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NOTE

Inhibition of Lactic Acid Bacteria in Kimchi Fermentation by Nisin

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Abstract Sixty isolates of lactic acid bacteria found in kimchi, a traditional Korean dish of fermented vegetables, were tested for nisin sensitivity. Of the sixty isolates, all belonging to the genera *Leuconostoc, Lactobacillus*, and *Pediococcus*, fifty isolates were sensitive to nisin at a concentration of 100 IU/ml, and four isolates appeared to be resistant to nisin. This demonstrated that the nisin sensitivity of lactic acid bacteria found in kimchi varied considerably among isolates. In MRS broth containing nisin at concentrations of 100 to 300 IU/ml, the growth of sensitive isolates of *Leuconostoc mesenteroides* and *Lactobacillus plantarum* was inhibited for two to three days at 20°C. When nisin was added to kimchi preparations at a concentration of 100 IU/ml, the growth of lactic acid bacteria was delayed and reached a maximum two days later than that in kimchi without nisin. These results suggest the possible use of nisin in kimchi preparation, at recommended levels, to control the lactic acid fermentation. Scanning electron micrographs of a sensitive isolate *L. plantarum* revealed the formation of pores on cell surfaces followed by rapid cell wall destruction 1 h after the addition of nisin.

Key words: Nisin, lactic acid bacteria, kimchi

Kimchi is a traditional Korean food, consisting of vegetables fermented by lactic acid bacteria (LAB). Most kimchi is homemade, although commercial production has increased since the 1980s. It is important to prevent kimchi from overripening, especially kimchi commercially produced for export. It is difficult to control the fermentation of kimchi, because it is a spontaneous and incomplete process involving the complex interaction of several species of lactic acid bacteria. Therefore, the most important problem facing the kimchi industry is to develop an effective method of fermentation control thereby facilitating preservation. There have been several attempts to develop processes that prevent the over-acidification of kimchi [7, 14, 15, 17]. In practice, however, storage at low temperature is the only method of preservation that does not affect the organoleptic qualities of kimchi.

Recent studies have shown that lactic acid bacteria used in the production of wine [10, 24, 25] and beer [23] can be inhibited by nisin. Moreover, the possibility of controlling the lactic acid fermentation in sauerkraut by using a nisin-resistant strain in the presence of nisin, has been reported [4].

In this study, we investigated the effect of nisin on LAB isolated from kimchi and the overall effect of nisin on the fermentation, as a possible method of kimchi preservation. The inhibitory action of nisin on the cells was also observed using a scanning electron microscope (SEM).

Inhibitory Effect of Nisin against Lactic Strains from Kimchi

Nisin sensitivity of LAB isolates from kimchi was determined in MRS broth containing nisin. LAB were isolated from homemade and commercial kimchi by using MRS agar (Difco Laboratories, Detroit, MI, U.S.A.), Rogosa SL agar (Difco), and Phenyl Ethyl Alcohol-Sucrose agar [20]. Isolates were identified according to the criteria described in Bergey's Manual of Systematic Bacteriology [21]. Isolates were stored at -20°C in MRS broth with 20% glycerol, and the stock cultures were propagated twice in MRS broth for 18 h at 30°C before each experiment. A commercial nisin preparation with an activity of 1×10^6 IU/g was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. A filter-sterilized stock solution containing 12,000 IU/ml in 0.02 N HCl was prepared weekly. The MRS broth containing 100 IU/ml of nisin was inoculated with a 15 to 18 h culture of the LAB strain at a level of 10^7 cells/ml, and incubated at 20°C. Cell growth in the presence of nisin was observed by measuring the optical density of the culture broth at 600 nm. When no growth was observed after 4 days of incubation, the isolates were regarded as very sensitive. For sensitive isolates and weakly sensitive isolates,

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Table 1. Nisin sensitivity of lactic acid bacteria isolated from kimchi.

Genus	No. of isolates tested	No. of resistant isolates	No. of sensitive isolates			
			W.S.[a]	S[b]	V.S.[c]	total
Leuconostoc	16	2	2	8	4	14
Lactobacillus	32	2	3	20	7	30
Pediococcus	12	0	1	8	3	12

The sensitivity to nisin was tested in MRS broth with 100 IU/ml of nisin at 20°C. The growth was determined by measuring optical density of culture at 600 nm.

[a]W.S.: weak sensitive. Growth was detected after 48 h.

[b]S: sensitive; Growth was detected after 96 h.

[c]V.S.: very sensitive. Growth was not detected after 96 h.

growth was observed after 96 and 48 h of incubation, respectively. When growth was similar to that observed in MRS broth controls without nisin, the isolates were regarded as resistant.

Table 1 shows the nisin sensitivity of sixty LAB isolates from kimchi belonging to the genera *Leuconostoc, Lactobacillus*, and *Pediococcus*, tested for their sensitivity to nisin. Only 4 isolates appeared to be resistant to nisin; 6 were weakly sensitive. Fifty isolates, a significant majority, were either sensitive or very sensitive to nisin at a concentration of 100 IU/ml, which is the recommended level for use in food used by many countries [11]. These results suggest that nisin could be used to delay the lactic acid fermentation of kimchi. The presence of 30 species of LAB during the fermentation of kimchi has been reported [8, 18]. Two organisms, *Leuconostoc mesenteroides* and *Lactobacillus plantarum*, invariably predominate over a range of conditions. Therefore, three strains of both genera, including a nisin-resistant strain of each, were selected to investigate growth responses at various nisin concentrations.

For inhibition test, nisin was added to MRS broth at concentrations of 0, 50, 100, and 300 IU/ml, and the broth was inoculated with a 15 to 18 h culture of the LAB strain at a level of 10^7 cells/ml. The inoculated broth was incubated at 20°C, and cell growth was determined by measuring optical density.

A very sensitive strain, *Leu. mesenteroides* MA18, was completely inhibited at a level of 50 IU/ml of nisin (Fig. 1a). The growth of another strain, *Leu. mesenteroides* ME6, was delayed for only one day at 50 IU/ml, and for three days at 300 IU/ml (Fig. 1b). The sensitive strain *Lb. plantarum* MD15 was inhibited for two days at 50 IU/ml, and three days at 300 IU/ml of nisin (Fig. 2a). In contrast, the growth of both *Leu. mesenteroides* MC8 (Fig. 1c) and *Lb. plantarum* MC16 (Fig. 2c) was unaffected by nisin concentrations up to 300 IU/ml. In all cases, when the growth of sensitive strains commenced after one to three days of inhibition, the rate of growth was the same as for the controls, regardless of the initial

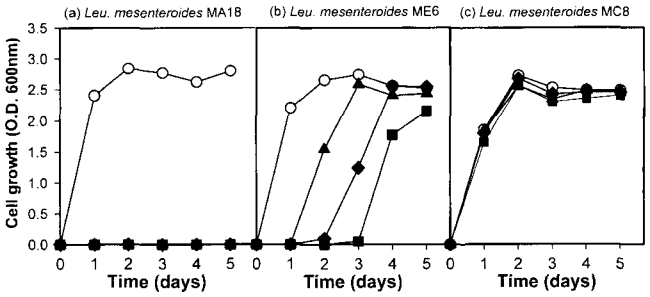

Fig. 1. The inhibitory effect of nisin on the growth of *Leuconostoc mesenteroides* from kimchi.

Cells were cultured in MRS broth at 20°C, using different concentrations of nisin: ○, 0 IU/ml; ▲, 50 IU/ml; ◆, 100 IU/ml; ■, 300 IU/ml.

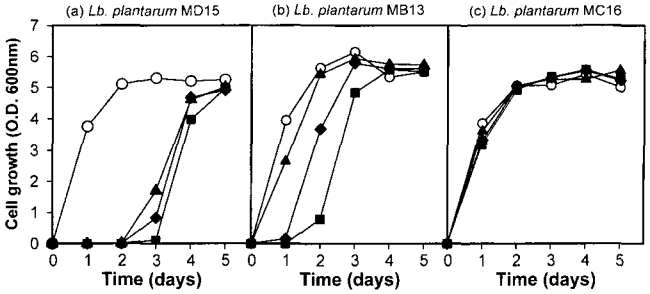

Fig. 2. The inhibitory effect of nisin on the growth of *Lactobacillus plantarum* from kimchi.

Cells were cultured in MRS broth at 20°C, using different concentrations of nisin: ○, 0 IU/ml; ▲, 50 IU/ml; ◆, 100 IU/ml; ■, 300 IU/ml.

nisin concentration. A similar phenomenon has been observed for *Lb. casei* [5], and it could be caused either by selection of resistant mutants or induction of a resistance mechanism. At present, kimchi is spontaneously fermented by the LAB normally found on the vegetables and other ingredients, both in homemade and commercially produced kimchi. With the development of a commercial process, the possible use of starter bacteria has been investigated [9, 13, 28]. Of the large variety of LAB species found in kimchi, strains of *Leu. mesenteroides* are considered to be the most suitable for use as a starter culture [13, 16, 19, 28]. These organisms initiate the fermentation of kimchi, attain maximum growth during the optimum ripening period, and subsequently decrease rapidly in numbers [16, 19]. Moreover, the optimal organoleptic qualities of kimchi are obtained when conditions are favorable for the growth of *Leu. mesenteroides*, rather than other LAB. The nisin-resistant strain of *Leu. mesenteroides* could therefore be used as a starter, to improve the quality of kimchi when nisin is present, as was studied, in part, by Breidt *et al.* [4].

Observation of Nisin-treated Cells by Scanning Electron Microscopy (SEM)

Leuconostoc mesenteroides ME6 and *Lactobacillus plantarum* MD15 were cultured overnight and transferred to MRS

broth for incubation at 30°C. When the cells reached exponential phase, nisin was added to a final concentration of 100 IU/ml. Samples were removed at appropriate intervals for observation by SEM. After fixation in 1% glutaraldehyde solution for 12 h at 4°C, the cells were post-fixed in 1% osmium tetraoxide for 2 h at room temperature. Samples were dehydrated in a graded ethanol series and dried with liquid CO_2 by the critical point drying. Specimens were sputter coated with silver and observed with a SEM (Hitachi S-2460N, Hitachi, Japan) at 12 to 16 kV.

The SEM micrographs clearly illustrate nisin-induced cell lysis of sensitive strains of *Leu. mesenteroides* (Fig. 3) and *Lb. plantarum* (Fig. 4). After 10 h of nisin treatment at a level of 100 IU/ml, most of the cells of *Leu. mesenteroides* ME6 were deformed and disrupted (Fig. 3b). Cell lysis proceeded more rapidly and drastically in *Lb. plantarum* MD15, where pores were observed on the cell surface after 1 h of treatment with nisin (Fig. 4b). After only 4 h, most, if not all cells were completely destroyed (Fig. 4c). The mode of action of nisin is known to involve changes in the permeability of the cytoplasmic membrane, due to its action as an

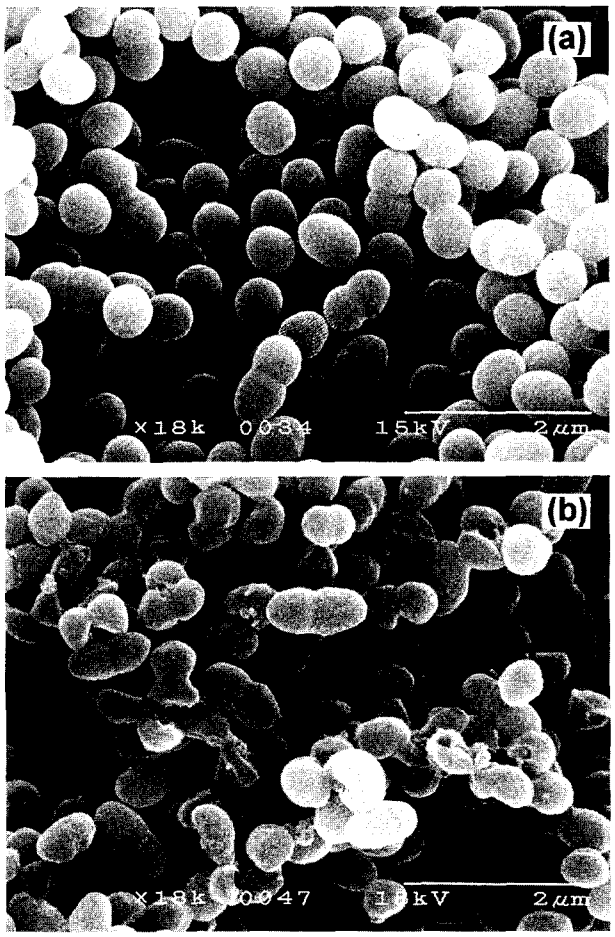

Fig. 3. Scanning electron micrographs of *Leuconostoc mesenteroides* ME6 after nisin treatment at a concentration of 100 IU/ml.
(a) Untreated cells. (b) Cells after 10 h of nisin treatment.

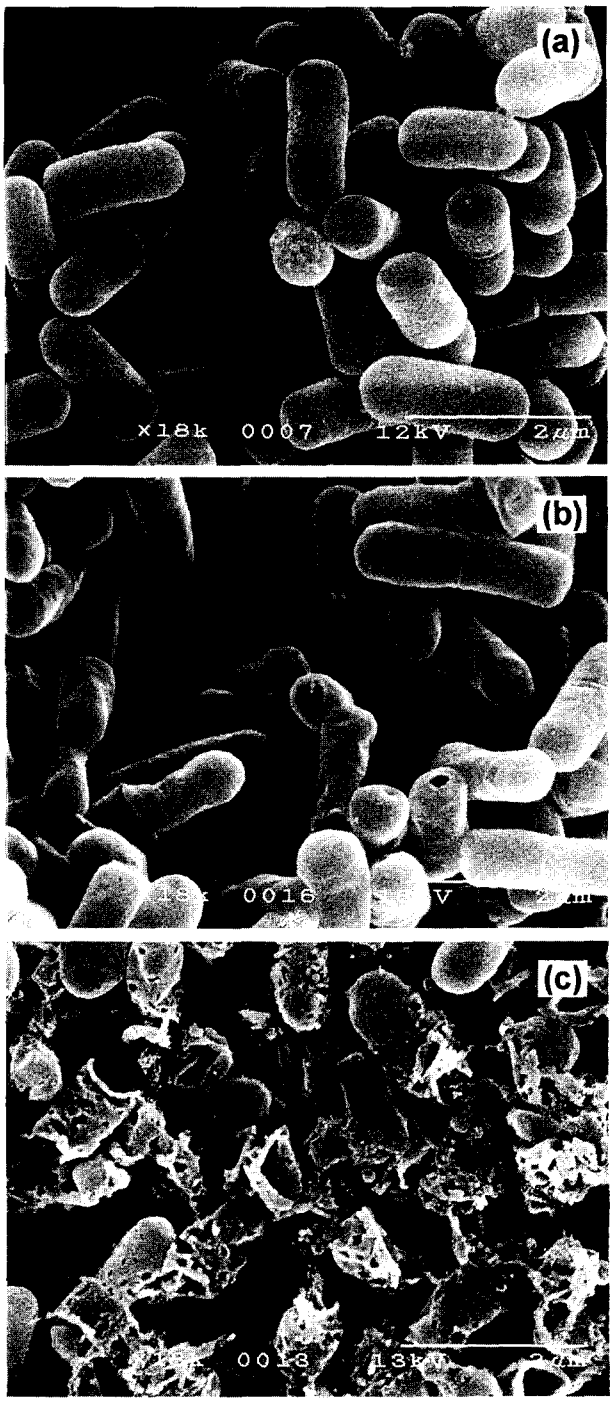

Fig. 4. Scanning electron micrographs of *Lactobacillus plantarum* MD15 after nisin treatment at a concentration of 100 IU/ml.
(a) Untreated cells. (b) Cells after 1 h of nisin treatment. (c) Cells after 4 h of nisin treatment.

ionophore [12, 27, 29]. Recent studies using *Listeria monocytogenes* have elucidated the mechanism [1, 6]. Relatively few studies of cell wall destruction using nisin have been reported [22, 26]. In Staphylococci, an autolytic system which could be induced by nisin has been reported [2, 3]. At present, it is not clear if a similar system exists in nisin-sensitive LAB strains. In this study, we present the first electron micrograph image (Fig. 3, Fig. 4) of a cell surface being destroyed by nisin, thus providing additional evidence for the lethal effect of nisin on LAB.

Effect of Nisin on the Fermentation of Kimchi

To investigate the influence of nisin on the fermentation of kimchi, nisin was incorporated into its preparation at a concentration of 100 IU/ml (Fig. 5). Mul-kimchi, a type of kimchi with water added, was prepared from 250 g of oriental radish, 10 g of green onion, 3 g of garlic, and 2 g of ginger. The oriental radish was sliced into ca. 2.0 × 2.0 × 0.4 cm pieces and the green onion was cut into 1 cm pieces. The garlic and the ginger were sliced into small, thin pieces. All the ingredients were added to 750 ml of brine containing 3% (w/v) NaCl and 100 IU/ml of nisin. The mixture was placed into a glass jar, capped, and incubated at 20°C. The total population of LAB was counted by the spread plate counting method on an MRS agar plate. The pH of the kimchi brine was measured by using a combination electrode and pH meter. Titrable acidity was determined by titration with 0.1 N standard NaOH to pH 8.3 and calculated on the basis of lactic acid.

Normally, the total LAB count increases slowly and attains a maximum level of 10^7 CFU/ml in two days.

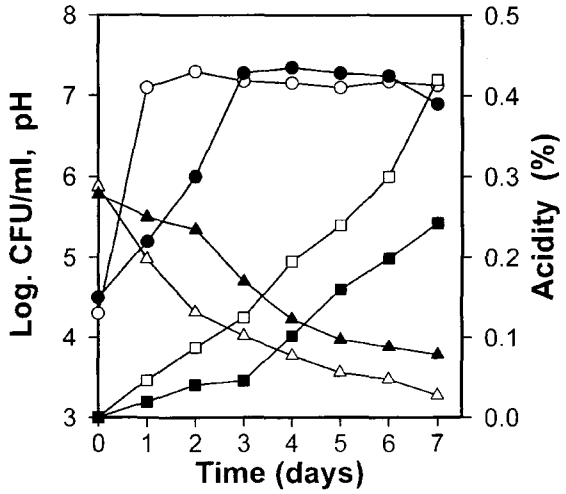

Fig. 5. The effect of nisin on the growth of lactic acid bacteria (●), pH (▲), and acidity (■) in kimchi fermentation.
Nisin was included in the preparation of kimchi at a concentration of 100 IU/ml and fermented at 20°C. Open symbols indicate the control without nisin.

The optimum ripening of kimchi for consumption corresponds to the period when its pH is approximately 4 to 5 [16, 19]. Our results showed that this pH occurred after one to three days of fermentation in controls, while in kimchi containing nisin it occurred after two to five days of fermentation. The results demonstrated that kimchi fermentation could be retarded by the addition of nisin, thus prolonging the period of ripening and delaying attainment of the optimum state.

In conclusion, these results suggest several potential applications for nisin in commercial kimchi production: controlling lactic fermentation, delaying the fermentation rate in the initial stage and/or prolonging the optimum period of ripening. Nisin-resistant strains of *Leu. mesenteroides* could be used in the presence of nisin to improve the orgarnoleptic quality of kimchi. To our knowledge, this paper is the first to demonstrate the process of rapid disruption of the cell wall of Lactobacilli by nisin, through the formation of pores on the cell surface. Though additional data are needed, it appears that, in at least some sensitive LAB strains, cell wall lysis may be an important function of nisin.

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