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RESEARCH NOTE

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Potential Probiotic Properties of Lactic Acid Bacteria Isolated from Kimchi

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Abstract Fourteen out of 87 strains of lactic acid bacteria isolated from Kimchi were found to be resistant against the action of artificial gastric and bile juices. In particular, *lactobacilli* KM 3, 7, 28, and 37 showed strong resistance and their viable cell counts at the initial stage remained the same even after 3 hours of cultivation in an artificial gastric juice. However, the survival rates of KM 14, 28, and 64 were found to be significantly enhanced in artificial bile juice. Based on the paper disc method, it was evident that isolated lactic acid bacteria showed antibacterial effect against *Listeria monocytogenes*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Vibrio vulnificus*, and *Salmonella typhimurium*. The isolated lactic acid bacteria were identified as *Lactobacillus plantarum* and *Leuconostoc mesenteroides*.

Key words: Kimchi, lactic acid bacteria, artificial gastric juice, artificial bile, antimicrobial activity

Introduction

Kimchi, one of the most famous traditional foods in Korea, is prepared by the fermentation of various ingredients including Korean cabbage, radish, salted fish (Jeot-gal), vegetables, and spices like garlic, ginger, green onion and red pepper. The unique taste of Kimchi is due to the presence of afore-mentioned ingredients and also due to the fermenting action of various microorganisms during the fermentation period (1). Lactic acid bacteria, the most important microorganism involved in Kimchi fermentation, has been widely employed for the vegetable fermentation and bio-agents (2, 3). Recently, lactic acid bacteria have been revaluated for their nutritional and pharmacological aspects, which have raised attention towards the functional effect of Kimchi. Lactic acid bacteria have been known to impart nutrition fortifying effect in the human body, and they also play physiological roles in mediating drug metabolism, antimutation, anticancer, gastric secretomotor and radiation resistance (4-6).

In recent years, the probiotic effects of lactic acid bacteria have gained interest in terms of their functional aspect. Probiotic, a growth promoting agent, is taken with obtained from living strains to maintain a balance of intestinal microflora by decreasing the growth of coliform bacillus. It has been also expected as a new type of antibacterial substance for resolving some of the complications in the use of antibiotics and the resistance of microorganism to antibiotic substances (7).

For commercial use, probiotics must satisfy safety as well as functional and technical issues, including viability, settlement, inhabitation, antibacterial agent creation, immunity hastening, antigenotoxic activation, pathogenic suppression, properties of organism, bacteriophage resistance and viability during the production process (8-11). Numerous studies have been carried out on the stabilization of gastrointestinal microflora, reduction of saprogenic products, prevention of degenerative disease, activation of immunity, mediation of anticancer activities, lowering of cholesterol, reduction of lactose intolerance, and suppression and prevention of constipation (12-16).

The present study deals with the separation and cultivation of probiotic organisms from Kimchi and on evaluating the resistance of probiotic organism against artificial gastric juice and pathogenics by examining the antibacterial activities to investigate potential utilization of the microorganisms as probiotics.

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Materials and Methods

Isolation of lactic acid bacteria Kimchi was provided from typical households in Mokpo, a city in southern part of Korea. A total of 87 lactic acid bacteria were collected from 5 different fermentation levels from 5 kinds of Kimchi. Prior to use, the isolated strains were incubated at 37°C for 24 hr in MRS agar plates (Difco, USA) containing 0.02% sodium azide (17). The pH values of Kimchi were measured by using a pH meter (520A, Orion Research Inc., USA).

Resistance of the isolated lactic acid bacteria against artificial gastric juice and bile juice An artificial gastric juice was created by adding 1% pepsin to the MRS broth adjusted to pH 2.5 by the addition of 1 N HCl as per the known method (18). The artificial bile juice was produced by adding 10% of sterilized oxgall (Difco, USA) solution to MRS broth medium (pH 2.5) containing 1% pancreatin.

After subculturing in MRS broth at 37°C for 24 hr, the isolated lactic acid bacteria were centrifuged at 3,000 rpm for 10 min, to collect the specific strains from the precipitate. The collected strains were cultivated in the artificial gastric juice or bile juice by shaking at 37°C for 3 hr. The viable bacteria were inoculated into the MRS agar medium and cultivated at 37°C for 24 hr in order to maintain survival characteristics in the juice (19).

Antibacterial properties of the isolated lactic acid bacteria Six kinds of tested microorganisms (*Listeria monocytogenes* ATCC 19111, *Escherichia coli* ATCC 10536, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Vibrio vulnificus* KCTC 2980, and *Salmonella typhimurium* KCTC 2515) were employed to observe the antibacterial activities of the isolated lactic acid bacteria. A paper disc method (20) was used to measure their activities.

Identification of the collected lactic acid bacteria Morphological, physiological and biochemical properties of the probiotic lactic acid bacteria, which exhibited strong resistance to the artificial gastric and bile juice, were determined by following the methods described in Bergey's Manual (23-26).

Results and Discussion

Resistance of the isolated lactic acid bacteria to the artificial gastric juice A total of 87 lactic acid bacteria were isolated from different stages of Kimchi fermentation, such as from the initial stage of acidification (pH 3.0-4.0), from the proper fermentation period to develop best Kimchi taste (pH 4.0-4.5), and from the typical home-made Kimchi in Mokpo and Jeonnam province. Among the isolated strains, 62 strains were found to be resistance against the artificial gastric juice (pH 2.5). The resistant strains were found to be present in all the five fermentation stages, which suggested that the lactic acid bacteria involved in the fermentation of Kimchi might be resistant in the artificial gastric and bile juices. The survival levels of fourteen isolated lactic acid bacteria which showed a relatively decent growth were investigated after shaking incubation in the artificial gastric juice for 3 hr (Table 1).

All of the tested strains exhibited survival levels of 10^9/mL in the initial stage of cultivation and 10^7-10^9/mL after cultivation. These results indicated that the survival levels generally decreased in a 1-2 log cycle, although each strain showed a slight difference in the survival rates. Especially, the strains of KM 3, 7, 28 and 37 showed relatively higher survival levels (56-77%) after shaking incubation in the artificial gastric juice for 3 hr.

Considering the fact that most of the microorganisms are destroyed by the acidic gastric juices (pH 1.4-2.0) in the stomach (21, 22), probiotic strains need to exhibit resistance under strongly acidic conditions to perform their various physiological functions in the human body. During the time of Kimchi intake, the lactic acid bacteria which are resistant to artificial gastric juices would survive in the stomach, although the survival rate of the various strains may decrease depending on the pH condition of the stomach.

Table 2 presents the changes in the viable bacterial cells of the resistance strains in the artificial gastric bile acid. The strains of KM 14, 28, and 64 presented significantly higher survival rates in the artificial bile juice. Probiotics also need to be resistant to the bile juice itself because the strains pass through not only the stomach but also through the duodenum in order to reach the intestinal tract (27). Thus, the resistance of the selected lactic acid bacteria to strong acid and the bile juice should enable them to remain active in the intestine.

Antibacterial activation of the isolated lactic acid bacteria Seven of the tested strains which were resistant to the artificial gastric and bile juices were selected for the antibacterial activation. Table 3 presents the antibacterial activities of the isolated lactic acid bacteria against the pathogenic strains. The selected lactic acid bacteria presented an antibacterial action against the pathogenic microorganisms. Especially, the KM 3 strain presented antibacterial activity against *Listeria monocytogenes*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, and *Salmonella typhimurium*.

It has been suggested that the intestinal lactic acid bacteria produces lactic acid and the acid suppresses the contamination or growth of acid-sensitive intestinal pathogenic bacteria, such as *Staphylococcus*, *Salmonella*, *Shigella*, and coliform, further leading to prevention of diarrhea or other intestinal diseases (28). In addition, the seven different kinds of lactic acid bacteria were also found to be resistant to 0-100 μg/mL of the general level of antibiotics such as niacin, rifampicin, streptomycin and tetracycline (data not shown). Consequently, the lactic acid bacteria isolated in this study were supposed to initiate the Kimchi production and probiotics with subsequent intestinal regulatory action.

Table 1. Viability of lactic acid bacteria isolated from Kimchi in the presence of artificial gastric acid

	Control (CFU/mL)	Pepsin (pH 2.5) (CFU/mL)	Viability (%)	pH of Kimchi origin
KM 3	4.2×10^8	2.6×10^8	61.9	
KM 7	3.7×10^8	2.4×10^8	64.9	3.0~3.5
KM 14	3.3×10^8	2.3×10^8	42.1	
KM 24	2.4×10^9	2.1×10^8	9.2	
KM 28	2.2×10^9	1.7×10^9	77.3	
KM 29	3.6×10^8	3.1×10^6	0.9	3.5~4.0
KM 33	5.5×10^9	1.1×10^9	37.1	
KM 36	1.1×10^9	4.1×10^7	3.7	
KM 37	6.2×10^9	3.5×10^9	56.5	4.0~4.5
KM 42	3.5×10^7	3.2×10^6	9.1	
KM 43	5.2×10^8	3.1×10^6	0.6	
KM 56	3.6×10^8	6.3×10^6	1.8	
KM 64	1.4×10^9	4.1×10^8	29.3	4.5~5.0
KM 87	3.2×10^8	3.7×10^6	1.2	

Table 2. Viability of lactic acid bacteria isolated from Kimchi in the presence of artificial bile juice (oxgall) after the treatment of artificial gastric acid for 2 hr at 37°C

	Artificial gastric acid (CFU/mL)	Artificial bile juice (CFU/mL)	Viability (%)
KM 3	5.4×10^8	5.6×10^8	103.7
KM 7	3.2×10^8	2.8×10^8	87.5
KM 14	2.6×10^8	3.2×10^8	123.1
KM 28	3.1×10^8	3.5×10^8	112.9
KM 33	2.4×10^8	2.3×10^8	95.8
KM 37	2.7×10^8	2.8×10^8	103.7
KM 64	1.9×10^8	2.2×10^8	115.8

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

Microorganism	Isolated lactic acid bacteria [1]						
	KM 3	KM 7	KM 14	KM 28	KM 33	KM 37	KM 64
Listeria monocytogenes ATCC 19111	14.5[2]	12.0	12.5	10.0	9.5	10.0	12.0
Escherichia coli ATCC 10536	12.5	10.5	13.5	10.5	10.5	10.0	12.0
Bacillus subtilis ATCC 6633	14.0	12.5	-	11.5	10.5	11.0	10.5
Staphylococcus aureus ATCC 6538	16.5	-	12.0	11.0	10.0	-	-
Vibrio vulnificus KCTC 2980	14.0	10.5	10.5	-	-	-	12.5
Salmonella typhimurium KCTC 2515	15.0	12.0	12.0	-	10.5	-	-

[1]Lactic acid bacteria isolated from the different home-made Kimchi
[2]Inhibition zone diameter (mm)

Identification of the isolated lactic acid bacteria Table 4 presents the physiological and biochemical properties of the seven strains which were resistant to the artificial gastric and bile juices, as well as the antibacterial properties of the pathogenic strains. All the isolated strains were non-spore, Gram positive bacteria and exhibited negative properties on catalase and oxidase.

Moreover, the strains showed the general characteristics of lactic acid bacteria in terms of morphological, cultivational, and physiological properties. The isolated homo fermentative bacteria, KM 3, 7, 14, 28, and 33 showed the general characteristic properties of *L. plantarum* such as production of acid from gluconate, arabinose and ribose instead of glycerol and rhamnose (23, 24). The KM 37 and 64 strains were suggested to be hetero lactic acid fermentative bacteria, because gas and dextrin were generated from glucose and sucrose, respectively. However, physiological differences were found between the two strains, which produced acids only from sucrose, trehalose, arabinose, and melibiose, and not from rhamnose and xylose (25, 26). It was strongly suggested that these strains were *Leu. mesenteroides* ssp. *mesenteroides* (26).

In conclusion, the 5 kinds of *L. plantarum* and 2 kinds of *Leu. mesenteroides* that were isolated from Kimchi in the present study, were resistant to the action of artificial gastric and bile juices. These bacteria presented high antibacterial properties against some pathogenic micro-

Table 4. Morphological and physiological characteristics of the homo-fermentative and hetero-fermentative lactobacilli isolated from Kimchi

Characteristics	KM 3	KM 7	KM 14	KM 28	KM 33	KM 37	KM 64
Cell form	rod	rod	rod	rod	rod	cocci	cocci
Spherical	-	-	-	-	-	-	-
Cell arrangement	+	+	+	+	+	+	+
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	DL	DL	D(-)	D(-)
Growth at 15	+	+	+	+	+	+	+
Growth at 45	-	-	-	-	-	-	-
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	+	+	+	+	+	-	-

Table 4. Continued

Characteristics	KM 3	KM 7	KM 14	KM 28	KM 33	KM 37	KM 64
Glycerol	-	-	-	-	-	-	-
Lactose	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+
Melezitose	+	+	+	+	+	-	-
Melibiose	+	+	+	+	+	-	-
Raffinose	+	+	+	+	+	+	+
Rhamnose	-	-	-	-	-	-	-
Ribose	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	-	-
Sorbose	+	+	+	+	+	-	-
Sucrose	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+
Xylose	-	-	-	-	-	-	-
Identified as	*Lactobacillus plantarum*					*Leuconostoc mesenteroides*	

organisms. Consequently, it was suggested that lactic acid bacteria could be employed as probiotic agent after confirming their further applicability.

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