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Lactic acid bacteria directly degrade *N*-nitrosodimethylamine and increase the nitrite-scavenging ability in kimchi

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ABSTRACT

To investigate whether lactic acid bacteria (LAB) play a role in reducing the concentrations of *N*-nitrosodimethylamine (NDMA) and its precursors during kimchi production, experimental kimchi prepared with added *Lactobacillus sakei*, *Lactobacillus curvatus*, and *Lactobacillus brevis* was periodically monitored for 20 days to analyze the concentrations of NDMA, nitrite, dimethylamine (DMA), nitrate, and biogenic amines. LAB species in MRS broth with and without NDMA or $NaNO_2$ were grown and NDMA and nitrite concentrations studied. The amounts of NDMA, nitrite, DMA, nitrate, and biogenic amines remaining in the LAB-fortified kimchi decreased significantly relative to that of the control kimchi. The effects of *L. sakei* and *L. curvatus* on the reduction of NDMA concentration in kimchi were higher than that of *L. brevis*. These LAB species might be indirectly reducing the amounts of NDMA in LAB-fortified kimchi by inhibiting the formation of NDMA precursors originating from kimchi. Interestingly, LAB were found to directly degrade NDMA during culture in MRS broth containing NDMA.

1. Introduction

Humans can be exposed to *N*-nitrosamines (NAs) from many environmental sources, as well as endogenously. In particular, *N*-nitrosodimethylamine (NDMA), a very toxic NA possessing carcinogenic, mutagenic, and teratogenic potentials, has been most commonly detected in food products, and it is also synthesized *in vivo* by intestinal microbiota (Choi & Valentine, 2002). The long-term effects of exposure to NDMA, even at low doses, include organ damage and neoplastic transformation of cells (Souliotis, van Delft, Steenwinkel, Baan, & Kyrtopoulos, 1998). NDMA is formed from dimethylamine (DMA) and nitrite, its immediate precursors, both *in vitro* and *in vivo*. *In vivo*, this carcinogen is formed by the reaction of nitrite with secondary amines under the acidic conditions of the stomach, potentially leading to digestive cancer (Chung, Lee, & Sung, 2002).

The presence of nitrites in foods is associated with an increased risk of cancer (DellaValle et al., 2013). Approximately 80% of dietary nitrates are derived from vegetable consumption, and high nitrate and nitrite levels are detected in Chinese cabbage and radish (Kang et al., 2016). Nitrate itself is relatively non-toxic, but nitrite production through nitrate reduction by commensal bacteria during food processing or in the mouth and gastrointestinal tract may produce harmful health effects. In humans, nitrate (5–10%) is converted into toxic nitrite by salivary or gastrointestinal reduction reactions; thus, endogenous NAs can primarily be formed after the intake of foods containing secondary amines and nitrite by reduction of nitrate (Chung et al., 2002; Dubrow et al., 2010).

In salted, fermented, smoked, and canned seafood, the action of endogenous enzymes breaks down trimethylamine *N*-oxide into volatile DMA and formaldehyde (Teklemariam, Tessema, &

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Abayneh, 2015). In Korea, salted and fermented seafood (Jeot-gal) is made by adding 20–30% (w/w) salt to shrimp, anchovy, etc., followed by mixing and storage for fermentation. The salted and fermented seafood contains high DMA levels after fermentation (Lee, Lee, & Lee, 2012). In addition, biogenic amines, which are also considered precursors of NA, occur in commercial kimchi and salted and fermented seafood (Lee et al., 2012; Mah, Kim, No, & Hwang, 2004).

Kimchi is a traditional Korean food prepared by fermenting vegetables and added spices for a certain period at ambient temperature (Jeong et al., 2013). Its major ingredients are *Baechu* (Chinese cabbage) and radish, which contain high nitrate and nitrite levels (Kang et al., 2016). Common ingredients of kimchi are salted and fermented seafood such shrimp and anchovy. Thus, kimchi is a food containing high nitrites and amines, which might lead to NDMA formation during manufacture and storage.

Kimchi fermentation is mainly carried out by numerous microorganisms such as lactic acid bacteria (LAB), which are naturally present in the raw materials used. LAB have various benefits such as modulation of immunological parameters, reduction of inflammation, and antimicrobial activity (Kang et al., 2016; Lee et al., 2012). LAB such as *Lactobacillus sakei*, *Lactobacillus curvatus*, and *Lactobacillus brevis* may either initiate or play a part in the fermentation of kimchi. *L. curvatus* and *L. brevis* have been observed during early fermentation and later fermentation, respectively; moreover, *Leuconostoc mesenteroides* dominates in early in fermentation, and *L. sakei* becomes predominant later (Cho et al., 2009).

Nowak, Kuberski, and Libudzisz (2014) reported that probiotic LAB degrade NDMA. However, little is known about the NDMA degradation potential of LAB. Here, *L. sakei*, *L. brevis*, and *L. curvatus* were evaluated for their ability to deplete nitrite and degrade NDMA in MRS broth. The effect of these LAB on NDMA and its precursors (nitrate, nitrite, secondary amines, and biogenic amines) in kimchi during storage has not yet been reported; therefore, this was also examined in this study.

2. Materials and methods

2.1. Chemicals and reagents

NDMA and *N*-nitrosodipropylamine (NDPA) were purchased from Chem Service Inc. (West Chester, PA, USA). DMA, *N*-(1-naphthyl)ethylenediamine dihydrochloride, sodium nitrite ($NaNO_2$), sodium nitrate ($NaNO_3$), sulfanilamide, potassium hexacyanoferrate(II) trihydrate [$K_4(Fe(CN)_6)$], sulfuric acid (H_2SO_4), phosphoric acid (H_3PO_4), zinc sulfate ($ZnSO_4$), carbon disulfide (CS_2), copper(II) sulfate pentahydrate, sodium hydroxide (NaOH), ammonium hydroxide (NH_4OH), sodium sulfate, acetic acid, trichloroacetic acid (TCA), putrescine, spermidine, tyramine, tryptamine, cadaverine, 1,7-diaminoheptane, sodium carbonate, proline, dansyl chloride, HCl, and ethyl ether were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Acetonitrile, dichloromethane (DCM), and chloroform were purchased from Merck Co. (Darmstädt, Germany).

2.2. Bacterial strains and growth conditions

L. sakei (KCTC13416, from Korean kimchi), *L. curvatus* (KCTC3767, from milk), and *L. brevis* (KCTC13094, from Korean kimchi) 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).

2.3. Bacterial counts for preparing kimchi seasoning mixtures

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 each 0.1 mL of the diluted bacterial suspensions was plated on MRS agar plates in triplicate and incubated at 37 °C for 24 h. The resulting bacterial counts, recorded as log colony-forming units (CFU) per milliliter, were used to calculate *L. sakei*, *L. curvatus*, and *L. brevis* numbers required for preparing kimchi seasoning mixtures with LAB.

2.4. Assessment of the NDMA degradation activity of LAB

NDMA degradation was assessed by quantifying residual NDMA after bacterial culturing in NDMA-containing MRS broth.

The LAB species were inoculated at 10^9 CFU/mL into 10 mL MRS broth containing 2 μg/mL or 10 μg/mL NDMA in each test tube. The control samples were prepared with MRS broth containing each strain of LAB without NDMA (negative control) and with MRS broth containing NDMA alone (positive control). The culture tubes were incubated at 15 °C with shaking (180 rpm) for 7 days, after which NDMA concentrations in each tube were determined.

2.5. LAB culture in MRS broth containing $NaNO_2$

Bacterial inoculum prepared with freshly cultured LAB strains was added (each at 10^9 CFU/mL) to the culture tube with 10 mL MRS broth containing 100 μg/mL $NaNO_2$. The tubes were then incubated for 2 different culturing periods (3 or 7 days). The culture tubes were incubated at 15 °C with shaking (180 rpm) as described above. In addition, we tested the incubation temperatures resembling the initial fermentation (13.5 °C) and ripening conditions (−1 °C) required for kimchi preparation. Accordingly, the bacterial incubation temperature was set at −1 °C after initial incubation at 13.5 °C for 2 days.

The control samples were prepared with MRS broth containing each LAB species without $NaNO_2$ (negative control) and with MRS broth containing 100 μg/mL $NaNO_2$ alone (positive control = control). The cultures were collected by centrifugation at 3000×*g* for 15 min, and the resulting supernatant was used for nitrite analysis. Each test was performed in triplicate.

2.6. Kimchi preparation

Heads of *Baechu* (Chinese cabbage) grown in Pyeongchang, Korea, were cut in half and soaked in a solution of 10% (w/v) salt solution for 16 h. The soaked Chinese cabbages were washed thrice 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, water, and LAB (Table 1). Each strain of LAB was incorporated into the seasoning mixture at 10^6 CFU/g (see subsection 2.3). The seasoning mixture for control kimchi was prepared without the LAB species (Table 1). The negative control was kimchi without the LAB and salted and fermented seafood (anchovy and shrimp) containing abundant secondary amines (Lee et al., 2012), which can react with nitrite for NDMA formation (Chung et al., 2002). The seasoning mixtures were added to the salted Chinese cabbage, and the 5 kinds of kimchi samples were filled in polyethylene bags, fermented at 13.5 °C for 2 days, and stored at −1.0 °C in a kimchi refrigerator (RP20H3010HY; Samsung, Seoul, Korea) for 20 days.