J. Microbiol. Biotechnol. (1999), **9**(1), 22–31

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Improvement of Kimchi Fermentation by Using Acid-Tolerant Mutant of *Leuconostoc mesenteroides* and Aromatic Yeast *Saccharomyces fermentati* as Starters

KIM, YOUNG-CHAN, EUN-YUNG JUNG, HYUNG-JOO KIM, DAI-HYUN JUNG, SEONG-GIL HONG, TAE-JONG KWON, AND SANG-MO KANG*

Department of Microbiological Engineering, Konkuk University, Seoul 143-701, Korea

Received: April 13, 1998

Abstract *Saccharomyces fermentati* and *Leuconostoc mesenteroides* were isolated from a traditional kimchi, and then the *Leu. mesenteroides* was mutated to the acid-tolerant mutant *Leu. mesenteroides* M-100. In the result of growth properties in MRS broth with various pHs adjusted with HCl and acid solution (latic acid:acetic acid = 1:2), an acid-tolerant mutant *Leu. mesenteroides* M-100 showed more increased ability for growth than its wild strain at various temperatures. The strains were used as starters for the fermentation of kimchi. The experiments were performed with classified experimental groups (Group I, control kimchi; Group II, addition of YK-19 only; Group III, addition of M-100 only; Group IV, addition of mixture of M-100 and YK-19), and their pH, total acidity, reducing sugars content, organic acid productivity, organoleptic tests, and microfloral changes were compared. As a result, in pH and acidity, the optimal ripening period of Group IV was about 13.5 days (i.e. from the 8.5 to 22 days of fermentation). This result indicates that the optimal ripening period of Group IV was about 1.5 times longer than that of Group I. Furthermore, Group IV was edible to 28 days of fermentation. In addition, high contents of succinc acid was observed in Group IV. Group IV was also highly ranked on the organoleptic test. During the fermentation of kimchi, the number of *Leuconostoc* sp. in group I reduced after 7 days; however, the number of *Leuconostoc* sp. in Group II, III, and IV decresed after 14 days of fermentation. An especially high number of *Leu.* sp. was observed in Group IV, and this gave better flavor of kimchi than any other during the whole fermentation period. Citric acid, tartaric acid, succinic acid, fumaric acid, and lactic acid were detected in the kimchi, and a significant increase in the concentration of lactic acid during fermentation was observed in the all experimental groups.

*Corresponding author
Phone: 82-2-450-3524; Fax: 82-2-450-3517;
E-mail: kangsm@kkucc.konkuk.ac.kr

Key words: *Leuconostoc mesenteroides, Saccharomyces fermentati*, kimchi, starter, fermentation

Kimchi is one of Korea's traditional fermented vegetable foods. The major preparation procedure includes lactic acid fermentation by the addition of various kinds of vegetables, spices, and edible salt to Chinese cabbage and Chinese radishes. kimchi is generally acknowledged to be more nutritious than other fermented vegetable foods such as sauerkraut, pickles, and Japanese tsukemoto. In addition it is also known that the fermentation processes of kimchi involve more complex biochemical and microbiological processes compared to other fermented foods [16]. During the entire stage of natural fermentation of kimchi, lactic acid producing bacteria is a major population, and after prolonged fermentation, various and excess organic acids are produced by the lactic acid bacteria species. Those excess organic acid productions in kimchi fermentation are called the acidification of kimchi. After that stage, other microorganisms including yeasts grow on the surface of kimchi, and that growth causes the softening of the texture of the ingredients [3, 8, 22]. Therefore, generally, it can be defined that the edible taste of kimchi is obtained before the texture-softening (i.e. edible period), and "the good taste" of kimchi is obtained before the acidification (i.e. optimum ripening period).

A hetero-fermentative type bacterial species, *Leu. mesenteroides*, is a major bacterial population of kimchi from the initial to the middle stage of fermentation [9]. During those stages, those bacteria produce various constituents such as lactic acid, acetic acid, alcohol, CO_2, mannitol, and dextran which are associated with the taste of kimchi, and the number of the bacteria reaches the highest during the optimum ripening period [4, 9, 20].

However, the number of *Leu. mesenteroides* decreases sharply when the pH of kimchi is decreased to 4.0. On the other hand, a homo-fermentative type lactic acid bacteria, *Lactobacillus plantarum*, which has a strong pH tolerance under high organic acid concentrations, has been continuously increased in their number during kimchi fermentation to the last stage [18]. It has been reported that the acidification of kimchi is mainly caused by that bacteria species [16]. Because the acid production from the hetero-fermentative type lactic acid bacteria is lower than that of the homo-fermentative type lactic acid bacteria, the addition of an acid-tolerant mutant strain of *Leu. mesenteroides* as a starter of a kimchi fermentation may inhibit the rapid pH decrease and lactic acid production during kimchi fermentation. Therefore, a previous report [24] suggested that a mutant strain of *Leu. mesenteroides* which has more stable growth ability in acidic conditions is able to increase the edible period of kimchi.

In the later stage of kimchi fermentation, the low acid level in the kimchi, probably inhibits the growth of yeasts which grow on the surface of kimchi and consume the lactic acid as carbon sources. The addition of *Saccharomyces fermentati* YK-19 producing good flavors as a starter of kimchi fermentation inhibits rapid pH decrease and lactic acid production during kimchi fermentation [7, 13]. This kind of yeast strains can consume lactic acid which is produced by acid producing bacteria, and produces aromatic compounds rather than bad smell.

In a previous research [24], we investigated the role of a HCl-tolerant mutant as a starter which had been mutated by N'-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) for the kimchi fermentation. In this paper, the retardation of acidification and the prolongation of the edible period of kimchi were examined when an acid-tolerant mutant *Leu. mesenteroides* M-100 and *S. fermentai* YK-19 [13] having the producing capacity of aromatic substances were used for kimchi fermentation as starters.

MATERIALS AND METHODS

Microorganisms and Growth Conditions

Leu. mesenteroids and *S. fermentati* were isolated from a traditionally prepared kimchi and identified [13, 24]. Both microorganisms were subcultured weekly using MRS (Difico) and YM [5], respectively. The strains had been pre-cultured at 10°C for adaptation before they were used as starters. *Leu. mesenteroides* were mutated by N'-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) according to a conventional lactic acid bacteria mutation method [17, 24], and an acid-tolerant mutant *Leu. mesenteroides* M-100 was selected by testing the growth of mutated strains in the MRS broth which was adjusted to pH 3.8 using acid solution (lactic acid:acetic acid = 1:2).

Growth Properties at Various pHs and Temperatures

A wild strain Mw and its acid-tolerant mutant strain M-100 of *Leu. mesenteroides* were inoculated into several MRS broths which were adjusted to pH 3.5, 3.8, 4.0, and 4.5 with HCl and acid solution (latic acid:acetic acid =1:2). The effects of temperature on growth of both strains were tested at 10°C, 20°C, and 30°C. Growth levels were demonstrated with the pH of MRS broth and optical density at 660 nm at proper time intervals.

Preparation of Kimchi Sample

One kg of chinese cabbage was immersed in 12% (W/V) NaCl (80% refined salt) solution for twelve hours, and then washed three times with running water. Excess water in the sample was drained, and then the sample was cut into 4×5 cm pieces. Then, spices and other vegetables (0.8 g garlic, 2.5 g green onion, 2.5 g red pepper, 0.5 g ginger, and 1.0 g salted anchovy) were mixed in to the sample [15]. The mixed sample was then divided and 100 g put into 10 plastic bags. The bags were sealed after the air was removed. The final concentration of NaCl in the sample was adjusted to 3%. When the packed samples were prepared, the 0.01% (w/w) YK-19 strain and the 0.5% (w/w) M-100 strain (cell number: 10^8 cells/ml in both strains) were added to the prepared kimchi samples. The samples were then incubated at 10°C for the experimental period.

The Physico-chemical Analysis of Kimchi

The acidity of kimchi was measured by the AOAC method [1]. The required volume of NaOH to neutralize 10 ml kimchi broth was measured, and the volume of NaOH was then converted into lactic acid concentration of the kimchi sample. The salt concentration was determined as descibed by Mohr [20]. The reducing sugar was measured by the 3,5-dinitrosalicylic acid method [6]. For analysis of organic acids in the kimchi sample, 50 g of kimchi sample were homogenized with 80 ml of distilled water, and the homogenized sample was sonicated for 20 mins. The sonicated sample was then centrifuged for 30 mins (12,000×g), and then the supernatant was filtered using a syringe filter (0.45 μm) for HPLC analysis. The condition for the HPLC analysis was as described below. The analysis instrument was HP 1050 and Bio AMINEX HPX-87H column was used. The solvent, chart speed, and column oven temperature were H_2SO_4 pH 2.4, 0.6 ml/min, and 50°C, respectively.

Organoleptic Test

For the organoleptic (or sensory) test we used the multi-sample comparison method, The taste evaluation team was made up of 10 microbial engineering students from Konkuk University. They served for a six month period. The experimentally made kimchi was evaluated for the following factors: (1) Occurrence of green odor; (2) occurrence of sour odor; (3) occurrence of foul odor; (4) entire taste evaluation including taste and smell. The evaluation factors were scored on a 5-point scale (i.e. very bad ①; bad ②; not bad ③; good ④; and very good ⑤) [12, 14, 25]. For making a statistical treatment of the results, the significance was tested between averages by statistical analysis (SAS), and the significance was examined using Duncan's multiple comparison analysis method [21].

Distribution of Lactic Acid Bacteria

The method of Han *et al.* [10] was used to isolate and count lactic acid bacteria in the kimchi samples by genus. Firstly, *Leuconostoc* species were isolated, and then counted using phenylethyl alcohol-sucrose agar. *Streptococcus* species and *Pediococcus* were isolated and counted using M-enterococcus agar. Modified LBS medium containing acetic acid and sodium acetate which inhibits growth of *Pediococcus* species was also used for the isolation and the counting of *Lactobacillus* species. Total viable cell counting was performed by using plate count agar (PCA, Difco).

RESULTS AND DISCUSSION

Growth Properties of Wild and Mutant Strain of *Leu. mesenteroides* at Various pHs and Temperatures

The results of growth properties of wild strain Mw and acid-tolerant mutant M-100 of *Leu. mesenteroides* at various pHs and temperatures are presented in Figs. 1–3. In the case of wild strain Mw, when the pHs of MRS broth were adjusted with HCl, growth did not occur in the temperature range of 20°C and 30°C at pH 3.5 and slightly occurred at pH 3.8. At 10°C, growth only slowly occurred at pH 4.0. However, when broth pHs were adjusted with acid solution (latic acid:acetic acid=1:2), Mw did not grow at pH 3.8 and showed slight growth at pH 4.0 at all tested temperatures. In contrast with Mw, when broth pHs were adjusted with HCl, M-100 showed slightly increased growth levels at pH 3.5 at all tested temperatures. When the pHs of MRS broth were adjusted with acid solution (latic acid:acetic acid=1:2), M-100 grew at pH 3.8, except at 10°C.

These results correspond well with the report of Mcdonald *et al.* [18] which suggested that *Leu. mesenteroides's* growth-limit external pHs varied with the kinds of orgainc acids existing in the growth conditions. From these acid-tolerant differences between Mw and M-100, we thought that the acid-tolerant mutant M-100 would show an increased growth level in competitive coexistence with *Lac. plantarum* and have prolonged growth to the last stage of kimchi fermentation.

Changes of pH and Total Acidity

kimchi samples were added with the acid-tolerant mutant strain M-100 of *Leu. mesenteroides* and *S. fermentati* YK-19 producing various aromatic compounds as starters and fermented at 10°C. The changes of pH and total acidity in the kimchi sample are presented in Fig. 4.

In the control group (i.e. without starters), the initial pH of sample was 5.71, and the pH value continuously decreased to 3.99 during 14 days of fermentation. However, the pH in that sample was slightly increased after 14 days. This increase in pH is a typical side effect of the texture softening caused by the growth of yeasts on the surface of the kimchi and polygalacturonase of the yeast [3].

The acidity in experimental Group I showed a sharp increase to 0.62% after 10 days of fermentation, and then the acidity slightly increased to 0.73% after 17 days of fermentation. After 17 days of fermentation (i.e. the later period of fermentation), the acidity in the sample reached 0.94%. The initial pH of experimental Group II (YK-19 added group) was 5.77, and the pH decreased to 3.91 after 28 days of fermentation. The initial acidity in experimental Group II was 0.14%. The acidity rapidly increased to 0.83% at 21 days of fermentation, and slowly reached 0.94% at 28 days of fermentation.

Although the acidity in experimental Group III (M-100 added group) and Group IV (YK-19 and M-100 mixture added group) indicated 0.53% (Group III) and 0.54% (Group IV) in 10 days of fermentation, those groups showed less acid production than Group I during the whole fermentation period.

Generally, the optimum ripening period of kimchi is known to be when the total acidity is between 0.4~0.7% [11]. In the case of Group I in these experiments, the optimum ripening period was 9.5 days, from days 8.0 to 17.5 days of fermentation (acidity 0.4~0.75%). Groups II and III showed longer optimum ripening periods (i.e. 10.5 days and 11 days, respectively) than that of Group I. However, the longest optimum ripening period was obtained from Group IV. Experimental Group IV had 13.5 days (i.e., from 8.5 to 22 days) optimum ripening period with a pH range of 4.05 to 4.6 and total acidity of 0.4 to 0.75%. Furthermore, the taste of Group IV was good at 0.9% acidity. Therefore, it can be shown that the addition of a starter mixture (i.e. mixture of YK-19 and M-100 strains) to kimchi fermentation had a strong inhibition effect against the acidification of kimchi during the fermentation, and also extended the shelf life of kimchi.

Reducing Sugars

Figure 5 shows the changes of reducing sugar content in the samples during the fermentation of kimchi. Initial reducing sugar contents of Groups I, II, III, and IV were

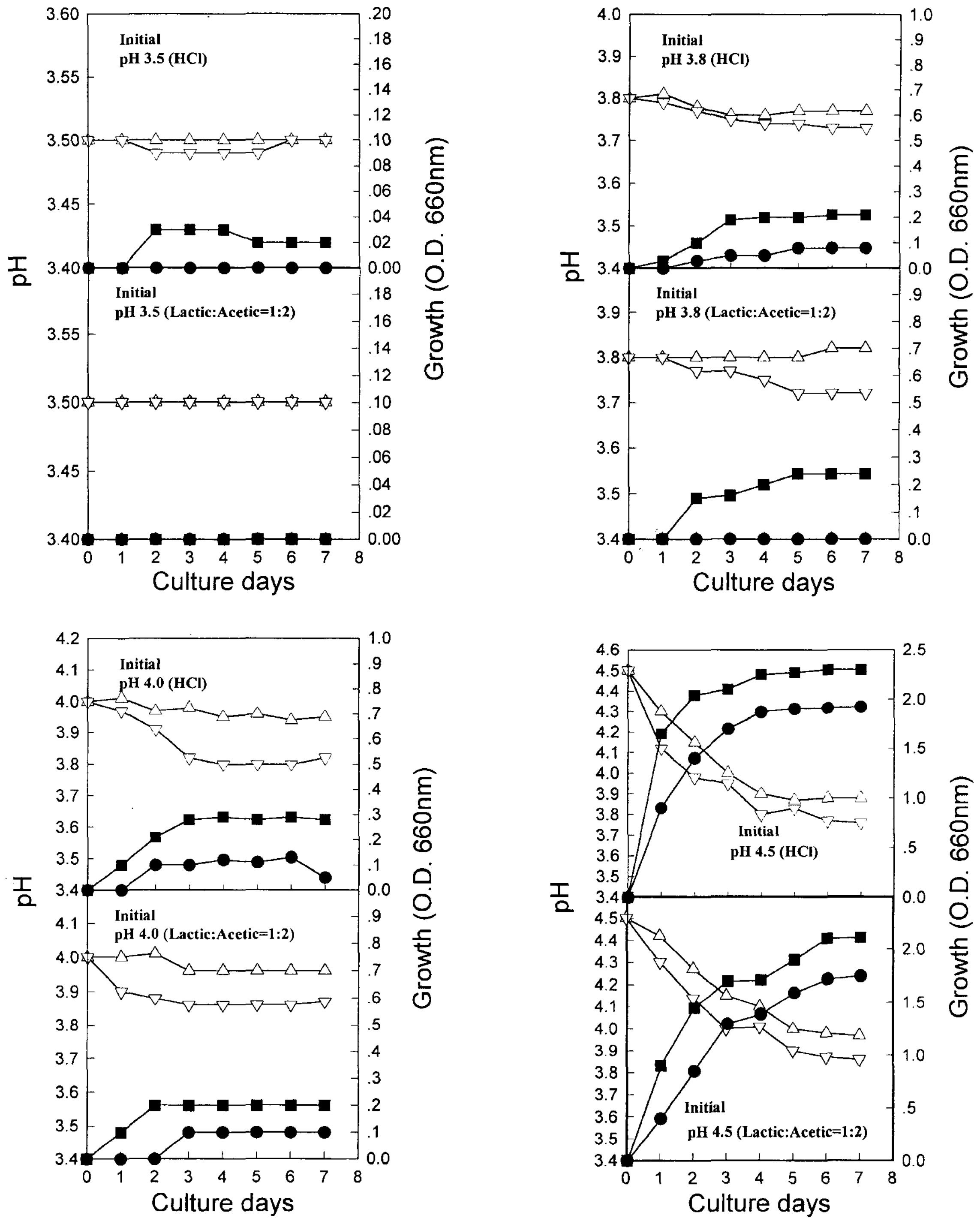

Fig. 1. Cell growth of wild and mutant strains of *Leu. mesenteroides* in MRS broth adjusted with HCl and acid solution (lactic acid:acetic acid = 1:2) at 30°C.

Symbols: —△—, pH changes of *Leu. mesenteroides* Mw; —▽—, pH changes of *Leu. mesenteroides* M-100; —●—, optical density changes of *Leu. mesenteroides* Mw; —■—, optical density changes of *Leu. mesenteroides* M-100.

20.8 mg/ml, 21.9 ml, 19.3 ml, and 20.5 ml, respectively, and the reducing sugar contents of all groups gradually increased for 3 days of fermentation, and then decreased after 3 days. After 10 days of fermentation, the reducing sugar contents in most experimental groups rapidly decreased. The final reducing sugar contents in Groups I, II, III, and IV were 3.9 mg/ml, 3.9 mg/ml, 5.3 mg/ml, and 4.8 mg/ml, respectively. A reason for the temporal increase

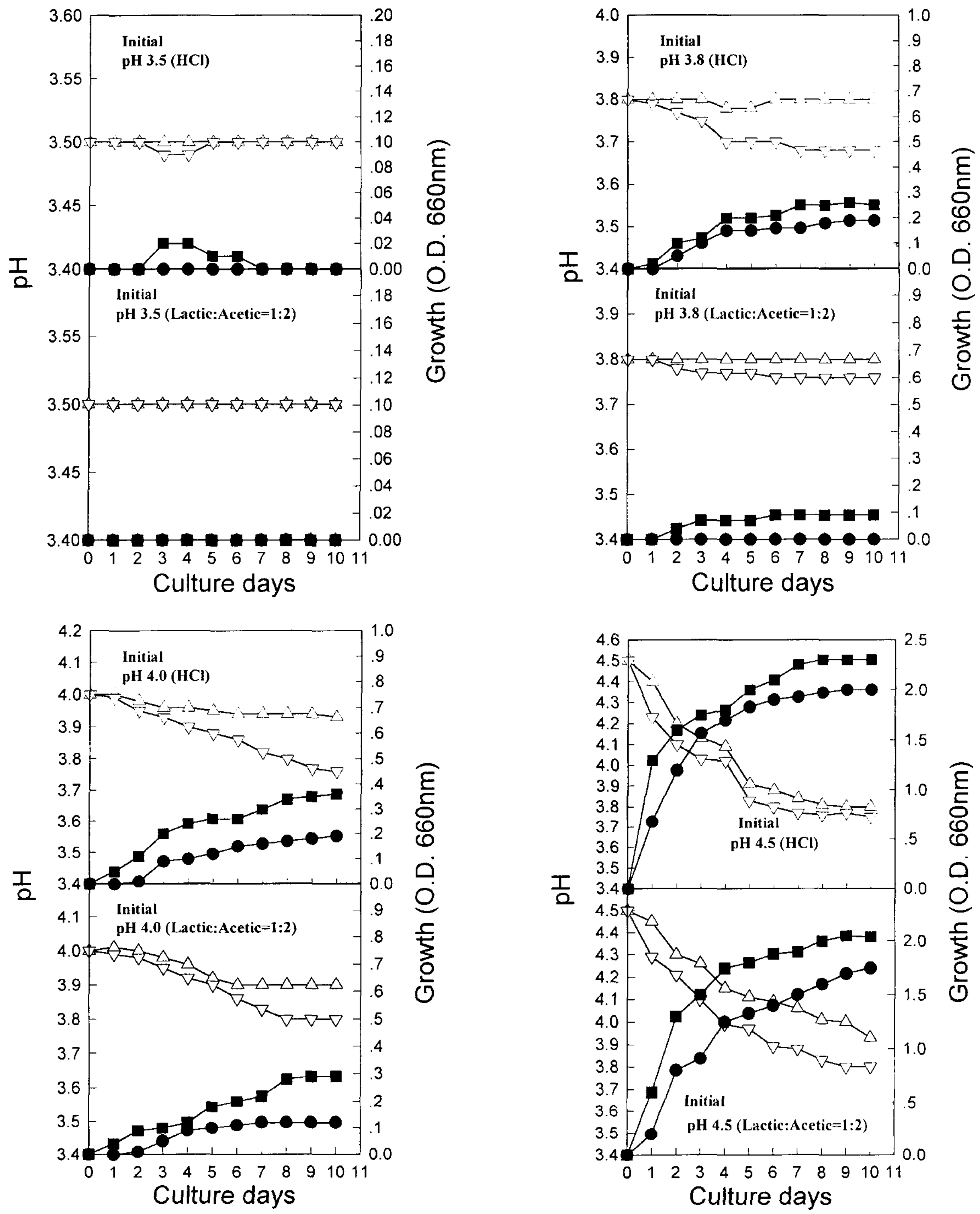

Fig. 2. Cell growth of wild and mutant strains of *Leu. mesenteroides* in MRS broth adjusted with HCl and acid solution (lactic acid:acetic acid = 1:2) at 20°C.
symbols: —△—, pH changes of *Leu. mesenteroides* Mw; —▽—, pH changes of *Leu. mesenteroides* M-100; —●—, optical density changes of *Leu. mesenteroides Mw*; —■—, *optical density changes of Leu. mesenteroides* M-100.

of reducing sugar contents in each group may be the result of the decomposition and/or the elution of compounds containing the reducing groups of cabbage and spices. When the population of lactic acid bacteria reached a high level, those compounds containing reducing sugar were converted to lactic acid, acetic acid, alcohol, carbon dioxide, etc, by lactic acid bacteria, and then the sugar contents decreased again [4, 16, 20]. There was no

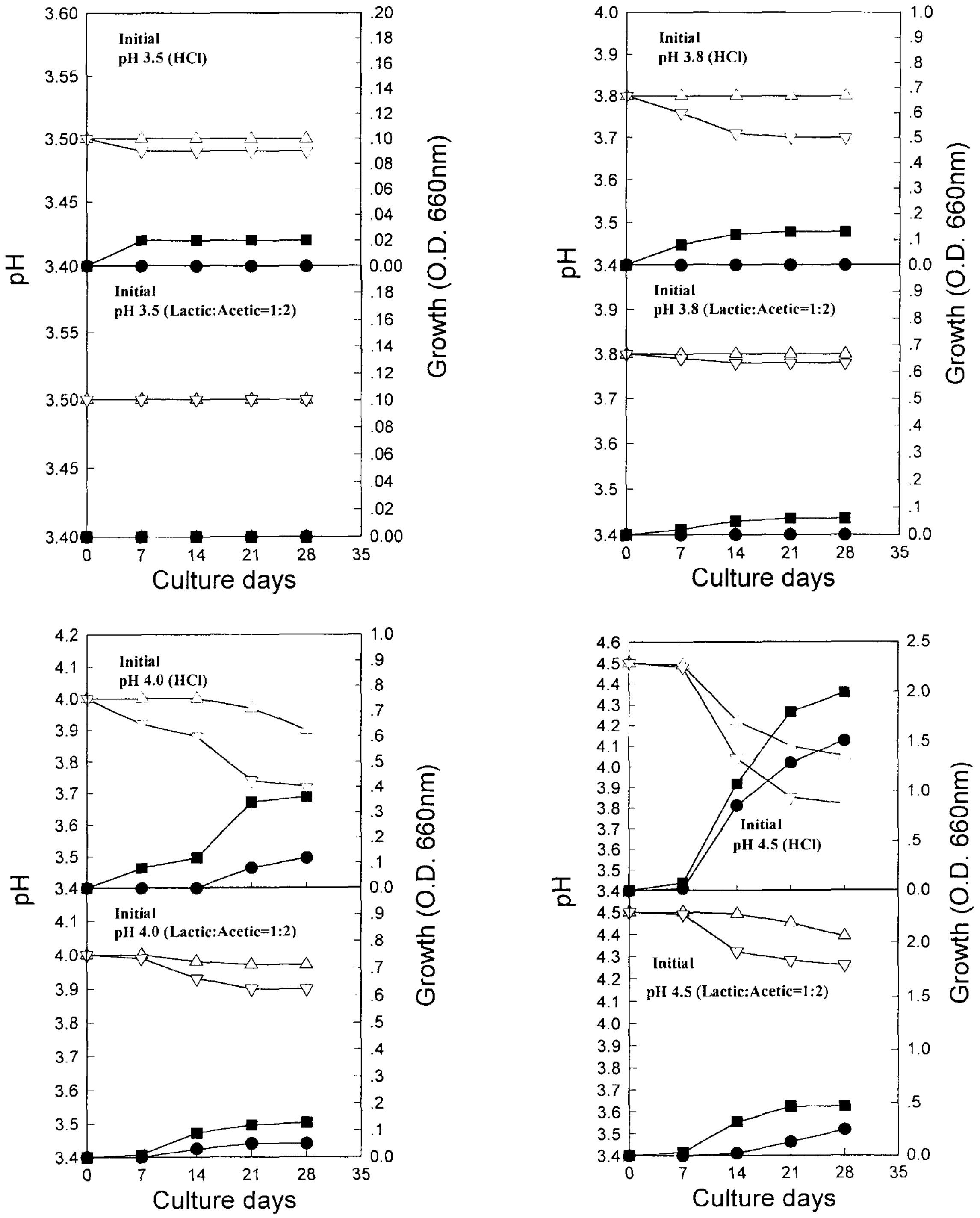

Fig. 3. Cell growth of wild and mutant strains of *Leu. mesenteroides* in MRS broth adjusted with HCl and acid solution (lactic acid:acetic acid = 1:2) at 10°C.

symbols: —△—, pH changes of *Leu. mesenteroides* Mw; —▽—, pH changes of *Leu. mesenteroides* M-100; —●—, optical density changes of *Leu. mesenteroides* Mw; —■—, optical density changes of *Leu. mesenteroides* M-100.

significant difference in the changes of reducing sugar content among the experimental groups; however, Group I had the lowest content of reducing sugars content during the fermentation.

Organic Acid

The variation of organic acid content in each group is listed in Table 1. The kinds of major organic acid which were detected in the experimental groups were citric acid,

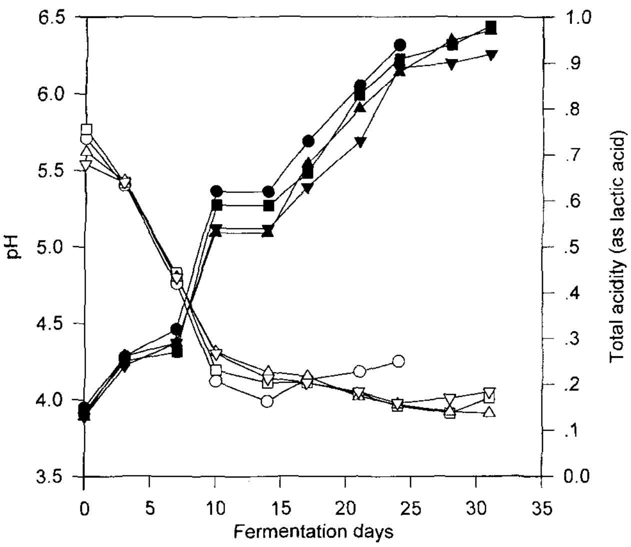

Fig. 4. Changes in pH and total acidity during kimchi fermentation at 10°C.

symbols: —●—, pH changes of control kimchi; —■—, pH changes of kimchi added with *S. fermentati* YK-19; —▲—, pH changes of kimchi added with *Leu. mesenteroides* M-100; —▼—, pH changes of kimchi added with *S. fermentati* YK-19 and *Leu. mesenteroides* M-100; —○—, total acidity changes of control kimchi; —□—, total acidity changes of kimchi added with *S. fermentati* YK-19; —△—, total acidity changes of kimchi added with *Leu. mesenteroides* M-100; —▽—, total acidity changes of kimchi added with *S. fermentati* Yk-19 and *Leu. mesenteroides* M-100.

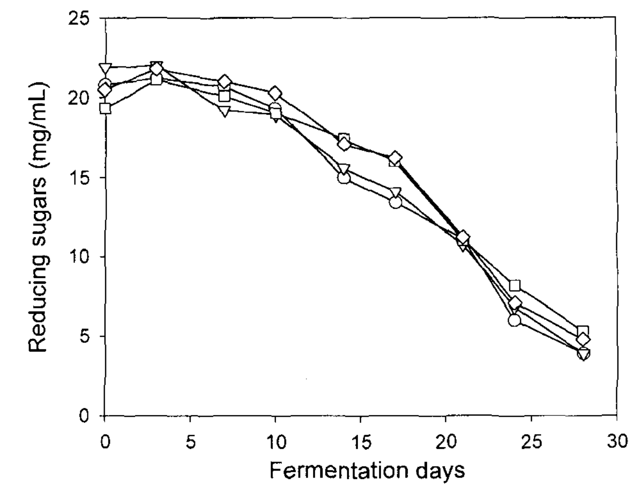

Fig. 5. Changes of reducing sugar during kimchi fermentation at 10°C.

Symbols: —○—, reducing sugar content of control kimchi; —▽—, reducing sugar content of control kimchi added with *S. fermentati* YK-19; —□—, reducing sugar content of kimchi added with *Leu. mesenteroides* M-100; —◇—, reducing sugar content of kimchi added with *S. fermentati* YK-19 and *Leu. mesenteroides* M-100.

tartaric acid, malic acid, succinic acid, fumaric acid, acetic acid, etc. The lactic acid content in every group maintained the highest value during the whole fermentation period.

Table 1. Changes of major organic acids during kimchi fermentation at 10°C for 28 days. Samples: Group I, control kimchi group; II, *S. fermentati* YK-19-added kimchi group; III, *Leu. mesenteroides* M-100-added kimchi group; IV, *S. fermentati* YK-19 and *Leu. mesenteroides* M-100-added kimchi group.

Fermentation period (days)	Sample Group	Organic acids content (w/v%)						
		Citric acid	Tartaric acid	Malic acid	Succinic acid	Lactic acid	Fumaric acid	Acetic acid
0	I	0.051	0.028	0.200	0.095	0.047	0.0005	0.045
	II	0.041	0.036	0.216	0.853	0.031	0.0005	0.023
	III	0.044	0.031	0.201	0.959	0.027	0.0005	0.050
	IV	0.044	0.023	0.241	1.185	0.036	0.0007	0.040
7	I	0.035	0.008	0.109	0.121	0.332	0.0007	0.147
	II	0.039	0.009	0.115	0.178	0.294	0.0006	0.113
	III	0.022	0.012	0.114	0.059	0.325	0.0007	0.119
	IV	0.019	0.009	0.120	0.111	0.457	0.0009	0.148
14	I	*	0.009	0.080	0.038	0.676	0.0008	0.204
	II	*	0.009	0.083	0.042	0.631	0.0006	0.193
	III	*	0.009	0.090	0.048	0.692	0.0008	0.229
	IV	*	0.008	0.091	0.040	0.629	0.0006	0.212
21	I	*	0.009	0.059	0.044	0.706	0.0006	0.229
	II	*	0.008	0.074	0.035	0.725	0.0007	0.219
	III	*	0.012	0.069	0.027	0.705	0.0006	0.232
	IV	*	0.012	0.079	0.041	0.701	0.005	0.238
28	I	*	0.013	0.083	0.056	0.681	0.008	0.287
	II	*	0.008	0.212	0.045	0.725	0.006	0.219
	III	*	0.009	0.113	0.042	0.698	0.006	0.228
	IV	*	0.012	0.107	0.059	0.722	0.004	0.266

* Not detected.

Group IV also had a high succinic acid concentration. Previous researches [14, 20] confirmed that succinic acid can be made by a hetero-fermentative bacteria such as *Leu. mesenteroides*. Therefore, the high content of succinic acid in Groups III and IV was probably caused by the addition of the mutant strain M-100 to both groups.

In all experimental groups, the increase of lactic acid concentration was observed during the initial stage of fermentation. In the later stages of fermentation, the concentrations of lactate were gradually decreased, except in experimental Group IV. Another paper suggested that the most favorable taste of kimchi was obtained when high contents of lactic acid and succinic acid were produced during kimchi fermentation [19]. Malic acid was initially observed in all experimental groups, and gradually decreased in its concentrations. Citric acid was not observed in any of the experimental groups after 14 days. In the present study, the texture-softening of kimchi was not observed in Group IV. This result is mainly caused by the strains of YK-19 and M-100, and their inhibition actions toward acid producing bacteria and yeasts in the fermentation.

Organoleptic Test

Table 2 shows the results of the organoleptic test of each experimental group. There was no difference in the degree of texture among the experimental groups. In the case of odor, Group I produced a slightly stronger odor than the other groups ($p<0.05$). A sour taste of almost the same values were found in all experimental groups in the initial stage of the fermentation, and there were prominent differences in sour taste among the groups after 17 days of fermentation (average values $p<0.05$). This result was the same as the results of the difference of total acid production among Groups I, II, III, and IV. In the case of total acceptability, Group I showed the highest score until 7 days of fermentation, and then Group II produced the highest score until 10 days of the fermentation. After 10 days of fermentation, the total acceptability of Group IV was good until 28 days of the fermentation. These results are mainly due to the consumption of organic acid by the strain YK-19 and the competition of growth between the strain M-100 and *Lactobacillus* sp. Therefore, the addition of the strains YK-19 and M-100 to the kimchi preparation prohibit other bacteria from over-production of acid during the fermentation, and also produced a tastier kimchi.

The Total Number of Microbes, Lactic Acid Bacteria, and Yeasts

Figure 6 shows a change of total number of microorganisms during the fermentation of kimchi. The number of *Leuconostoc* sp. dramatically increased from the initial fermentation period in all experimental groups. The number of *Leuconostoc* sp. in Groups II, III, and IV reached the highest levels (7.9×10^8, 1.5×10^9, 7.8×10^9 cells/ml, respectively) at 14 days of fermentation, whereas that of Group I was 3.8×10^8 cells/ml at 7 days. Generally, at along 14 days of fermentation, the number of *Leuconostoc* sp. went up to its highest level, with a good taste. It indicates the general characteristic of kimchi that the taste improves as the number of *Leuconostoc* sp. gets higher. Furthermore, as strain YK-19 was added, the number of *Leuconostoc* sp. increased more than that of Group I (control kimchi). It may be a result from the death rate of *Leuconostoc* sp. slowing down because YK-19 inhibited the production of acid so that a better environment was offered for *Leuconostoc* sp. [13]. Even though, the mutant strain M-100 growing at pH 3.8 has more acid-resistance than a wild strain, it is not strong enough to survive under acidic conditions compared to *Lactobacillus* sp.

Table 2. Sensory evaluation of kimchi added with starters during fermentation at 10°C for 28 days. Samples: Group I, control kimchi group; II, *S. fermentati* YK-19-added kimchi group; III, *Leu. mesenteroides* M-100-added kimchi group; IV, *S. fermentati* YK-19 and *Leu. mesenteroides* M-100-added kimchi group.

Fermentation period (days)	Flavor				Sourness				Texture				Total acceptability			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
0	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	5.00	5.00	5.00	5.00	4.50	4.50	4.50	4.50
3	4.00	4.00	4.00	4.00	3.90	3.90	3.90	3.90	4.50	4.50	4.50	4.50	4.20	4.15	4.10	4.15
7	4.23	4.18	4.10	4.25	4.28	4.15	4.16	4.26	4.20	4.20	4.50	4.50	4.24	4.15	4.10	4.10
10	4.00	4.00	4.05	4.15	4.20	4.20	4.00	4.00	4.10	4.00	4.00	4.00	4.12	4.29	4.20	4.15
14	3.95	3.98	4.00	4.15	4.00	4.25	4.35	4.30	3.95	3.90	4.00	4.00	3.80	3.80	4.15	4.27
17	3.40	3.45	3.50	3.50	3.24	3.74	4.11	4.20	3.12	3.20	3.25	3.20	3.30	3.40	3.85	3.90
21	2.95	3.00	3.00	3.25	2.55	2.63	3.05	3.10	3.00	3.00	3.00	3.10	2.80	2.90	3.20	3.40
24	2.50	2.55	2.60	2.70	2.45	2.50	2.73	2.95	2.75	2.80	2.80	2.95	2.75	2.75	2.86	3.00
28	2.00	2.12	2.20	2.20	2.15	2.23	2.31	2.40	2.40	2.50	2.50	2.50	2.33	2.32	2.40	2.45
Mean ± S.D.	3.50 ± 0.85^a	3.53 ± 0.81^b	3.55 ± 0.78^b	3.63 ± 0.78^b	3.47 ± 0.89^a	3.57 ± 0.88^b	3.68 ± 0.78^c	3.73 ± 0.73^d	3.67 ± 0.88	3.67 ± 0.86	3.73 ± 0.87	3.75 ± 0.85	3.56 ± 0.78^a	3.58 ± 0.78^b	3.71 ± 0.71^c	3.77 ± 0.68^d

Standard deviations with the same superscript (a,b,c, and d) in a row are not significantly different ($P<0.05$).

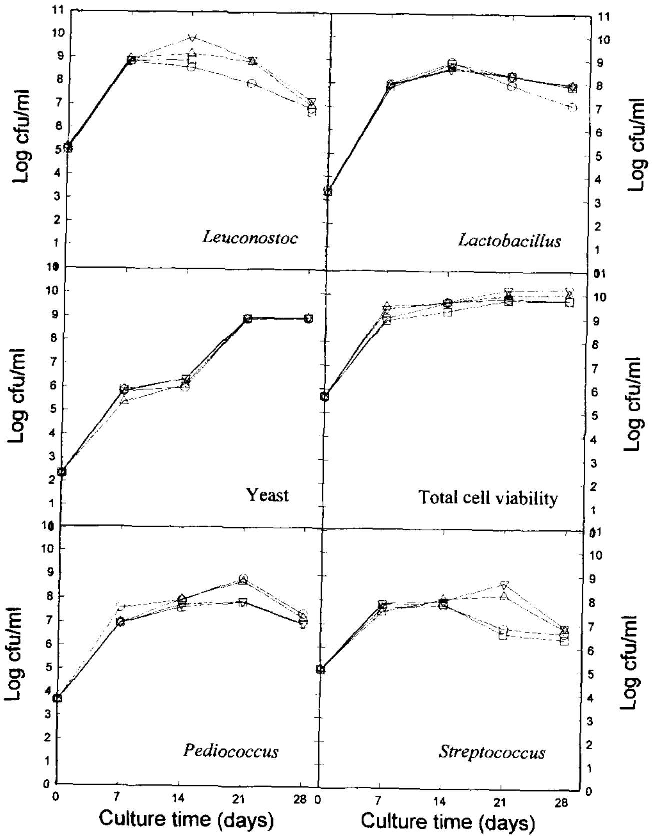

Fig. 6. Microbial changes of lactic acid bacteria and yeast during kimchi fermentation at 10°C.

Symbols: —○—, microfloral changes of control kimchi; —□—, microfloral changes of kimchi added with *S. fermentati* YK-19; —△—, microfloral changes of kimchi added with *Leu. mesenteroides* M-100; —▽—, microfloral changes of kimchi added with *S. fermentati* YK-19 and *Leu. mesenteroides* M-100.

The number of *Lactobacillus* sp. rapidly increased until 7 days of fermentation, and then, except for Group I, showed almost the same number until 28 days of fermentation, and the numbers in Groups III and IV decreased after 21 days of fermentation. At 21 days of fermentation, Group I presented the highest number of *Lactobacillus* sp. After the acidification and texture-softening of kimchi, the number of *Lactobacillus* in all experimental groups decreased.

In the case of *Streptococcus* sp., the highest number was obtained in Groups IV at 21 days (5.0×10^8 cells/ml), while the numbers in Groups I, II, and III at 21 days of fermentation were much lower than Groups I. Like *Leuconostoc* sp., *Streptococcus* sp. is known to supply good taste to kimchi [23]. In this view point, the population of *Streptococcus* sp. in Group IV could be effected by the addition of starter which causes the same sequence as the case for *Leuconostoc* sp. in the kimchi.

The population of *Pediococcus* increased until 21 days of fermentation, and then slightly decreased. It is an acidification bacteria [2] and lower populations of the bacteria were observed in Groups III and IV due probably to the addition of the starter.

The number of yeasts increased until 28 days of fermentation in all experimental groups and maintained their number at the final stage of fermentation. In the case of total number of bacteria and yeasts in the experimental groups, the numbers were rapidly increased until 7 days of fermentation and the numbers were maintained during the whole period of fermentation. This result showed that the addition of starter into the fermentation is not directly related to the variation of total number of microorganisms in the kimchi.

Addition of the Leu. mesenteroides M-100 to the kimchi preparation may induce the prolonged acidification of kimchi because of its low production of lactic acid and increased growth in comparison with *Lac. plantarum*. Also *S. fermentati* may lengthen the edible period of kimchi by reducing lactic acid and acetic acid in the later period of kimchi fermentation. Then, the good flavor in the starter-added kimchi groups may be due to the various aromatic compounds, CO_2, and succinic acid produced by *Leu. mesenteroides* M-100 and *S. fermentati* YK-19.

Acknowledgments

This study was conducted as a part of the G7 project supported by the Ministry of Science and Technology, Korea.

References

1. AOAC. 1995. Fruits and products acidity (titrable) of fruit products. *Official Methods of Analysis*. Chapter 37, p. 10. 16th ed. AOAC International, U.S.A.
2. Back, W. and E. Stackebrandt. 1978. DNS/DNS-Homologiestudien innerhalb der Gattug *Pediococcus*. *Arch. Microbiol.* **118:** 79–85.
3. Choi, K. C. 1978. Studies on the yeasts isolated from kimchi. *Kor. J. Microbiol.* **16:** 1–10.
4. Chyun, J. H. and H. S. Rhee. 1976. Studies on the volatile fatty acids and carbon dioxide producted in different kimchis. *Kor. J. Food Sci. Technol.* **8:** 90–94.
5. Difco Laboratories. 1984. p. 689. *Difco Manual*. 10th ed. Detroit, Michigan, U.S.A.
6. Gail, L. M. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **31:** 426.
7. Ha, S. S. 1960. Studies on the effects of polygalacturonase and film-forming microbes on the salt deterioration of the pickled vegetables. *Bulletin of the Scientific Research Institute* **5:** 13
8. Han, H. U., C. R. Lim, and H. K. Park. 1990. Commensalism between *B. custersii* and *K. oxytoca* in

kimchi spoilage. *Bulletin of the Institute for Basic Science*, Inha University. **11:** 171–178.
9. Han, H. U., C. R. Lim, and H. K. Park. 1990. Determination of microbial community as an indicator of kimchi fermentation. *Kor. J. Food Sci. Technol.* **22:** 26–32.
10. Han, H. U. and H. K. Park. 1991. Differential counts of lactic acid bacterial genera on bromophenol blue medium. *Bulletin of the Institute for Basic Science*, Inha University. **12:** 89–94.
11. Hong, S. I., J. S. Park, and N. H. Park. 1995. Quality changes of commercial kimchi products by different packaging methods. *Kor. J. Food Sci. Technol.* **27:** 112–118.
12. Kang, K. O., H. J. Sohn, and W. J. Kim. 1991. Changes in chemical and sensory properties of *Dongchimi* during fermentation. *Kor. J. Food Sci. Technol.* **23:** 267–271.
13. Kim, H. J., C. S. Lee, Y. C. Kim, and S. M. Kang. 1996. Identification of yeasts isolated from kimchi for kimchi starter. *Kor. J. Appl. Microbiol. Biotechnol.* **24:** 430–438.
14. Kim, H. O. and H. S. Rhee. 1975. Studies on the nonvolatile organic acids in kimchis fermented at different temperatures. *Kor. J. Food Sci. Technol.* **7:** 74–81.
15. Kim, M. H., M. S. Shin, D. Y. Jhon, Y. H. Hong, and H. S. Lim. 1987. Quality chracteristics of kimchis with different ingredients. *J. Kor. Soc. Food Nutr.* **16:** 268–277.
16. Lee, C. W., C. Y. Ko, and D. M. Ha. 1992. Microfloral changes of the lactic acid bacteria during kimchi fermentation and identification of the isolates. *Kor. J. Appl. Microbiol. Biotech.* **20:** 102–109.
17. MacFaddin, J. F. 1980. *Biochemical Test for Identification of Medical Bacteria*. 2th ed. Williams and Ilhius, London.
18. Mcdonald, L. C., H. P. Fleming, and H. M. Hassan. 1990. Acid tolerance of *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. *Appl. Environ. Microbiol.* **56:** 2120–2124.
19. Rhee, H. S. 1977. Studies on the nonvolatile organic acids in kimchi with different contents of salts. *Kor. J. Food Sci. Technol.* **9:** 69–81.
20. Ryu, J. Y., H. S. Lee, and H. S. Rhee. 1984. Changes of organic acids and volatile flavor compounds in kimchi fermented with different ingredients. *Kor. J. Food Sci. Technol.* **16:** 169–173.
21. SAS. 1988. *SAS User's Guide.*
22. So, M. H. and Y. B. Kim. 1995. Identification of psychrotrophic lactic acid bacteria isolated from kimchi. *Kor. J. Food Sci. Technol.* **27:** 495–505.
23. Speckman, R. A. and E. B. Collins. 1968. Diacetyl biosynthesis in *Streptococcus diacetylactis* and *Leuconostoc citrovorum*. *J. Bacteriol.* **95:** 174–180.
24. Yang, W. S., Y. C. Kim, E. Y. Jung, Y. G. Han, and S. M. Kang. 1995. Strain improvement of *Leuconostoc mesenteroides* for kimchi fermentation and effect of starter. *Kor. J. Appl. Environ. Biotechnol.* **23:** 461–471.
25. Yoon, J. S. and H. S. Rhee. 1977. A study on the volatile flavor components in kimchis. *Kor. J. Food Sci. Technol.* **9:** 116–122.