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Identification of *Lactobacillus ruminus* SPM0211 Isolated from Healthy Koreans and Its Antimicrobial Activity against Some Pathogens

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The intestinal microbiota are important to the host with regard to resistance they impart against bacterial infections and their involvement in mediating metabolic functions. Lactic acid producing bacteria such as *Lactobacillus* play an important physiological role in these matters. The aim of the present study was to isolate *Lactobacillus* sp. that inhibits enteric pathogens. Initially, 17 isolates from healthy Koreans were collected on *Lactobacillus* selective medium. Resistance of the isolates to antibiotics including rifampicin, streptomycin, clindamycin and vancomycin was measured. One of the isolate was identified as *Lactobacillus ruminus* on the basis of bacterial cell morphology, cultural characteristic and biochemical characteristics, 16S rRNA sequence analysis and PCR-RAPD. Antimicrobial activity of the bacterium against Vancomycin Intermediate Resistant *Staphylococcus aureus* (VISA) and Vancomycin-Resistant *Enterococci* (VRE) was measured. About 10^4 cells of VISA or VRE were mixed with 1, 5, and 9 mL of *L. ruminus* SPM 0211 and the final volume was adjusted to 10 mL with brain heart infusion (BHI) broth. The cell suspension was incubated for 3, 6, 9, and 24 h, serially diluted and then plated on BHI agar plates. As numbers of *L. ruminus* SPM 0211 were increased, viable cell count of VISA and VRE decreased. The strongest antimicrobial activity of SPM 0211 was observed after 9 h incubation in any mixture, almost completely inhibiting the growth of these two bacteria. The results suggest that the freshly isolated *L. ruminus* SPM 0211 may be used as a pro-biotic microbe that prevents the colonization of enteric pathogens and can thereby promote good gastrointestinal health.

Key words: Vancomycin, *Lactobacillus ruminus*, 16S-rRNA sequence, PCR-RAPD, Vancomycin Intermediate resistant, *Staphylococcus aureus*, Vancomycin-resistant, *Enterococci*

INTRODUCTION

Lactobacillus sp. are nonpathogenic, facultative anaerobes or microaerophile and gram positive bacteria (Lee *et al.*, 1999). *Lactic acid bacteria* (LAB) can be divided into 5 genera: *Streptococcus*, *Lactobacillus*, *Leuconostoc*, *Bifidobacteria*, and *Pediococcus* (Yu *et al.*, 2003). The microbe plays important roles in maintaining good human health, specifically with respect to intestinal environment by inhibiting the growth of harmful bacteria by lowering the pH of the intestines, improvement of diarrhea or constipation cases, synthesis of vitamins, lowering the level of blood cholesterol and by functioning as a medicine for intestinal disorders (Breslaw *et al.*, 1973; Mitsuokaet *et al.*, 1990; Modleret *et al.*, 1990; Rhee *et al.*, 2002). LAB wards off disease by suppressing harmful bacteria in the intestines through the propagation of macrophages (Sekine *et al.*, 1985). It contributes greatly in the intestinal regulation due to its specific proteins that can combine strongly to the mucosa and epithelia.

Amongst the other genera, *Lactobacillus* genus produces acidophillin, which has an inhibitory activity against *Shigella*, *Salmonella*, *Staphylococcus*, and harmful diarrhea related bacteria (Yokoyamaet *et al.*, 1979; Mcdonel *et al.*, 1980; Choi *et al.*, 1999).

In case of the long-term use of antibiotics for treatments

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in diseases such as tuberculosis and Hansen's disease, intestinal normal flora are disrupted which results in antibiotic-associated diarrhea. In the case of antibiotic-associated diarrhea, the use of probiotics with therapeutic agents is recommended (Yamashita *et al.*, 1985). In these cases, therapeutic agents should not kill the used probiotic strains. But if the microbe in this probiotic preparation is sensitive to some antibiotics, its therapeutic effect cannot be expected. Therefore, this study is conducted in order to develop antibiotic-resistant *lactic acid bacteria.*

On the other hand, the quantity of antibiotics used in fruit farms and live stock feeds are at risk (Park *et al.*, 2000). The abuse of antibiotics enhances the occurrence of antibiotic-resistant bacteria. The stronger the antibiotic with bactericidal activity to the antibiotic resistant bacteria is developed, the faster the occurrence of new antibiotic-resistant bacteria arises. The first report on *Staphylococcus aureus* with intermediate-level resistance to vancomycin (VISA) broke out in Korea in 1997, raising the threat of incurable *staphylococcus* disease. On the other hand, the first vancomycin-resistant *S. aureus* (VRSA) was reported in Michigan in June 2002 (Fridkin *et al.*, 2001; Olivier *et al.*, 2002; Catherine *et al.* 2003). The recent appearance of antibiotic resistant-bacteria to various kinds of antibiotics is a big threat to the human population. Actually, quinolone resistant *salmonella* were discovered in a rooster feeding on feeds along with quinolone antibiotics. In most of the cases, a person who eats *salmonella* infected chicken could be a victim for *salmonella* food poisoning.

Also, MRSA (Methicillin-Resistant *Staphylococcus aureus*) and VRE (Vancomycin-Resistant *Enterococci*) are one of the causes for nosocomial infections which resulted in high mortality rates (Park *et al.* 2002; Kim *et al.*, 2003). The rapid spread of antibiotic resistant bacteria is increasing and its becoming more varied (Song *et al.,* 2002).

To develop antibiotic-resistant probiotic strains, we isolated several *Lactic acid bacteria* (LAB) from fecal samples of healthy Koreans. One of the isolated strains, which possess high resistance to several antibiotics, was identified as *Lactobacillus ruminus* SPM 0211 by 16S-rRNA sequencing. To develop this strain as a probiotic, MIC values of several antibiotics including vancomycin and growth inhibitory activity against several harmful bacteria were examined.

MATERIALS AND METHODS

Bacterial strains and media

Lactobacillus ruminus SPM0211, which was isolated in this study, was used as a probiotic strain and VISA # 11-13, and VRE #14 which were stored in our lab (Lee *et al.*, 2002) were used as pathogenic strains. *Lactobacillus fermentum* KCTC 3112, *Lactobacillus plantarum* KCTC 1048, *Lactobacillus reuteri* KCTC 3594, *Lactobacillus casei* KCTC 2180 and *Lactobacillus ruminus* KCTC 3601 purchased from KCTC were used as reference strains. General anaerobic medium (GAM) and Blood-Liver (BL) medium were purchased from Nissui Pharm. Co. Ltd., (Japan). The other reagents were of analytical grade.

Isolation and identification of *Lactobacillus* sp.

Fecal samples of 17 healthy Koreans (20-30 years old) were collected by BBL's anaerobic sample collection and transportation in order to maintain anaerobic condition and were used within 24 h. Fecal samples were serially diluted 10 fold from 10^{-1} to 10^{-8} and 100 μL of appropriate dilutions was spread onto the selective BL (Blood-Liver, Nissue) agar (Kang *et al.*, 2004). After 48 h of incubation in anaerobic condition (Bactron Anaerobic Chamber, Sheldon Manufacturing Inc., U.S.A.); brown, milky, and light brown colonies with 0.5-2 mm in diameter were selected for further studies (Bae *et al.*, 2004). After the isolation of pure culture, microscopic observation was followed to identify the isolated colonies. To identify the isolated *Lactobacillus* sp., at the level of species, 16S-rRNA sequencing was preformed by Bioleaders (Daejeon, Korea) (Matsuki *et al.*, 1998; Mullie *et al.*, 2003; Venema *et al.*, 2003).

PCR-RAPD analysis of *Lactobacillus ruminus* SPM 0211

Genomic DNA was extracted by using DNA extraction kit with some modifications (Promega, Co. Ltd., Madison, America). Four primers of 20 mer were synthesized by Seoulin Bioscience Institute, Seoulin Bioscience, Co. Ltd., Seoul, Korea (Table I). PCR reactions were carried out in 50 μL reaction mixtures containing the DNA template (20 to 50 ng of purified DNA), 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM of each of the dNTP's, 200 ng primer and 2.5 unit *Taq* DNA polymerase (Promega, Co. Ltd., Madison, U.S.A.) (Ha *et al.*, 1999; Yu *et al.*, 2003; Ha *et al.* 2004). The reaction mixture was overlaid with a thin layer of sterile mineral oil to prevent evaporation. DNA amplification was performed in a programmable DNA thermal cycler (Perkin-Elmer Cetus, Inc., U.S.A.). The cycling parameters used were initial denatura-

Table I. Description of oligonucleotide sequences of 4 microbial uniprimers

Primers	Sequence (5'-3')
Microbial Uniprimer1	5'-ATCCAAGGTCCGAGACAACC-3'
Microbial Uniprimer2	5'-CCCAGCAACTGATCGCACAC-3'
Microbial Uniprimer3	5'-GTGTGCGATCAGTTGCTGGG-3'
Microbial Uniprimer4	5'-AGGACTCGATAACAGGCTCC-3'

tion at 94°C, annealing for 1 min at 55°C, and extension for 2 min at 72°C (Kang *et al.*, 2002). After the last cycle, the PCR tubes were incubated at 72°C for 7 min and were further held at 4°C. Amplified products were electrophoresed on a 2% agarose gel in TAE buffer and DNA bands were visualized by staining with ethidium bromide. Amplication reaction was performed twice to check the consistency and reproducibility of the method.

Measurement of minimum inhibitory concentrations (MICs)

Minimum Inhibitory Concentration of several antituberculosis agents against *L. ruminus* SPM0211 and reference *Lactobacillus* sp. strains were tested. The following antibiotics were tested; vancomycin (Eli Lilly Benelux, Brussels, Belgium), clindamycin (Yuhan), ciprofloxacin (Ildong), isoniazid (Yuhan), ethambutol (Ildong), rifampicin (Chongkundang), streptomycin (Chongkundang), and cycloserine (Donga). The minimum inhibitory concentrations (MICs) of various antibiotics was determined by the agar dilution method based on the guidelines made by National Committee for Clinical Laboratory Standards with some modifications (The inoculum size was 10^7 cells/mL) (NCCLS) (Chun *et al.*, 1994; National Committee for Clinical Labortory Standards, 1988; Kim *et al.* 2002; Jones *et al.*, 2005).

Inhibitory effect of *Lactobacillus ruminus* SPM 0211 on the growth of vancomycin intermediate resistant *Staphylo-coccus aureus* (VISA) and vancomycin-resistant *Enterococci* (VRE)

Inhibitory effect of *Lactobacillus ruminus* SPM 0211 on the harmful pathogens was tested. *Lactobacillus ruminus* SPM 0211 was cultured on GAM broth (Nissue), and VISA and VRE were incubated in BHI broth for 18 h at 37°C. The co-cultures of *L. ruminus* with VISA or VRE were achieved as follows; culture broth of VISA (Clinical isolate of VISA) was diluted to 10^4 cells/mL and mixed with GAM broth containing 1, 5, and 9 mL of *L. ruminus* SPM0211 culture. After 3, 6, 9, and 24 h of incubation, appropriate volumes of cultures were taken, plated onto BHI agar plates and viable cells were counted.

RESULTS

Isolation and identification of *Lactobacillus ruminus* SPM 0211

According to the 16s rRNA sequencing, SPM 0211 shows 99% homologous characteristics to that of *Lactobacillus ruminus*. We designated this strain as *L. ruminus* SPM 0211 (Fig. 1).

PCR-RAPD analysis of isolated *L. ruminus* SPM 0211

L. ruminus SPM0211 and 5 types of strains of *Lactobacillus* were analyzed with four uniprimers and the RAPD profiles were compared. As shown in Fig. 2 to Fig. 5, *Lactobacillus ruminus* SPM 0211 exhibited absolutely different band patterns when compared with the other *Lactobacillus* sp. With PCR-RAPD using Microbial Uniprimer 3, we found the distinguishing band of about 350 bp (Fig. 4) and from PCR-RAPD with Microbial Uniprimer 4, we found distinguishing band of about 650 bp (Fig. 5). According to these results *L. ruminus* SPM0211 showed different PCR profiles when compared with other *Lactobacillus* sp..

Measurement of minimum inhibitory concentrations

The MICs of several antibiotics, including vancomycin against these 6 strains were tested. The high level resistance pattern to vancomycin means that these 6

(a) gaattcactagtgsttagaaaggaggtgatccagccgcaggttctcctacggctacctgttacgacttcaccccaat
catctgtcccaccttaggcggctggctccaaaaggttaccccaccgacttcgggtgttacaaactctcatggtgtgac
gggcggtgtgtacaaggcccgggaacgtattcaccgcgacatgctgattcgcgattactagcgattccgacttcatgc
aggcgagttgcagcctgcaatccgaactgagaacggctttaagagattagcttgccctcgcgagttagcgactcgttg
taccgtccattgtagcacgtgtgtagcccaggtcataaggggcatgatgatttgacgtcatccccaccttcctccggt
ttgtcaccggcagtctcgccagagtgcccaacttaatgatgg

(b) gaattagattagagtttgatcctggctcaggacgaacgctggcggcgtgcctaatacatgcaagtcgaacgaagcttt
ctttcaccgaatgcttgcattcaccgaaagaagcttagtggcgaacgggtgagtaacacgtaggcaacctgcccaaaa
gaggggataacacttggaaacaggtgctaataccgcataaccatgaacaccgcatgatgttcatgtaaaagacggct
tttgctgtcacttttggatgggcctgcggcgtattaacttgttggtggggtaacggcctaccaaggtgatgatacgta
gccgaactgagaggttgatcggccacattgggactgagacacggcccaaactcctacgggaggcagcagtagggaatc
ttccacaatggacgaaagtctgatggagcaacgccgcgtgaatg

Fig. 1. 16s-rRNA sequence analysis of *Lactobacillus ruminus* SPM 0211. (a) *Lactobacillus ruminus* SMP 0211-16S rRNA gene 3'-term, (b) *Lactobacillus ruminus* SMP 0211-16S rRNA gene 5'-term

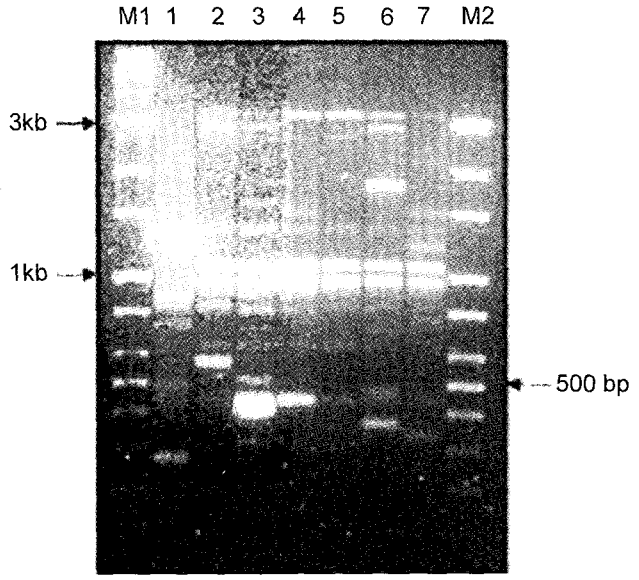

Fig. 2. Genomic fingerprinting patterns obtained by using Microbial Uniprimer 2. lane M1: 1kb ladder, lane 1: *Lactobacillus plantarum* KCTC 1048, lane 2: *Lactobacillus casei* KCTC 2180, lane 3: *Lactobacillus fermentum* KCTC 3112, lane 4: *Lactobacillus suebicus* KCTC 3549, lane 5: *Lactobacillus reuteri* KCTC 3594, lane 6: *Lactobacillus ruminus* KCTC 3601, lane 7: *Lactobacillus ruminus* SPM0211, lane M2: 100bp ladder.

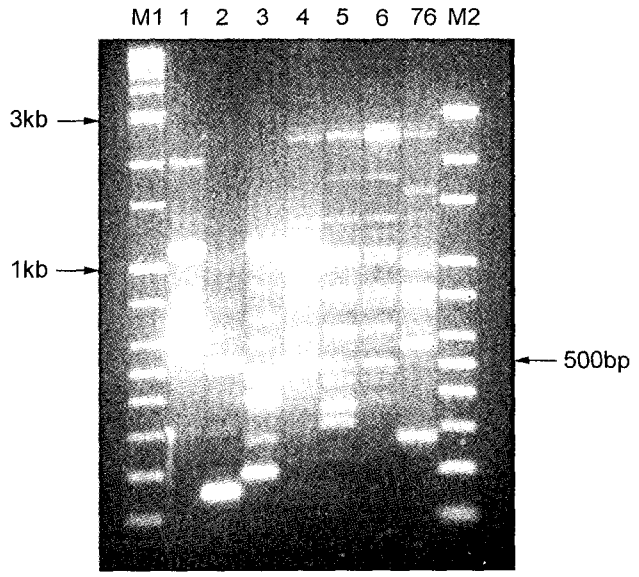

Fig. 3. Genomic fingerprinting patterns obtained by using Microbial Uniprimer 3. lane M1: 1kb ladder, lane 1: *Lactobacillus plantarum* KCTC 1048, lane 2: *Lactobacillus casei* KCTC 2180, lane 3: *Lactobacillus fermentum* KCTC 3112, lane 4: *Lactobacillus suebicus* KCTC 3549, lane 5: *Lactobacillus reuteri* KCTC 3594, lane 6: *Lactobacillus ruminus* KCTC 3601, lane 7: *Lactobacillus ruminus* SPM0211, lane M2: 100 bp ladder.

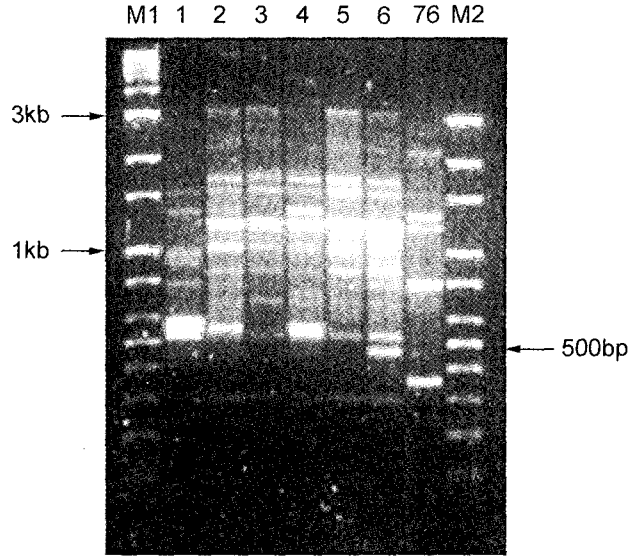

Fig. 4. Genomic fingerprinting patterns obtained by using Microbial Uniprimer 4. lane M1: 1kb ladder, lane 1: *Lactobacillus plantarum* KCTC 1048, lane 2: *Lactobacillus casei* KCTC 2180, lane 3: *Lactobacillus fermentum* KCTC 3112, lane 4: *Lactobacillus suebicus* KCTC 3549 lane 5: *Lactobacillus reuteri* KCTC 3594, lane 6: *Lactobacillus ruminus* KCTC 3601, lane 7: *Lactobacillus ruminus* SPM0211 and lane M2: 100 bp ladder.

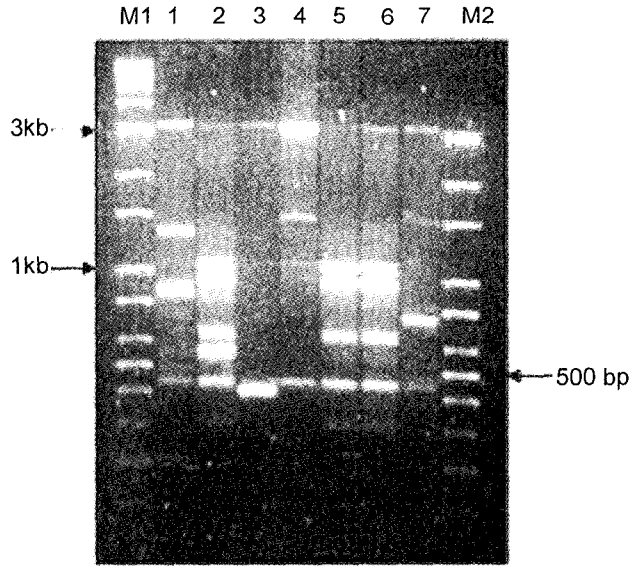

Fig. 5. Genomic fingerprinting patterns obtained by using Microbial Uniprimer 5. lane M1: 1kb ladder, lane 1: *Lactobacillus plantarum* KCTC 1048, lane 2: *Lactobacillus casei* KCTC 2180, lane 3: *Lactobacillus fermentum* KCTC 3112, lane 4: *Lactobacillus suebicus* KCTC 3549, lane 5: *Lactobacillus reuteri* KCTC 3594, lane 6: *Lactobacillus ruminus* KCTC 3601 lane 7: *Lactobacillus ruminus* SPM0211 and lane M2: 100 bp ladder.

strians were typical *Lactobacillus* sp. (Woodford *et al.*, 1995). All of the tested strains showed high resistance to INAH, Ethambutol and cycloseirn (>100 μg/mL) and the MIC values of streptomycin against 6 strains were 3.12 - >100 μg/mL. Also all of the tested strains were sensitive to rifampicin and ciprofloxacin (Table II).

Table II. Minimum inhibitory concentrations of several antibiotics against *Lactobacillus* sp.

Strains	MIC(μg/mL)							
	VAN	CLIN	CIF	INAH	ETM	RIF	SM	CYCS
Lactobacillus fermentum KCTC 3112	>100	1.6	25	>100	>100	0.8	25	>100
Lactobacillus plantarum KCTC 1048	>100	100	3.12	>100	>100	0.8	50	>100
Lactobacillus reuteri KCTC 3594	>100	3.12	25	>100	>100	0.1	3.12	>100
Lactobacillus casei KCTC 2180	>100	50	3.12	>100	>100	0.8	25	>100
Lactobacillus ruminus KCTC 3601	>100	50	3.12	>100	>100	0.4	25	>100
Lactobacillus ruminus SPM 0211	>100	25	3.12	>100	>100	6.25	>100	>100

ETM: ethambutol, RIF: rifampicin, SM: streptomycin, CYCS: cycloserine, CLIN: clindamycin, CIF: ciprofloxacin, VAN: vancomycin, INAH: isoniazide

Growth inhibition test of VISA and VISA by *Lactobacillus ruminus* SPM 0211

To test the inhibitory effect of *L. ruminus* on the growth of VISA and VRE, co-cultures of *L. ruminus* with VISA and *L. ruminus* with VRE were performed. When 10^4 cells of VISA were cultured with 5×10^7 cells (5 mL of culture broth) of *L. ruminus* SPM0211 and 9×10^7 cells (9 mL of culture broth) of *L. ruminus* SPM0211, there were no differences in the number of VISA between the initial and the final incubation. This means that the inhibitory effect of *L. ruminus* SPM0211 on the growth of VISA was bacteriostatic. When 9×10^7 cells of *L. ruminus* were cultured with VRE, VRE were completely inhibited by *L. ruminus* after 9 h. Hence, it can be concluded that *L. ruminus* SPM 0211 may be bacteriocidal against VRE (Fig. 6, 7).

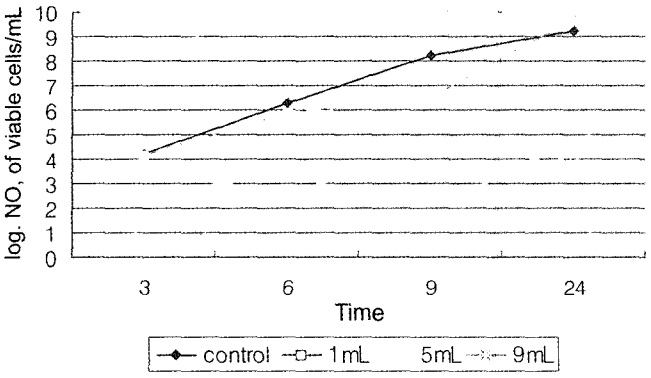

Fig. 6. Growth inhibition of *Vancomycin intermediate Staphylococcus aureus* (VISA) by *Lactobacillus ruminus* SPM 0211. *S. aureus* was incubated with *L. ruminus* SPM 0211 culture broth and growth was measured by viable cell counting.

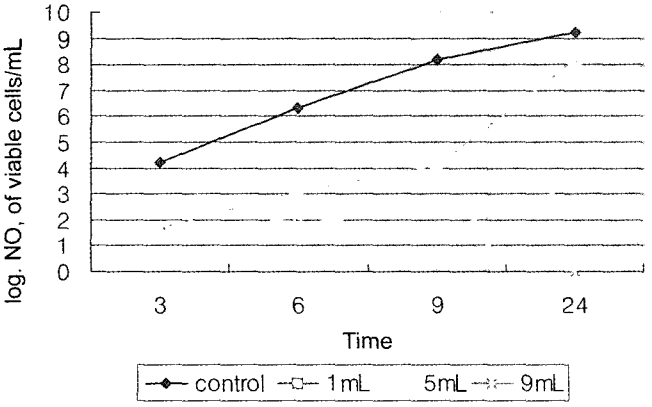

Fig. 7. Growth inhibition of Vancomycin-Resistant *Enterococci* (VRE) by *Lactobacillus ruminus* SPM 0211. Enterococci was incubated with *L. ruminus* SPM 0211 culture broth and growth was measured by viable cell counting.

DISCUSSION

In our present study, the isolated lactic acid bacteria from healthy Koreans showed very good probiotic activity. We have isolated some lactic acid bacteria and one of them was identified as *L. ruminus* by 16S-rRNA sequence analysis. The genetic characteristics of the strains were identified through the PCR-RAPD which enabled us to discriminate the genera and its species. Amongst them, we found out that *Lactobacillus ruminus* SPM 0211, possess the highest level of resistance against several antibiotics. It also exhibited antibiosis against antibiotic-resistant bacteria. The *L. ruminus* SPM 0211 isolated from this study showed high-level of resistance to various antibiotics. All *Lactobacillus* strains were found to grow even in the presence of 100 μg/mL of vancomycin, isoniazide, ethambutol and cycloserine, but showed high sensitivity to rifampicin and ciprofloxacin. Above all, the *L. ruminus* SPM 0211 showed moderate tolerance to rifampicin when compared with other *Lactobacillus* strains. The broad-spectrum resistance of *L. ruminus* SPM0211 against several antituberculosis agents can be good characteristics of this strain to function as probiotics. Long-term use of antibiotics could result in some intestinal disorders and hence probiotic supplements can be used as auxiliary agents.

The genetic characteristic trait of *L. ruminus* was analyzed through the PCR-RAPD analysis. *L. runminus* 0211's RAPD profiles were different from the other *Lactobacillus* type strains as detected in the Microbial

Uniprimer 3, 4. In this aspect, we strongly agreed that *L. ruminus* SPM 0211 had a distinguishing characteristic when compared with the other *Lactobacillus* genus strains.

We also tested inhibitory effect of *L. ruminus* SPM 0211 on the growth of antibiotic-resistant pathogens, like VISA (Vancomycin Intermediate Resistant *Staphylococcus aureus*) and VRE (Vancomycin-Resistant *Enterococcui*). When we co-cultured 10^4 cells of the VRE with 9×10^7 cells of *L. ruminus* SPM 0211, we found that *Lactobacillus ruminus* SPM 0211 showed bacteriostatic activity on the growth of VISA and *Lactobacillus ruminus* SPM 0211 showed bacteriocidal activity on the growth of VRE.

Therefore, we concluded that *Lactobacillus ruminus* SPM0211 may be used as a potent probiotic strain, which inhibits the growth of antibiotic-resistant pathogens like VISA and VRE. It is also believed that *Lactobacillus ruminus* SPM0211 can also improve or help in relieving gastrointestinal disorders.

REFERENCES

Bae, H. G., Kim, S. O., Park, J. S., Kang, B. Y., Choi, S. S., Kang, J. Y., and Ha, N. J., Analysis of Genetic Diversity of *Bifidobacterium* sp. Isolated from Korean Adults Fecal Samples. *Yakhak Hoieji*, 48(1), 20-26 (2004).

Breslaw, E. S., Kleyn D. H., *In vitro* digestibility of protein in yoghurt at various stages of processing. *J. Food Sci.*, 38, 1016 (1973).

Catherine, L. and Henry, F. C., *Staphylococcus aureus* with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. *Antimicrobial Agents and Chemotherapy*, 47(10), 3040-3045 (2003).

Choi, E. H., Kim, H. Y., Kim, Y. H., Kim, W. K., Oh, S. J., and Kim, Y. H., Effects of Selected Oligosaccharides on Fecal Microflora and Lipid Constitution in Rats. *Korean Nutrition society J.*, 32(3), 221-229 (1999).

Committee on Treatment if International Union against Tuberculosis and Lung Disease: Antituberculosis regimens of chemotherapy. *Bull. Int. Union Tuberc. Lung Dis.*, 63, 60 (1988).

David, S. J., Jasmina, D., and Sean, P. G., The resistance of polyvinylpyrrolidone–Iodine–poly(-caprolactone) blends to adherence of *Escherichia coli*. *Biomaterials*, 26(14), 2013-2020 (2005).

Fridkin, S. K., Vancomycin-intermediate and -resistant *Staphylococcus aureus*: What the infectious disease specialist needs to know. *Clinical Infectious Diseases*, 32(1), 108-115 (2001).

Ha, K. H., Characterization of Vibrio parahaemolyticus Isolated from Diarrheal Patients and Environmental Sources by RAPD-PCR. *J. Bacteriol. Virol.*, 34(1), 45-58 (1999).

Ha, N. J., Park, J. S., Yun, J. H., Jang, D. H., Kang, B. Y., Chung, M. J., Kim, S. D., Hong, O. P., and Choi, S. S., Genomic Fingerprinting of *Bifidobacterium* sp. Isolated from the Feces of Healthy Korean Adults using Microbial Microbial Uniprimer Kit. *FASEB J.*, A992 (2004).

Kang, B. Y., Yun, J. H., Chung, M. J., Kim, S. D., Hong, O. P., Kang, J. Y., Choi, S. S., and Ha, N. J., Isolation, Identification and Characterization of potential probiotic *Bifidobacterium* sp. from Healthy Korea Adults. *J. Microbiol.*, 42(3), (2004).

Kim, B. J., Gong, J. Y., Lee, C. S., and Lee, Y. H., Development of Remedy by for the Infectible Disease by Resistant Bacteria; Development of New Antibiotics with Antimicrobial Activity against Resistant Bacteria. *Korea Research Institute Chemical Technology*, 1-207 (2003).

Kim, S. H., Keon, N. H., Kim, J. Y., Lim, J. Y., Bae, W. K., Kim, J. M., Noh, K. J., Hur, J., Jung, W. K., Park, K. T., Lee, J. E., Ra, J. C., and Park, Y. H., Antimicrobial Activity of Natural Product Made by Opuntia ficus-indica var. saboten Against *Salmonella* sp. and *Escherichia coli* O157:H7. *J. Fd. Hyg. Safety*, 17(2), 71-78 (2002)

Lee, J. W., Epidemiological typing of Vancomycin-Resistant Streptococcus equines Isolated from Animal Foods by Microbial Uniprimer Kit™. (2002).

Lee, K. M., Yun, J. H., Choi, S. S., and Ha, N. J., Comparison of environmental growth condition in *Bifidobacterium* sp. parent and It's mutants. *J. Life Sci.*, 12, 113-121 (2004).

Lee, S. H., Park, N. Y., and Choi, W. J., Changes of the Lactic Acid Bacteria and Selective Inhibitive Substances against Homo and Hetero *Lactic Acid bacteria* Isolated from Kimchi. *Kor. J. Appl. Microbiol. Biotechnol.*, 27(5), 410-414 (1999).

Matsuki, T., Watanabe, K., Tanaka, R., and Oyaizu, H., Rapid identification of human intestinal *bifidobacteria* by 16*S* rRNA-targeted species- and group-specific primers. *FEMS Microbiology Lett.*, 167, 113-121 (1998)

Mcdonel, J. L., Clostridium perfringens toxins (Type A, B, C, D, E). *Pharmacol. Ther.*, 10, 617-655 (1980).

Mitsuoka, T., Bifidobacteria and their role in human health. *J. Ind. Microbiol.*, 6, 263-268 (1990).

Modler, H. W., Mckeller, R. C., and Yaguchi, M., Bifidobacteria and bifidogenic factors. *Can. Inst. Food Sci. Technol. J.*, 23, 29-41 (1990).

Mullie, C., Odou, M. F., Singer, E., Romond, M. B., and Izard, D., Multiplex PCR using 16S rRNA gene-targeted primers for the identification of *bifidobacteria* from human origin. *FEMS Microbiology Lett.*, 222, 129-136 (2003).

National Committee for Clinical Labortory Standards (1988).

Olivier, D., Claire, N., Baudouin, B., Christiane, K., Sophie, B. D., and Marc, J. St., Emergence of vancomycin-intermediate *Staphylococcus aureus* in a Belgian hospital: microbiological and clinical features. *J. Antimicrobial Chemother.*, 50(3), 383-391 (2002).

Park, J. C., Kim, I. S., Kwon, S. K., Noh, J. M., Lee, S. M., Park, J. P., Lee, W. K., and Ryu, S. R., Prevalence of Antibiotic-Resistant Strains among Bacteria Isolated from Bovine

Mastitis, Swine Diarrhea, and Swine Pneumonia. *Kor. J. Appl. Microbiol. Biotechnol.*, 28(4), 189-194 (2000).

Park, S. G., Park, S. K., Jung, J. H., and Jin, Y. H., Antibiotic Susceptibility of Salmonella sp. Isolated From Diarrhoea Patients in Seoul From 1996 to 2001. *J. Fd. Hyg. Safety*, 17(2), 61-70 (2002).

Rhee, Y. K., Han, M. J., Choi, E. C., Kim, D. H., Hypocholesterolemic activity of Bifidobacteria isolated from a healthy Korean. *Arch. Pharm. Res.*, 25, 681 (2002).

Sekine, K., Toida, T., Saito, M., Kuboyama, M., Kawashima, T., and Hashimoto, Y., A new morphologically characterized cell wall preparation (whole peptidoglycan) from *Bifidobacterium infantis* with a higher efficacy on the regression of an established tumor in mice. *Cancer Res.*, 45, 1300 (1985).

Song, J. H., Emerging Infectious Disease due to Micorbial Adaptation: Emergence and Spread of Antimicrobial Resistance. *Korean Society of Infection Disease, Infection and chemotherapy*, 31(1), 79-87 (2002).

Chun, U. H., Park, B. S., and Cho, J. S., Optimum Conditions for the Protoplast Formation of *Lactobacillus plantarum* and *Lactobacillus plantarum*. *Korean J. Biotechnol. Bioeng.*, 9(2), 191-199 (1994)

Venema, K. and Maathuis, J. H., A PCR-based method for identification of *bifidobacteria* from the human alimentary tract at the species level. *FEMS Microbiology Lett.*, 224, 143-149 (2003).

Woodford, N., Johnson, A. P., Morrison, D., and Speller, D. C. E., Current perspectives on glycopeptide resistance. *Clin. Microbial Rev.*, 8, 585-615 (1995).

Yamashita, M., Hatano, M., Matsui, T., and Kumon, Y., Effects of Bifermin-Radmistered in combination with antibiotics on the fecal flora. *Bifidobacteria Microflora*, 4, 23 (1985).

Yokoyama, M. T. and Carlson, J. R., Microbial metabolites of tryptophan in the intertinal tract with special reference to skatole. *Am. J. Chin. Nutr.*, 32, 173-178 (1979).

Yu, H. J., Lee, S. S., Lee, D. S., and Kim, H. B., Isolation of *Lactobacillus plantarum* HB1 from Tongchimi and Its Nitrite-Scavenging Effect. *Kor. J. Microbiol.*, 39(3), (2003).