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Comparison of Microbial Diversity of Korean Commercial *Makgeolli* Showing High β-Glucan Content and High Antihypertensive Activity, Respectively

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We measured physiological functionalities, including antihypertensive angiotensin I-converting enzyme inhibitory activity and immun-stimulating β-glucan content for sixty kinds of *Makgeolli* that is commercially available from the market. As a result, we selected R-12 commercial raw *Makgeolli*, with a high content of immuno-stimulating β-glucan, and R-14 commercial raw *Makgeolli*, exhibiting high antihypertensive activity. Due to the similarities in their overall physicochemical properties and raw materials used for fermentation, we compared the microbial flora in order to investigate the reason for the differences in their functionalities. Nested PCR and denaturing gradient gel electrophoresis for yeasts and bacteria were performed for analysis of microbial diversity of two different kinds of *Makgeolli* (i.e., R-12, R-14), which showed immuno-stimulating β-glucan content and exhibited a very high level of antihypertensive activity, respectively. Analysis of the 18S rDNA amplicon revealed a major presence of the yeast strain *Pichia burtonii* in every *Makgeolli* sample. Analysis of the 16S rDNA amplicon revealed a predominance of lactic acid bacteria, and the most frequent lactic acid bacteria were *Lactobacillus ingluviei, L. fermentum,* and *L. harbinensis, and Lactobacillus* sp. Among these, *L. harbinensis* was detected only in R-12 and *L. ingluviei* was found only in R-14. Different functionalities from the individual commercially available *Makgeolli* may be attributed to actions of different microbial flora during fermentation.

KEYWORDS : Commercial *Makgeolli*, Denaturing gradient gel electrophoresis, Lactic acid bacteria, Microbial diversity, Yeasts

Recently, public demand for traditional Korean wine or *Makgeolli* has shown a significant increase due to a growing perception of superiority of its nutraceuticals and physiological functionalities. The main ingredient of *Makgeolli* is cooked rice, which is mixed with *nuruk* (*koji*), containing amylolytic fungi, ethanol-producing yeast, and lactic acid bacteria, to initiate the alcohol fermentation process [1]. After fermenting for a period of one week, water is usually added to dilute the *Makgeolli*, so that its final alcohol content is approximately 6% to 7%, with a pH level around 3.4 to 4.0, since organic acids, including succinic acid and lactic acid, are produced by the actions of the yeast and lactic acid bacteria [2, 3].

Of particular interest, antihypertensive angiotensin I-converting enzyme (ACE) inhibitory activities have been found in several alcoholic beverages, including sake and its by-products, as well as traditional Korean wine [4, 5]. β-glucans, polysaccharide of D-glucose linked by β type glycosidic bonds, are known to activate the immune system [6]. In addition, consumption of β-glucan has been reported to result in reduction of blood cholesterol concentration [7]. However, no attempt has yet been made to determine the β-glucan contents of *Makgeolli*. We determined the physiological functionalities, including antihypertensive ACE inhibitory activity and β-glucan content, in 60 different kinds of commercially available *Makgeolli* obtained from all over the country. According to the results, R-12 raw *Makgeolli* showed high β-glucan content (35.6%), but moderate ACE inhibitory activity (73.7%). In contrast, R-14 showed very high ACE inhibitory activity (89.0%), but moderate β-glucan content (10.7%). It is interesting to note that, although rice was used as raw material for both R-12 and R-14, each exhibited totally different functionalities. Table 1 provides a summary of the overall physicochemical properties and functionalities of these *Makgeolli*. The ethanol content of the raw *Makgeolli* was 6.0%, with similar total acid contents of 0.20% (R-12) and 0.18% (R-14). Therefore, using PCR-denaturing gradient gel electrophoresis (PCR-DGGE) analysis, we attempted to compare the differences between

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Table 1. Physicochemical properties and functionalities of bioactive *Makgeolli* used in this study

Commercial *Makgeolli*	Ethanol content (%)	pH	Total acid (%)	Volatile acid (%)	Residual sugar (mg/mL)	ACE inhibitory activity (%)	β-Glucan content (%)
R-12	6.0	3.6	0.20	0.004	3.15	73.7	35.6
R-14	6.0	4.0	0.18	0.007	1.49	89.0	10.7

ACE, antihypertensive angiotensin I-converting enzyme.

highly β-glucan-containing R-12 commercial raw *Makgeolli* and antihypertensive R-14 commercial raw *Makgeolli*, in terms of microbial diversity, according to the methods described in a previous paper [8].

Fungal analysis in the functional *Makgeolli*. To investigate fungal diversity of the two types of functional *Makgeolli*, nested PCR-DGGE was performed for analysis of the V3 region of 18S rDNA [9-13]. As shown in Fig. 1A, two clear 18S rDNA bands of the two types of *Makgeolli* were observed on the DGGE gel. Bands that formed on the gel were recovered and analysis was performed for identification. DNA sequencing and subsequent BLAST analysis confirmed that every DNA band originates from the *Pichia burtonii* (Table 2). Of particular interest, we detected no other fungi or yeasts in the two types of *Makgeolli*. Alcoholic fermentation is typically carried out by complex microorganisms derived from yeast and bacteria from *nuruk* or *koji* and yeast seed cultures [14]. Some papers have reported on discovery of *P. burtonii* in murcha in Nepal, Bhutan, and the Himalayan regions of India; regi in Indonesia; loog-pang in Thailand; bubod in the Philippines; and Chinese yeast in Taiwan as a starter for rice wine-making [15, 16]. However, this is the first report to demonstrate the exclusive presence of *P. burtonii*, without *Saccharomyces cerevisiae*, in antihypertensive R-14 *Makgeolli* and high β-glucan-containing R-12 *Makgeolli*, which were used in this study.

Bacteria in the functional *Makgeolli*. Many lactic acid bacteria, including *Lactobacillus paracasei*, *L. hilgardii*, and *L. arizonensis*, have been identified in *Makgeolli* [17]. Nested PCR-DGGE analysis of the V2 region of 16S rDNA was performed for investigation of bacterial diversity in the two types of *Makgeolli*. Fig. 1B shows typical DGGE patterns of amplicons for 16S rDNA. Clear bands for the 16S rDNA fragments that formed on the DGGE gel were identified by direct DNA sequencing after performance of PCR for re-amplification of the excised bands. A list of identified bands is shown in Table 2. We detected *L. fermentum*, *L. harbinensis*, and *Lactobacillus* sp. in the R-12 and *L. ingluviei*, *L. fermentum*, and *Lactobacillus* sp. in the R-14 *Makgeolli*. DNA bands common to all *Makgeolli* samples were *L. fermentum* and *Lactobacillus* sp. However, *L. ingluviei* was found only in the antihypertensive R-14 commercial raw *Makgeolli* and *L. harbinensis* was detected only in highly β-glucan-containing R-12 commercial raw *Makgeolli*. The R-12.1 and R-12.3 bands were identified as *L. fermentum* and the R-14.1, and R-14.4 bands were identified as *L. ingluvie*; however, they were observed at different positions on the DGGE gel. Sequence heterogeneity between multiple copies of the 16S rDNA gene of any given strain may cause these multiple banding patterns [18]. Meanwhile, the presence of antihypertensive ACE inhibitory activity

Table 2. Identification of microorganisms from Korean functional *Makgeolli* showing high β-glucan content (R-12) and high antihypertensive activity (R-14) by PCR-DGGE

Bands		Putative species	Related GenBank sequence	Identity (%)
Yeast	R-12.1	*Pichia burtonii*	AB158656.1	385/388 (99)
	R-12.2	*P. burtonii*	AB158656.1	383/385 (99)
	R-14.1	*P. burtonii*	AB158656.1	385/388 (99)
	R-14.2	*P. burtonii*	AB158656.1	383/385 (99)
Bactiera	R-12.1	*Lactobacillus fermentum*	JN792459.1	187/197 (95)
	R-12.2	*L. harbinensis*	AB681318.1	187/197 (95)
	R-12.3	*L. fermentum*	EU886731.1	188/198 (95)
	R-12.4	*Lactobacillus* sp.	AB681518.1	180/198 (91)
	R-14.1	*L. ingluviei*	JQ395039.1	195/197 (99)
	R-14.2	*L. fermentum*	JN792459.1	187/197 (95)
	R-14.3	*L. fermentum*	EU886731.1	188/198 (95)
	R-14.4	*L. ingluviei*	JQ395039.1	179/197 (91)
	R-14.5	*Lactobacillus* sp.	AB681518.1	180/198 (91)

PCR-DGGE, PCR-denaturing gradient gel electrophoresis.

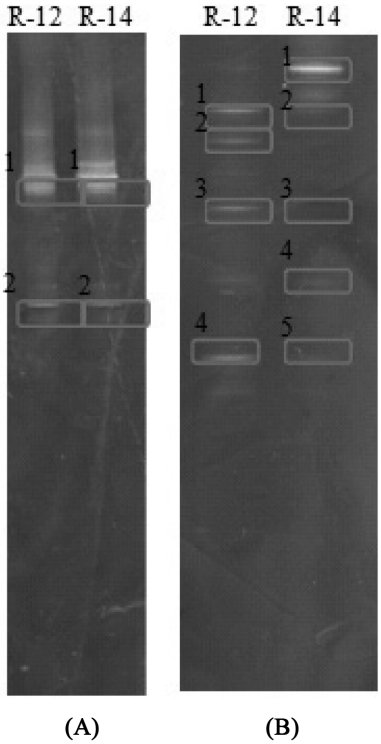

Fig. 1. Yeast (A) and Bacteria (B) community of Korean functional *Makgeolli* as revealed by PCR-denaturing gradient gel electrophoresis. Lanes corresponding to two *Makgeolli* samples are indicated by R-12 and R-14 at the top. Numbers on the gel represent bands that were recovered for identification. A summary of the identities of the excised 18S rDNA fragments (A) and 16S rDNA fragments (B) is shown Table 2.

has been reported in several alcoholic beverages, including Japanese rice wine, sake, and Korean rice wine, and most ACE inhibitors are peptides that originate from raw materials during fermentation [4, 5]. In addition, findings reported from several clinical trials have suggested that consumption of milk that has been fermented with *S. cerevisiae* and *L. helveticus* may result in suppression of blood pressure in hypertensive individuals [19, 20]. This effect is attributed not to viable probiotic cells themselves but to the action of the ACE inhibitor-like peptides produced during fermentation. Based on these reports, we cannot exclude the possibility that the high level of antihypertensive biological activity found in R-14 *Makgeolli* may be attributed to peptides produced by the proteolytic action of microorganisms, including *L. ingluviei*, on the raw materials during fermentation of *Makgeolli*. Similarly, *L. harbinensis* may play a role in the high level of β-glucan detected in R-12 commercial raw *Makgeolli*. However, conduct of further study to determine the nature of antihypertensive ACE inhibitory activity and origin of β-glucan found in *Makgeolli* products will be necessary.

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References

1. Seo MY, Lee JK, Ahn BH, Cha SK. The changes of microflora during the fermentation of Takju and Yakju. Korean J Food Sci Technol 2005;37:61-6.
2. Lee SJ, Kwon YH, Kim HR, Ahn BH. Chemical and sensory characterization of Korean commercial rice wines (yakju). Food Sci Biotechnol 2007;16:374-80.
3. Lee WK, Kim JR, Lee MW. Studies on the changes in free amino acids and organic acids of Takju prepared with different *koji* strains. J Korean Agric Chem Soc 1987;30:323-7.
4. Saito Y, Wanezaki K, Kawato A, Imayasu S. Structure and activity of angiotensin I converting enzyme inhibitory peptides from sake and sake lees. Biosci Biotechnol Biochem 1994;58:1767-71.
5. Kim JH, Lee DH, Choi SY, Lee JS. Characterization of physiological functionalities in Korean traditional liquors. Korean J Food Sci Technol 2002;34:118-22.
6. Miura NN, Ohno N, Aketagawa J, Tamura H, Tanaka S, Yadomae T. Blood clearance of (1-->3)-beta-D-glucan in MRL lpr/lpr mice. FEMS Immunol Med Microbiol 1996; 13:51-7.
7. Othman RA, Moghadasian MH, Jones PJ. Cholesterol-lowering effects of oat β-glucan. Nutr Rev 2011;69;299-309.
8. Min JH, Nam YG, Ju JI, Jung JH, Lee JS Kim HK. Changes of Yeast and Bacterial Flora during Fermentation and Storage of Gugija-Liriope tuber *Makgeolli* using PCR-DGGE. Korean J Microbiol Biotechnol 2012;40:111-6.
9. Sheffield VC, Cox DR, Lerman LS, Myers RM. Attachment of a 40-base-pair G+C-rich sequence (GC-clamp) to genomic DNA fragments by polymerase chain reaction results in improved detection of single-base changes. Proc Natl Acad Sci U S A 1989;86:232-6.
10. Ercolini D. PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. J Microbiol Methods 2004; 56:297-314.
11. Thanh VN, Mai LT, Tuan DA. Microbial diversity of traditional Vietnamese alcohol fermentation starters (bahn men) as determined by PCR-mediated DGGE. Int J Food Microbiol 2008;128:268-73.
12. Endo A, Okada S. Monitoring the lactic acid bacterial diversity during shochu fermentation by PCR-denaturing gradient gel electrophoresis. J Biosci Bioeng 2005:99;216-21.
13. Cocolin L, Bisson LF, Mills DA. Direct profiling of the yeast dynamics in wine fermentations. FEMS Microbiol Lett 2000; 189:81-7.
14. Jo KY, Ha DM. Isolation and identification of the lactic acid bacteria from *nuruk*. Agric Chem Biotechnol 1995;38:95-9.
15. Takeuchi A, Shimizu-Ibuka A, Nishiyama Y, Mura K, Okada S, Tokue C, Araj S. Purification and characterization of an α-amylase of *Pichia burtonii* isolated from the traditional starter

”Murcha” in Nepal. Biosci Biotechnol Biochem 2006;70: 3019-24.
16. Hesseltine CW, Rogers R, Winarno FG. Microbiological studies on amylolytic oriental fermentation starters. Mycopathologia 1988;101:141-55.
17. Jin J, Kim SY, Jin Q, Eom HJ, Nam NS. Diversity analysis of lactic acid bacteria in Takju, Korean rice wine. J Microbiol Biotechnol 2008;18:1678-82.
18. Nübel U, Engelen B, Felske A, Snaidr J, Wieshuber A, Amann RI, Ludwig W, Backhaus H. Sequence heterogeneities of genes encoding 16 rRNAs in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. J Bacteriol 1996;178:5636-43.
19. Sanders ME. Considerations for use of probiotic bacteria to modulate human health. J Nutr 2000;130:384S-90S.
20. Takano T. Milk derived peptides and hypertension reduction. Int Dairy J 1998;8:375-81.