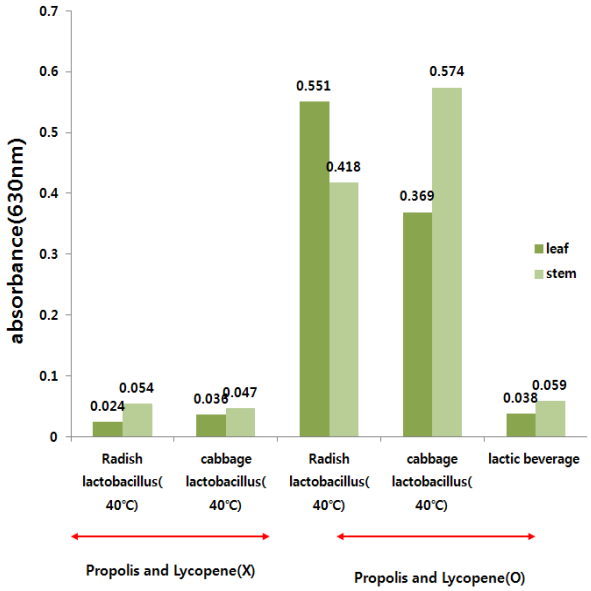
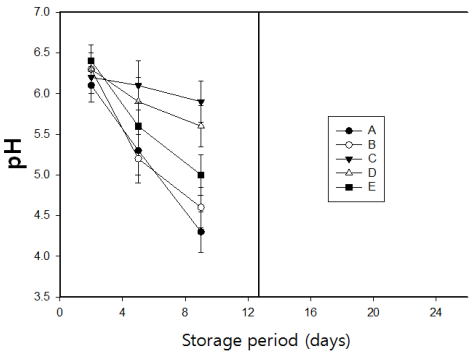


Figure11. Change of lactobacillus engrafted into leaves and stems after treatment with propolis and lycopene of lactobacillus that had lost its vitality

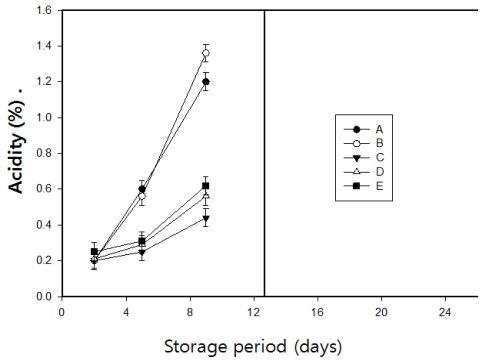


A: Kimchi produced after applying lycopene and lactobacillus fragment on lactobacillus of radish cultivated in soil temperature of 40 ℃
B: Kimchi produced after applying lycopene and lactobacillus fragment on lactobacillus of cabbage cultivated in soil temperature of 40 ℃
C: Kimchi produced after applying lactobacillus of radish cultivated in soil temperature of 40 ℃
D: Kimchi produced after applying lactobacillus of cabbage cultivated in soil temperature of 40 ℃
E: Kimchi produced after using lactobacillus contained in lactic beverage for lactobacillus fragment

Figure 12. Quality test of kimchi through pH test on kimchi treated with propolis and lycopene

According to Fig. 12, pH value decreases as fermentation of kimchi proceeds by lactobacillus, and

when kimchi was produced with lactobacillus, treated with lycopene and lactobacillus fragment, of radish and cabbage cultured in the soil temperature of 40 ℃, the decrease of pH was similar to that with lactobacillus of radish and cabbage cultured in the normal soil temperature. Therefore, it was confirmed that injecting lycopene and fragment of lactobacillus of radish and cabbage cultured in normal temperature can recover the growth of lactobacillus. However, when lactobacillus fragment of lactic beverage was used, the growth of lactobacillus was not able to be recovered even if lycopene was injected.



A: Kimchi produced after applying lycopene and lactobacillus fragment on lactobacillus of radish cultivated in soil temperature of 40 ℃

B: Kimchi produced after applying lycopene and lactobacillus fragment on lactobacillus of cabbage cultivated in soil temperature of 40 ℃

C: Kimchi produced after applying lactobacillus of radish cultivated in soil temperature of 40 ℃

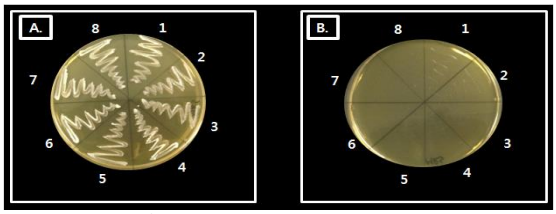
D: Kimchi produced after applying lactobacillus of cabbage cultivated in soil temperature of 40 ℃

E: Kimchi produced after using lactobacillus contained in lactic beverage for lactobacillus fragment

Figure 13. Quality test of kimchi, that propolis and lycopene was applied, through acidity test

When kimchi was produced after lycopene and lactobacillus fragment was applied to lactobacillus of radish and cabbage cultured in soil temperature of 40 ℃, Fig. 13 shows that the acidity, which shows the level of fermentation, was similar to acidity of kimchi containing lactobacillus of radish and cabbage cultured in normal soil temperature. This result presents that lycopene and lactobacillus fragment recover the vitality of lactobacillus.

Experiment 8) Growth Change in Soil and Recovery or Lactobacillus that Has Lost Vitality by Temperature Change



A: Soil culture of lactobacillus, treated with lycopene and propolis, that has lost its vitality by the temperature change

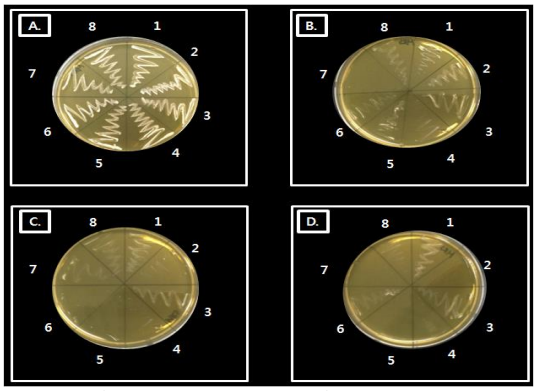
B: Soil culture of lactobacillus has lost its vitality by the temperature change

Figure 14. Soil culture of lactobacillus, treated with lycopene and propolis, that has lost its vitality by the temperature change

As shown in Fig. 14, lactobacillus, treated with lycopene and propolis, that had lost its vitality grew in a soil with no problem, but lactobacillus that had lost its vitality by temperature change could not grow in a soil.

Experiment 9) Change of Engrafting Ability on Cabbage of Lactobacillus Treated with Lycopene and Propolis

As shown in Fig. 15, when lactobacillus, treated with propolis and lycopene, with lower vitality was injected into the soil, lactobacillus in leaves and stems of grown plants was detected normally, but when the same lactobacillus without treatment of propolis and lycopene was injected into the soil, lactobacillus was not found in leaves and stems.



A: Culture of lactobacillus in leaves of plants in the soil containing lactobacillus, treated with lycopene and propolis, that had lost its vitality by the temperature change

B: Culture of lactobacillus in stems of plants in the soil containing lactobacillus, treated with lycopene and propolis, that had lost its vitality by the temperature change

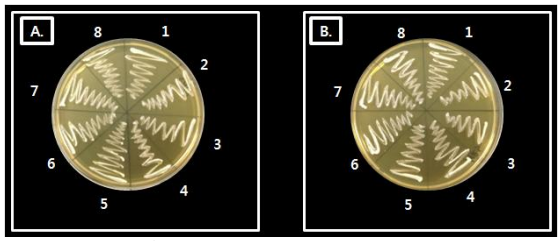
C: Culture of lactobacillus in leaves of plants in the soil containing lactobacillus that had lost its vitality by the temperature change

D: Culture of lactobacillus in stems of plants in the soil containing lactobacillus that had lost its vitality by the temperature change

Figure 15. Culturing lactobacillus of leaves and stems after soil culture of lactobacillus, treated with lycopene and propolis, that has lost its vitality by the temperature change

Experiment 10) Operation of Vitalized Lactobacillus for Next Generation

In case of lactobacillus whose vitality was decreased and treated with propolis and lycopene, it maintained its vitality and growth even if it was injected into the soil after it grew in the soil and was cultured again in the media, according to Fig. 16.



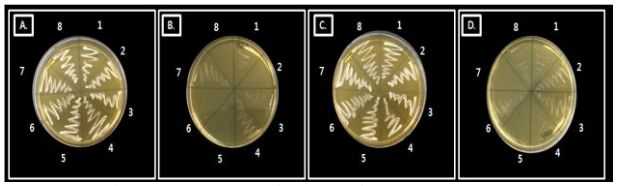
A: Soil Culture of Vitalized Lactobacillus

B: Soil Culture of Lactobacillus that was 1) Cultured in Soil and Separated 2) Cultured Again in MRS Media

Figure 16. Result of re-culture of vitalized lactobacillus

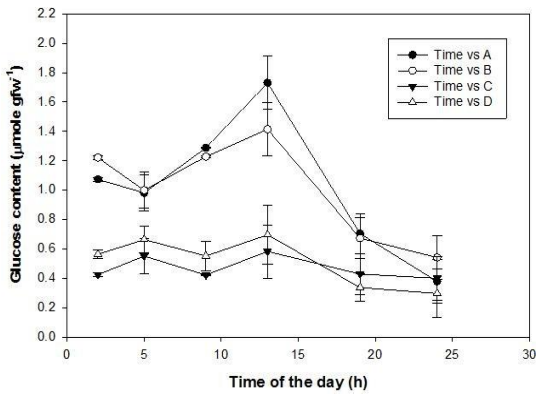
Experiment 11) Relationship between Change of Metabolism Material of Plants and Engraftment on Plants

Plants grew in the soil containing lactobacillus that had recovered its vitality by lycopene and propolis showed similar metabolism as that containing lactobacillus that had never lost its vitality, as shown in Fig. 17. According to Fig. 18, as the content of glucose in plants is higher, more unstable the plant is, and when lactobacillus that had not recovered its vitality or animal lactobacillus was injected into the soil, glucose content of the plants grew in those cases was higher than the plants grew in the soil with lactobacillus that had recovered its vitality by lycopene and propolis. Therefore, it was checked that metabolism of plants can be changed by lactobacillus. Sucrose, the intermediate to converted into different matter during the metabolism process, produces different substances for plants throughout the process of synthesis in daytime and decomposition at night. As shown in Fig. 19, higher sucrose content was maintained in the plants grew in the soil containing lactobacillus with low vitality or animal lactobacillus, showing that decomposition and production was not processing well. This is the result that confirms that lactobacillus can cause the change in metabolite of plants, thus it can cause the growth of the plants. In conclusion, lactobacillus is the significant factor deciding the quality of radish and cabbage, and it influences not only the fermentation but also the growth of radish and cabbage.



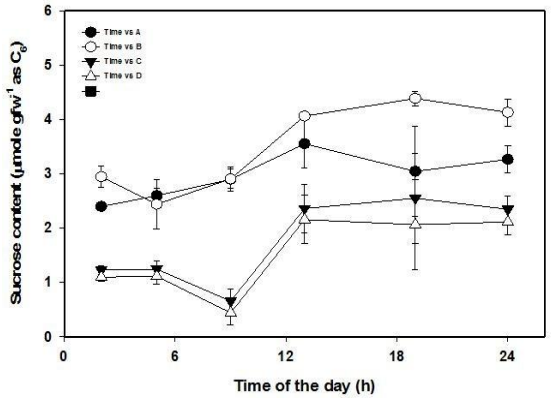
A. Engraftment on leaves of plants after the lactobacillus who has recovered its vitality was injected into the soil
B. Engraftment on leaves of plants after the lactobacillus who has not recovered its vitality was injected into the soil
C. Engraftment on stems of plants after the lactobacillus who has recovered its vitality was injected into the soil
D. Engraftment on stems of plants after the lactobacillus who has not recovered its vitality was injected into the soil

Figure 17. Culture of lactobacillus in order to check the production of metabolite of plants and change of engraftment ability of lactobacillus treated with lycopene and propolis on cabbage



A. Injection of lactobacillus that has not recovered its vitality
B: Injection of animal lactobacillus
C. Injection of lactobacillus that has recovered its vitality
D. Injection of normal lactobacillus

Figure 18. Comparing glucose content in order to check the production of metabolite of plants and change of engraftment ability of lactobacillus treated with lycopene and propolis on cabbage



A. Injection of lactobacillus that has not recovered its vitality
B: Injection of animal lactobacillus
C. Injection of lactobacillus that has recovered its vitality
D. Injection of normal lactobacillus

Figure 19. Comparing sucrose content in order to check the production of metabolite of plants and change of engraftment ability of lactobacillus treated with lycopene and propolis on cabbage

IV. Conclusion

The existence of lactobacillus on the surface of cabbage and radish is the reason that those vegetables can be made into kimchi. Although this type of lactobacillus is the type that commonly found in regular type of soil and this cannot be engrafted alive on the surface of the radish and cabbage, when radish and cabbage are raised after this lactobacillus was cultured on the sterilized soil, this lactobacillus is detected on leaves and stems of those vegetables. If lactobacillus separated cultured from radish and cabbage exists in soil, while no change is detected for other bacteria in a normal soil condition, the lactobacillus helps other bacteria to grow normally when the soil is in condition that other bacteria is difficult to grow. This was also checked throughout the analysis of different substances appearing by nitrogen circulation. Lactobacillus separated from radish and cabbage with these characteristics loses its vitality when the temperature rises and cannot engraft on leaves and stems of cabbage and radish, thus it cannot help soil bacteria grow even the soil bacteria is in condition difficult to proliferate. In other words, temperature change of the soil caused by the climate change cause the negative impact on the growth of lactobacillus of radish and cabbage. Moreover, lactobacillus that has lost its vitality because it was exposed to the temperature change, the vitality was greatly lower during the process of fermentation to become kimchi. This was confirmed by the change of pH and acidity of kimchi produced with those types of lactobacillus. Thus, it was checked that change of soil temperature caused by the climate change impedes the vitality of lactobacillus and the engraftment of lactobacillus on cabbage and radish, and the radish and cabbage contain lactobacillus with low vitality, which make difficult to produce normal kimchi.

In order to solve this problem, according to the research on ‘Stability of Korean Lactobacillus about Propolis and Spice, and the Selection of Lactobacillus that has Outstanding Antibacterial Activation Ability in order to Enhance the Storage of Kimchi’, based on the

fact that propolis enhances the vitality of lactobacillus, lycopene, anti-oxidative component of tomato, was extracted and injected, and fragmentation of lactobacillus was injected (injecting fragments of lactobacillus is the application of 'probiotics,'the phenomenon that proliferation of lactobacillus can be accelerated when fragments or component of same type of lactobacillus, of Seigen Korea Inc.). As a result the vitality of lactobacillus was able to be recovered. As the application of this research, because the decrease of vitality of lactobacillus can cause negative impact on the quality of kimchi, it was confirmed that environmental change can cause the change in our dietary life, and this it thought to be the time to explain the Korean characteristic of fermentation food, kimchi. The fact that kimchi produced with radish and cabbage grown in other countries cannot have the same taste as traditional kimchi made in Korea can be explained by the characteristics of lactobacillus existing in cabbage and radish. Furthermore, as lactobacillus influences not only the quality of kimchi but also the growth of plants, it shows lactobacillus well engrafted on plants causes positive effect on the growth of the plants. This will also be the one question that should be proved more specifically throughout the experiment.

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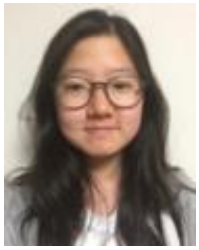

Alberta Yoo was born in Columbus, Ohio in 1998 and will be graduating from Yongsan International School of Seoul in 2017. Recipient of two grants from Korean Society of Civil Engineers and one from Seoul National University's Department of Civil & Environmental Engineering, Alberta has focused her research on environmental and agricultural studies, publishing *The Influence of Wastewater in Cultivation of Antibiotic Resistant Bacteria within the Han River* in *Wiset Junior Science & Technology Research Reports.* Alberta has also conducted applied environmental research, inventing emergency zipper bag of disposable antibiotic composition with Zeolite and active charcoal and patenting the invention under Korean Intellectual Property Organization. Alberta hopes to continue studying environmental engineering in college and promote sustainable development through her research.