SCREENING OF LACTCIC ACID BACTERIA FROM KIMCHI TO DEPLETE NITRITE

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***Abstract*—The aim of this study was to develop a new starter for depletion of nitrite. Our approach started with 35 acid-producing isolates from Kimchi, and then PCR screening was used to identify 26 *Lactobacillus* strains. Nitrite level was measured by using MRS broth added nitrite of concentration 200 μg/ml for 4 days, at 20 and 37℃, respectively. In MRS broth, nitrite was inoculated with 0.1 ml of the selected lactic acid bacteria cultures to give an initial levels of 10^6 cells per ml. Among them, one bacterium that showed a strong depletion activity on nitrite was identified and further characterized, using 16S rRNA sequencing and API 50CHL system. Because this isolate was identified as a *Lactobacillus plantarum*, it was name as *L. plantarum* LKC0502. LKC0502 depleted over 90% of nitrite in 2 and 4 days of growth at 20 and 37℃, respectively. However, the difference between culture temperature (20, 37℃) and sodium chloride concentration (0, 2, 4, 6, 8%) did not shown.**

Index Terms-Lactobacillus plantarum, Nitrite depletion, Lactic acid bacteria, Kimchi,

I. INTRODUCTION

Kimchi is a very popular Korean traditional fermentative food made from such ingredients as red pepper, garlic, ginger, fermented fish sauce, and other spices in salted Chinese cabbage. The fermented ingredients form a harmonious blend of vegetables and spices with organic acids producing the characteristic taste and texture of kimchi. Kimchi is also associated with restoring the balance of the intestinal flora by lactic acid bacteria participating in the fermentation. It is also a source of dietary fiber, vitamins, and minerals, supplying vital nutrients and probiotics by the continuous metabolic action of microbes (Lim, 1993). The use of nitrite and nitrate in the manufacturing of meat products is commonly expressed as “curing”. The verb “to cure” means to correct, restore, and treat. In most countries the use of both substances, usually added as potassium or sodium salts, is limited. Either the ingoing or the residual amounts are regulated by laws (Honikel, 2008). Nitrite, which causes met-hemoglobinemia and becomes an important precursor to *N*-nitrosamines, is produced by the reactions of secondary and tertiary amines and is found in various foods ingested habitually in daily life. Nitrite is a common food additive used for fixing color, improving texture, and inhibiting the growth of *Clostridium botulinum* (Bosch et al., 1995). This paper presents the lactic acid bacteria isolated from kimchi cultured in the lactobacilli MRS broth had an activity to deplete the nitrite.

II. MATERIAL AND METHODS

A. Isolation, identification and culturing of lactic acid bacteria from kimchi

Cultures of lactic acid bacteria were isolated from two commercial samples of packed kimchi. These samples represented products from two different food companies. Lactobacilli MRS agar (Difco) was used for primary isolation of the cultures. Random colonies on countable plates were isolated and purified on BCP agar (Difco). Isolates which were gram-positive, catalase-negative and non-motile were retained and identified using standard procedures (Cavett, 1963). Tests used for identification included carbohydrate fermentations and 16S rRNA sequencing.

B. Depletion of nitrite in broth culture

Test tubes containing 9 ml of MRS broth were prepared. Nitrite was added from filter-sterilized stock solution to give a final concentration of 200 200 μg/ml. sterilized distilled water was added to control tubes. The

tubes were inoculated with 0.1 ml of an 18-h-old culture of the selected strain to obtain an initial cell density of approximately 1 X 10^6 cell per ml. The inoculated tubes were incubated at 5–36°C for 0–10 days under aerobic conditions. The depletion of sodium nitrite was determined by measuring initial and final nitrite concentrations at 538 nm. Initial and final nitrite concentrations were determined using a colorimetric method (Ito et al., 1979). All analyses were done in triplicate.

III. RESULTS AND DISCUSSION

Thirty-five lactic acid bacteria were isolated from Kimchi, and one of these isolates showed effective nitrite-depletion activity in broth culture. The isolate was a Gram-positive strain of a coccus-type cell, and formed creamy, opaque, circular colonies on MRS plates. Isolated strain was confirmed bacteria of gram-positive, catalase-negative and non-motile. The isolate was identified as *L. plantarum* by examination of its metabolic characteristics as shown in Table 1. The 16S rRNA gene sequence (1,402 bp) of the isolate showed 98% homology with that of *L. plantarum* SC56.

Table 1. Carbohydrate utilization profile of the *L. plantarum* LKC0502 isolated, as determined using the API 50 CHL system

Carbohydrate	Reaction	Carbohydrate	Reaction
Glycerol	-	Salicine	+
Erythritol	-	Cellobiose	+
D-Arabinose	-	Maltose	+
L-Arabinose	+	Lactose	+
Ribose	+	Melibiose	+
D-Xylose	-	Saccharose	+
L-Xylose	-	Trehalose	+
Adonitol	-	Inuline	-
b-Methyl-xyloside	-	Melezitose	+
Galactose	+	D-Raffinose	-
D-Glucose	+	Starch	-
D-Fructose	+	Glycogene	-
D-Mannose	+	Xylitol	-
L-Sorbose	-	b-Gentiobiose	+
Inositol	-	D-Turanose	-
Manitol	+	D-Lyxose	-
Sorbitol	+	D-Tagatose	-
a-Methyl-D-mannoside	-	D-Fucose	-
a-Methyl-D-glucoside	+	Gluconate	+
N-Acetyl glucosamine	+	2 keto-gluconate	-
Amygdaline	+	5 keto-gluconate	-
Esculine	+		

+, positive; -, negative

The depletion of nitrite during incubation at 20 and 37°C was increased by the *L. plantarum* LKC0502 isolated from kimchi. LKC0502 depleted over 90% of nitrite in 2 and 4 days of growth at 20 and 37℃, respectively. However, the difference between culture temperature (20, 37℃) and sodium chloride concentration (0, 2, 4, 6, 8%) did not show (Figure 1). Park and Cheigh reported that nitrite increased during the initial stages of kimchi fermentation, and rapidly decreased after 5 days. Lactic acid bacteria to ferment kimchi degraded a great deal of nitrite. With these results, we affirmed that the nitrite decrease during fermentation of kimchi is due to degradation by the lactic acid bacteria. Awort et al. reported that the rapid degradation of nitrite in cabbage at 0 and 20℃ was due to the conversion of nitrite-N to gaseous nitrogen compounds, but according to Dodds and Collins-Thompson, the depletion of nitrite in foods was due to destruction of nitrite by nitrite-depleting bacteria, especially lactic acid bacteria.

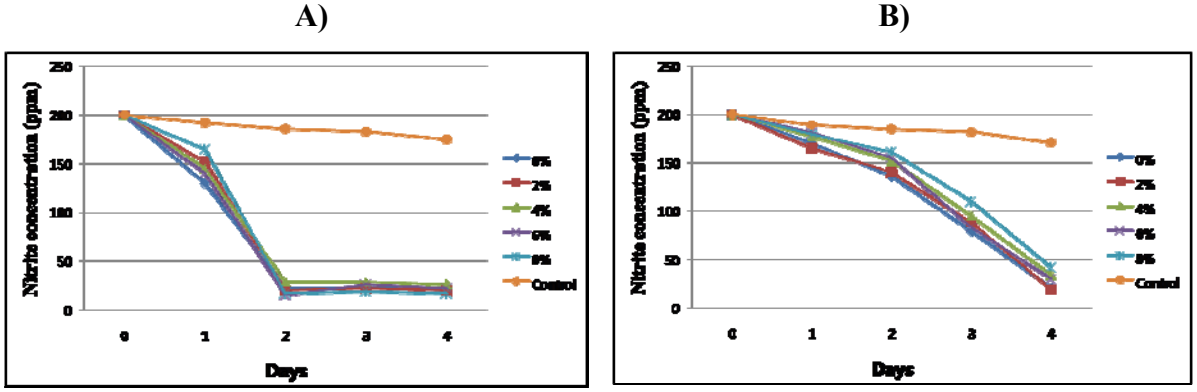

Figure 1. Depletion activity of nitrite by lactic acid bacteria isolated from Korean cabbage kimchi and *L. plantarum*LKC0502 strain during incubation at 20 and 37°C. Nitrite concentration 200 μg/ml, Sodium chloride concentration 0, 2, 4, 6, and 8%, respectively A) Incubated at 37℃, B) Incubated at 20℃

IV. CONCLUSIONS

The results showed that the depletion activity of nitrite to rapidly increased in MRS broth containing nitrite and sodium chloride by the *L. plantarum* LKC0502. However, it is necessary to evaluate the further property of nitrite depletion activity in the production of meat products such as sausage and ham by addition of the *L. plantarum* LKC0502.

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