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Kimchi probiotic bacteria contribute to reduced amounts of *N*-nitrosodimethylamine in lactic acid bacteria-fortified kimchi

Sang-Hyun Kim [a, 1], Sung Hyun Kim [b, 1], Kyung Hun Kang [c], Sanghyun Lee [d], Seoung Ju Kim [e], Jeong Gyun Kim [c], Mi Ja Chung [e, *]

[a] Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju 52828, Republic of Korea
[b] World Institute of Kimchi, Gwangju 61755, Republic of Korea
[c] Department of Seafood Science and Technology, Institute of Marine Industry, Gyeongsang National University, Tongyeong 53064, Republic of Korea
[d] Department of Integrative Plant Science, Chung-Ang University, Anseong 17546, Republic of Korea
[e] Department of Food Science and Nutrition, Gwangju University, Gwangju 61743, Republic of Korea

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ABSTRACT

The role of kimchi probiotic bacteria in reducing the amounts of *N*-nitrosodimethylamine (NDMA) and its precursors occurring in the kimchi-making process and digestion was assessed using *Leuconostoc carnosum* (LEC), *Leuconostoc mesenteroides* (LEM), *Lactobacillus plantarum* (LP), and *Lactobacillus sakei* (LS) grown in MRS broth containing NDMA or its precursors. The results showed that the four bacteria could directly deplete NDMA and nitrite levels in the MRS broth, and this effect was more pronounced in the presence of LP and LS than in LEC and LEM. The concentration of NDMA and its precursors (nitrite, dimethylamine, and biogenic amine) were significantly reduced in bacteria-fortified kimchi compared with the control kimchi, the extent of which depended on the respective bacterial load. Endogenous formation of NDMA by precursors in bacteria-fortified kimchi was demonstrated under simulated gastric digestion. Following digestion, the bacteria-fortified kimchi inhibited NDMA formation. These results suggest that probiotic bacteria may cause a significant decrease in NDMA occurring in kimchi, possibly by direct degradation and inhibition of NDMA formation. Therefore, such lactic acid bacteria could be used in the kimchi-making process to reduce NDMA levels, with an emphasis on LP as it exerted a greater reduction in NDMA concentration in the bacteria-fortified kimchi.

1. Introduction

Volatile nitrosamines (NAs), including *N*-nitrosodimethylamine (NDMA), *N*-nitrosopiperidine, are genotoxic and classified as human carcinogens (Chung, Lee, & Sung, 2002). NDMA has most commonly been detected in food products and is generally formed through reactions between secondary amines and nitrite under certain conditions (Chung et al., 2002). NDMA is also formed during digestion of foods that contain precursors of NDMA such as nitrite, nitrate, and amines (Choi, Chung, Lee, Shin, & Sung, 2007). Biogenic acids, such as putrescine and cadaverine, are converted into secondary amines, which react with nitrite to form NAs (Bulushi, Poole, Deeth, & Dykes, 2009). Kimchi is the one of the foods that has the potential to generate NAs.

Kimchi is a traditional fermented Korean side dish made up of vegetables. Chinese cabbage (*beachu*) is the main ingredient, and it contains high levels of nitrate and nitrite. Common sub-ingredients added during preparation are salted and fermented seafood, such as shrimps and anchovies, which contain high levels of amine, such as dimethylamine (DMA) and biogenic amines. Thus, kimchi-derived nitrite, nitrate, and amines are associated with the occurrence of NAs during kimchi preparation, fermentation, and digestion. However, the kimchi seasoning mixture (green onion, red pepper powder, garlic, and ginger) including phenolic compounds and allyl sulfur compounds, which are potent blocking agents of NAs formation (Chung et al., 2002) may inhibit nitrosamine

Abbreviations: LAB, lactic acid bacteria; *L.*, *Lactobacillus*; *Le.*, *Leuconostoc*; NAs, *N*-nitrosamines; NDMA, *N*-nitrosodimethylamine; NDPA, *N*-nitrosodipropylamine; TCA, trichloroacetic acid.

* Corresponding author. Department of Food Science and Nutrition, College of Health, Welfare and Education, Gwangju University, 277 Hyodeok-ro, Nam-gu, Gwangju 61743, Republic of Korea.
E-mail address: mijachung@gwangju.ac.kr (M.J. Chung).

[1] The first two authors contributed equally to this work and are considered co-first authors.

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formation in kimchi. The lactic acid bacteria (LAB) in kimchi may also restrain NAs formation.

Kimchi, made from Chinese cabbage, green onion, red pepper powder, garlic, ginger, and salted and fermented seafood, are fermented by a variety of microorganisms such as LAB. *Leuconostoc* (*Le*). *mesenteroides* is the predominant strains during the early stage of kimchi fermentation. *Lactobacillus* (*L.*) *plantarum* dominates during the middle and later stages of fermentation, and *L. sakei* becomes predominant later stages of fermentation (Cho et al., 2009). Hence, the major LAB contributing to kimchi fermentation includes *Le. mesenteroides*, *L. plantarum,* and *L. sakei*. *Le. carnosum* is found during the early stage of kimchi fermentation but is not detectable later (Cho et al., 2009). The *Le. carnosum*, *Le. mesenteroides*, *L. plantarum,* and *L. sakei* were used to produce the bacteria-fortified kimchi in this study.

Although previous studies showed that LAB degraded NDMA (Kim et al., 2017; Nowak, Kuberski, & Libudzisz, 2014), there have been no studies indicating that the LAB such as *Le. mesenteroides*, *L. plantarum,* and *Le. carnosum* contributing to kimchi fermentation destroy NDMA. The inhibitory effect of LAB on NDMA formation during kimchi digestion and under reaction conditions of nitrite and DMA have not been reported yet. The aim of the current study was to investigate the inhibitory effects of LAB (*Le. carnosum*, *Le. mesenteroides*, *L. plantarum, L. sakei*) on the formation of NDMA and its precursors in a LAB culture system and under real conditions during kimchi fermentation and digestion. In addition, we investigated direct NDMA degradation and nitrite scavenger activity of LAB during LAB culture in MRS broth containing NDMA or $NaNO_2$.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Le. carnosum (KCTC 3525), *Le. mesenteroides* (KCTC 3530), *L. plantarum* (KCTC 3104), and *L. sakei* (KCTC 3603) were obtained from the Korean Collection of Type Cultures (KCTC, Daejeon, Korea). All LAB strains were cultured overnight in MRS broth (#288130; Difco, Sparks, MD, USA).

2.2. Bacterial counts for kimchi seasoning mixture preparation

For bacterial enumeration, the optical density (OD) of the bacterial cultures was adjusted ($A_{600} = 0.5$). Tenfold serial dilutions of the OD-adjusted cultures were prepared with PBS and then 0.1 mL each of the diluted bacterial suspensions were plated onto MRS agar plates, in triplicate, and incubated at 37 °C (*L. plantarum* and *L. sakei*) or 25 °C (*Le. mesenteroides* and *Le. carnosum*) for 24 h. The resulting bacterial counts, recorded as log colony-forming units (CFU) per milliliter, were used to calculate *Le. carnosum*, *Le. mesenteroides*, *L. plantarum,* and *L. sakei* numbers required for preparing kimchi seasoning mixtures with LAB.

2.3. Assessment of NDMA degradation activity of LAB

NDMA degradation was assessed by quantifying residual NDMA after bacterial culturing in NDMA-containing MRS broth. The LAB strains were inoculated at 10^7 CFU/mL in 10 mL MRS broth containing 0.2 μg/mL NDMA in each test tube. The two types of the control samples were prepared; the one with MRS broth containing each stain of LAB without NDMA (negative control) and the other with MRS broth containing only NDMA without LAB (positive control). Culture tubes were incubated at 15 °C, with shaking (180 rpm), for 2 days after which NDMA concentrations were determined in each tube.

2.4. LAB culture in MRS broth containing $NaNO_2$ or DMA

Bacterial inoculum samples prepared with freshly cultured LAB strains were added (10^7 CFU/mL each) to culture tubes with 10 mL of MRS broth containing 10 μg/mL $NaNO_2$ or 100 μg/mL DMA. The tubes were then incubated at 15 °C, with shaking (180 rpm), for 2 different culturing periods: 1 day and 2 days to determine nitrite depletion and 2 days to determine DMA depletion.

The two types of the control samples were prepared; the one with MRS broth containing only $NaNO_2$ without LAB (positive control) and the other with MRS broth containing each stain of LAB without $NaNO_2$ (negative control). In addition, the control samples were prepared with MRS broth containing each strain of LAB without DMA (negative control) and with MRS broth containing only DMA without LAB (positive control). The cultures were collected by centrifugation at $3000 \times g$ for 15 min, and the resulting supernatant was used for nitrite and DMA analysis.

2.5. Kimchi preparation

Heads of *Baechu* (Chinese cabbage) grown in Pyeongchang, Korea, were cut in half and soaked in a 10% (w/v) salt solution for 16 h. The soaked Chinese cabbages were washed three times under running tap water and drained for 2 h. Seasoning mixtures were prepared by weight-based mixing of red pepper powder, garlic, ginger, green onion, salted and fermented anchovy, salted and fermented shrimp, sugar, and LAB (Table 1). Each strain of LAB was incorporated into the seasoning mixture at 10^5 CFU/g, 10^7 CFU/g, and 10^9 CFU/g (see subsection 2.3). The seasoning mixture for control kimchi was prepared without LAB strains (Table 1). The control kimchi without LAB contained salted and fermented seafood (anchovy and shrimp). The seasoning mixtures were added to the salted Chinese cabbage and the 5 types of kimchi samples were filled separately in polyethylene bags, fermented at 13.5 °C for 1 day in kimchi refrigerator (RP20H3010HY; Samsung, Seoul, Korea) and then stored together at 4 °C in a standard refrigerator (LRS35LMGLM2; Samsung, Seoul, Korea) for 20 days.

2.6. NDMA determination

Homogenized kimchi (10 g) or 10 mL MRS broth containing LAB, with or without NDMA (or precursors of NAs), was mixed with 1.0 mg/kg NDPA (1 mL, internal standard) and 0.1 M NaOH solution (10 mL) in a 50-mL tube using a high-speed vortex mixer (VM-10; Daihan Scientific Co., Wonju, Korea) for 3 min. The sample mixture was combined with Extrelut NT (12 g) using a high-speed vortex mixer (Daihan Scientific Co.) for 2 min and poured into a glass column. The sample tube was washed twice with 10 mL of the eluent (DCM/hexane, 9:1, v/v) and poured onto the column. An additional 40 mL of the eluent was used to complete the elution. The eluate was evaporated to a volume of 2–3 mL in a 30 °C water bath using a rotary evaporator (N-1200BV, Eyela, Tokyo, Japan).

For the cleanup, the NA-enriched sample was loaded onto a Sep-pak Florisil cartridge preconditioned with 6 mL hexane. The residues in the vial were washed with hexane (1 mL) three times and added to the cartridge for washing. The cartridge was washed with additional hexane (3 mL). Following the washing step, NA was eluted with 6 mL of DCM/hexane (95:5, v/v) solution in a Kuderna-Danish concentrator. The eluate was concentrated under nitrogen gas to a final volume of 1.0 mL, filtered through a 0.22-μm filter, and then analyzed using gas chromatography-tandem mass spectrometry (GC-MS/MS) with GC condition described by Kim et al. (2017).