

Antimicrobial Activities of Lactic Acid Bacteria Isolated from *Mul-Kimchi*

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물김치로부터 분리된 유산균의 항미생물 활성

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Abstract

Five strains out of twenty four strains of lactic acid bacteria isolated from *Mul-Kimchi* showed a resistance in the artificial gastric juice. These lactic acid bacteria were identified as *Lactobacillus plantarum* species. In particular, *lactobacilli* MUL-2, MUL-4 and MUL-18 showed a strong resistance and their viable cell counts of the initial stage were not changed after the 3 h cultivation in the artificial gastric juice. The isolated lactic acid bacteria showed the antimicrobial effect against *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Vibrio vulnificus*, and *Salmonella typhimurium* in the paper disc method.

Key words : *Mul-Kimchi*, lactic acid bacteria, artificial gastric juice, antimicrobial activity

Introduction

Mul-Kimchi is one of the most famous traditional foods in Korea, which is produced by fermentation of various ingredients including Korean cabbage, radish, vegetables, and spices (garlic, ginger, green onion, etc). Its unique tastes are attribute to both of spices and various actions of microorganisms in fermentation period(1). Lactic acid bacteria have been applied to milk production, vegetable fermentation products and bio-agents(2,3). Recently, the lactic acid bacteria have been reevaluated for their nutritional and pharmacological merits, which induced to concern on functional effect of *Kimchi*. These bacteria have been known for nutrition strengthening effects on the human body and physiological effects such as drug metabolism, antitumor, anticancer, gastric secretomotor and radiation resistance (4-6).

Commercially available lactic acid products should be satisfied on safety as well as functional issues including

viability, settlement, inhabitation, antibacterial agent creation, immunity hastening, antigenotoxic activation, pathogenic suppression, properties of organism, bacteriophage resistance and viability in the production process(7-10).

This study conducted on the separation and cultivation of lactic acid bacteria from *Mul-Kimchi*, resistance activity of it in the artificial gastric juice, and pathogenics by the antimicrobial activities.

Materials and Methods

Isolation of lactic acid bacteria from *Mul-Kimchi*

Mul-Kimchi was provided from average households in Mokpo, Korea. The isolated strains were incubated at 37°C for 24 hr in MRS agar (Difco, USA) including 0.02% sodium azide(11) before using. The pH values of *Mul-Kimchi* were measured using a pH meter (Orion Research Inc. 520A, USA).

Resistance against artificial gastric juice

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An artificial gastric juice was created by adding 1% pepsin in the MRS broth adjusted with 1N HCl to pH 2.5 (12). The isolated lactic acid bacteria were centrifuged at 3,000 rpm for 10 min, after sub-culturing at 37°C for 24 hr in the MRS broth to collect strains from the precipitate. After adding the artificial gastric juice warmed to 37°C, the collected strains were cultivated with shaking at 37°C for 3 hr. To strain culture, 0.02% of sodium azid was added and streaked into MRS agar medium to observe the colony.

In order to investigate the survival rate of the strains collected from the artificial gastric juice, the strains were diluted with 0.1% of sterilized peptone, after cultivation in the artificial gastric acid for 3 hr. This diluted solution was inoculated into the MRS agar medium and cultivated at 37°C for 24 hr. Then, a viable bacterial cell colony was counted and compared with that of the state before the cultivation.

Identification of lactic acid bacteria

Morphological, physiological and biochemical properties of the probiotic lactic acid bacteria, which showed a strong resistance against the artificial gastric were determined with Bergey's methods(13-16).

Antimicrobial activities of the isolated lactic acid bacteria

The 5 kinds of test microorganisms (*Listeria monocytogenes* ATCC 19111, *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538, *Vibrio vulnificus* KCTC 2980, and *Salmonella typhimurium* KCTC 2515) were used to observe the antimicrobial activities of the isolated lactic acid bacteria.

A paper disc method was used to measure the activities of the isolated bacteria as following : The isolated bacteria were incubated in MRS broth for 24 hr and the cultivated solution was coated on paper disc. The disc was placed on the overlaid agar plate containing each test strain, and then incubated for 24 hr. The antimicrobial activities were determined by clear zones around the disc(17).

Results and Discussions

Resistance for the artificial gastric juice

A total of 24 lactic acid bacteria were isolated from the ordinary home-made *Kimchi* in *Mokpo, Jeonnam*. Among the total 24 isolated strains, 5 strains presented a resistance in the artificial gastric juice(pH 2.5). In order to investigate the survival levels, 5 kinds of strains with a decent growth were collected from the isolated lactic acid bacteria after shaking

incubation in the artificial gastric juice for 3 hr in order to investigate the survival levels. Consequently, all tested strains presented 10^9 /mL of survival levels in the initial stage of cultivation and $10^7 \sim 10^9$ /mL of the levels after cultivation. These results indicated that the survival levels generally decreased in a 1~2 log cycle, although each strain shows a slight differences in the survival rates of the different strains. The strains of MUL-2, MUL-4 and MUL-18 showed a relatively higher survival levels(53.6~80.0%) after shaking incubation in the artificial gastric juice for 3 h. The survival levels of the isolated lactic acid bacteria were presented in Table 1. The lactic acid strains should have resistance in strong acid to present their various physiological functions in the human body, while most microorganisms are destroyed by the gastric juice(pH 1.4~2.0) in the stomach. In the case of the intake of *Mul-Kimchi*, the lactic acid bacteria which have a resistance to the artificial gastric juices would survive in the stomach, although the survival rate of the strain may decrease depending on pH condition of the stomach(7,9).

Table 1. Viability of lactic acid bacteria isolated from *Mul-Kimchi* in the artificial gastric acid

Lactic acid bacteria	Control (CFU/mL)	Pepsin(pH 2.5) (CFU/mL)	Viability(%)
MUL- 2	2.8×10^9	1.5×10^9	53.6
MUL- 4	3.1×10^8	2.1×10^8	67.7
MUL-18	1.5×10^8	1.2×10^8	80.0
MUL-21	3.8×10^9	2.3×10^6	0.06
MUL-23	2.1×10^9	3.5×10^7	1.7

Identification of the isolated lactic acid bacteria

Table 2 presented the physiological and biochemical properties of the 5 strains which showed a resistance in the artificial gastric. All isolated strains were the non-spore Gram positive bacteria and showed negative properties on catalase and oxidase. Moreover, the strains showed the general characteristics of lactic acid bacteria on morphological, cultivational, and physiological properties. The isolated homo fermentative bacteria, MUL-2, MUL-4 and MUL-18 showed production of acid from gluconate, arabinose and ribose instead of glycerol and rhamnose. Besides, it also showed an indigestive action against glucose, sucrose and arginine (13,14). All of which are general characteristic properties of what *Latobacillus plantarum*(15).

Table 2. Morphological and physiological characteristics of homo-fermentative lactobacilli isolated from *Mul-Kimchi*

Characteristics	Strains No.		
	MUL-2	MUL-4	MUL-18
Cell form	rod	rod	rod
Spherical	-	-	-
Cell arrangement (Pairs and chains)	+	+	+
Gram stain	+	+	+
Motility	-	-	-
Spore formation	-	-	-
Facultative anaerobic	+	+	+
Catalase	-	-	-
Oxidase	-	-	-
Gas from glucose	-	-	-
NH ₃ from arginine	-	-	-
Dextran from sucrose	-	-	-
Isomer of lactic acid	DL	DL	DL
Growth at 15°C	+	+	+
Growth at 45°C	-	-	-
Growth at pH 3.6	+	+	+
Growth at pH 9.6	+	+	+
Growth in 6.5% NaCl	+	+	+
Growth in 10% ethanol	+	+	+
Acid from			
Amygdalin	-	-	-
Arabinose	+	+	+
Arbutin			
Cellobiose	+	+	+
Esculin			
Fructose	+	+	+
Galactose	+	+	+
Glucose	+	+	+
Gluconate	+	+	+
Glycerol	-	-	-
Lactose	+	+	+
Maltose	+	+	+
Mannitol	+	+	+
Mannose	+	+	+
Melezitose	+	+	+
Melibiose	+	+	+
Raffinose	+	+	+
Rhamnose	-	-	-
Ribose	+	+	+
Salicin	+	+	+
Sorbitol	+	+	+
Sorbose	+	+	+
Sucrose	+	+	+
Trehalose	+	+	+
Xylose	-	-	-
Identified as	<i>Lactobacillus plantarum</i>		

Antimicrobial activities of *Lactobacillus plantarum* isolated from *Mul-Kimchi*

The 5 kinds of strains showed a resistance to the artificial

gastric were identified and selected. Table 3 presents the results of the antimicrobial activities of the isolated lactic acid bacteria to pathogenic strains. The identified lactic acid bacteria presented an antimicrobial action to the pathogenic microorganism, although the bacteria would have different antimicrobial properties to the pathogenic strains. Especially, the strain of MUL-18 presented a strong antimicrobial activity against *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, and *Salmonella typhimurium*.

It has been suggested that the intestinal lactic acid bacteria from lactic acid and acetic acid suppressed the contamination or the growth of the acid sensitive intestinal pathogenic bacteria, such as *Staphylococcus*, *Salmonella*, *Shigella*, and *Coliform*. It also prevented diarrhea, or other intestinal diseases. In addition, these strains play a role in increasing the resistance to infections by stimulating the immune system of the intestinal host(18). The lactic acid bacteria isolated in this study was supposed to use as a initiate for the *Mul-Kimchi* production and as probiotics for intestine regulation. These bacteria presented the strong antimicrobial properties for some pathogenic microorganism, and were expected to act in a seminal operation.

Table 3. Antimicrobial activities of the lactic acid bacteria isolated from *Mul-Kimchi*

Microorganism	Isolated lactic acid bacteria ¹⁾		
	MUL-2	MUL-4	MUL-18
<i>Listeria monocytogenes</i> ATCC 19111	+ ²⁾	+	+
<i>Escherichia coli</i> ATCC 10536	-	+	+
<i>Staphylococcus aureus</i> ATCC 6538	+	-	+
<i>Vibrio vulnificus</i> KCTC 2980	-	+	+
<i>Salmonella Typhimurium</i> KCTC 2515	-	+	+

¹⁾Lactic acid bacteria isolated from the different home-made *Mul-Kimchi*.

²⁾+ Positive (Clear zone diameter-above 2 mm), - Negative.

Acknowledgments

This work was supported by the Food Industrial Technology Research Center (RRC) of Mokpo National University, and the Ministry of Commerce, Industry and Energy.

요 약

전남 목포시의 각 가정에서 담근 물김치에서 총 24균주

의 유산균을 분리하였다. 이들 분리 유산균 중 5균주가 인공 위액에서 내성을 나타내었다. 이들 유산균은 *Lactobacillus plantarum*으로 동정하였고, 특히 물김치로부터 동정된 유산균 MUL-2, MUL-4, MUL-18은 인공위액에서 3시간 배양 후에도 초기 생균수에 비해 변화가 거의 없어 가장 강한 내성을 나타내었다. 이들 분리 유산균은 병원성 미생물인 *Listeria monocytogenes*, *Escherichia coli*, *Staphyrococcus aureus*, *Vibrio parahaemolyticus*, *Samonella typhimurium*에 대해 뚜렷한 항미생물활성을 나타내었다.

Reference

- Cheigh, H.C. and Park, K.Y. (1994) Biochemical, microbiological, and nutritional aspects of *Kimchi*. Crit. Rev. Food Sci. Nut., 34, 175-203
- Kim, D.S. (2002) Characteristics of the bacteriocin from *Lactobacillus* sp. Oh-B3. Kor. J. Microbiol. Biotechnol., 30, 184-188
- Ahn, D.K., Han, T.W., Shin, H.Y., Jin, I.N. and Ghim, S.Y. (2003) Diversity and antibacterial activity of lactic acid bacteria isolated from *Kimch*. Kor. J. Microbiol. Biotechnol., 31, 191-196
- Atrih, A., Rekhif, N., Milliere, J.B. and Lefebvre, G. (1993) Detection and characterization of a bacteriocin produced by *Lactobacillus plantarum* C19. Can. J. Microbiol., 39, 1173-1179
- Klaver, F.A.M. and Van der Meer, R. (1993) The assumed assimilation of cholesterol *Lactobacilli* and *Bifidobacterium bifidium* is due to their bile salt conjugating activity. Appl. Environ. Microbiol., 59, 1120-1124
- Rhee, C.H. and Park, H.D. (1999) Isolation and characterization of lactic acid bacteria producing antimutagenic substance Korean *Kimchi* Kor. J. Microbiol. Biotechnol., 27, 15-22
- Ahn, Y.T., Kim, Y.H., Jung, E.J., Lim, H.T., Kang, H.J. and Kim, H.U. (1999) Resistance of lactobacilli and bifidobacteria isolated from fermented milk products to low pH and bile acid. Kor. J. Anim. Sci., 41, 335-342
- Cho, M.K., Kim, K., Kim, C.H., Lee, T.K. and Kim, K.Y. (2000) Isolation and characterization of *Lactobacillus fermentum* YL-3 as a poultry probiotic. Kor. J. Appl. Microbiol. Biotechnol., 28, 279-284
- Lee, S.H. and No, M.J. (1997) Viability in artificial gastric and bile juice and antimicrobial activity of some lactic acid bacteria isolated from *Kimchi*. Kor. J. Appl. Microbiol. Biotechnol., 25, 617-622
- Xanthopoulos, V., Litopoulou-Tzanetaki, E. and Tzantakis, N. (2000) Characterization of *Lactobacillus* isolates from infant feces as dietary adjuncts. Food Microbiol., 17, 205-215
- Lee, N.K., Kim, H.W., Cho, S.Y. and Paik, H.D. (2003) Some probiotic properties of some lactic acid bacteria yeasts isolated from Jeot-gal. Kor. J. Microbiol. Biotechnol., 31, 297-300
- Kobayashi, Y., Tohyama, K. and Terashima, T. (1974) Tolerance of the multiple antibiotic resistant strain, *L. casei* PSR 3002, to artificial digestive fluids. Jpn. J. Microbiol. 29, 691-697
- Heinz, S.K. (1984) Gram-positive cocci. In Bergey's Manual of Systematic Bacteriology, Vol 2. Krieg, N.R., Holt, J.G. (ed.) Williams and Wilkins Co. Baltimore, M.D. 999-1103
- Otto, K. and Weiss, N. (1984) Regular, Nonsporulating Gram-positive Rods. In Bergey's Manual of Systematic Bacteriology. Vol 2. Krieg, N.R., Holt, J.G. (ed.) Williams and Wilkins Co., Baltimore, MD 1208-1260
- Stanley, T.W. and Holt, J.G. (1994) Bergey's Manual of Determinative Bacteriology. 9th ed. The Williams and Wilkins Co., Baltimore, MD. 535
- Holzappel, W.H. and Schillinger, U. (1981) The genus *Leuconostoc*. In The Prokaryotes, second ed., Bslows, A., Truper, H.G., Dworkin, M., Harder, W. and Schleifer. K.H.(ed.), Springer-Verlag. Berlin heidelberg 1572-1625
- Beuchat, L.R., and Brackett, R.E. (1990) Inhibitory effects of raw carrots on *Listeria monocytogenes* Appl. Environ. Microbiol., 53, 198-201
- Anand, S.K., Srinirasan, R.A. and Rao, L.K. (1985) Antibacterial activity associated with *Bifidobacterium bifido*. Cultured Dairy Products., 6, 24-28

(접수 2005년 2월 2일, 채택 2005년 5월 20일)