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Analysis of Microflora Profile in Korean Traditional *Nuruk*

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A variety of *nuruk* were collected from various provinces in Korea, and their microflora profiles were analyzed at the species level. A total of 42 *nuruk* samples were collected and when the viable cell numbers in these *nuruk* were enumerated, the average cell numbers of bacteria, fungi, yeast, and lactic acid bacteria from all *nuruk* were 7.21, 7.91, 3.49, and 4.88 log CFU/10 g, respectively. There were no significant differences in viable cell numbers of bacteria or fungi according to regions collected. *Bacillus amyloliquefaciens* and *B. subtilis* were the predominant bacterial strains in most samples. A significant portion, 13 out of 42 *nuruk*, contained foodborne pathogens such as *B. cereus* or *Cronobacter sakazakii.* There were various species of lactic acid bacteria such as *Enterococcus faecium* and *Pediococcus pentosaceus* in *nuruk*. It was unexpectedly found that only 13 among the 42 *nuruk* samples contained *Aspergillus oryzae,* the representative saccharifying fungi in *makgeolli,* whereas a fungi *Lichtheimia corymbifera* was widely distributed in *nuruk*. It was also found that *Pichia jadinii* was the predominant yeast strain in most *nuruk*, but the representative alcohol fermentation strain, *Saccharomyces cerevisiae,* was isolated from only 18 out of the 42 *nuruk*. These results suggested that a variety of species of fungi and yeast were distributed in *nuruk* and involved in the fermentation of *makgeolli.* In this study, a total of 64 bacterial species, 39 fugal species, and 15 yeast species were identified from *nuruk*. Among these strains, 37 bacterial species, 20 fungal species, and 8 yeast species were distributed less than 0.1%.

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Makgeolli is a traditional turbid rice wine in Korea, of which consumption is continuously increasing owing to the recent globalization campaign of Korean ethnic foods led by the Korean government [16]. It is usually brewed using rice as a main ingredient and *nuruk* as a fermentation agent [13, 22]. *Nuruk* is a starter culture made with wheat flour and is fermented spontaneously by various microorganisms such as fungi, yeasts, and bacteria as well as lactic acid bacteria inoculated from nature [4]. For *makgeolli* production, diverse types of microorganisms are involved in the saccharification, fermentation, and ripening processes. Fungi and bacteria such as *Bacillus subtilis* in *nuruk* saccharify the rice starch and thereby produce glucose, and subsequently yeast cells conduct alcoholic fermentation using glucose to produce ethanol and carbon dioxide [14].

In *nuruk*, fungi such as *Aspergillus* sp., *Rhizopus* sp., and *Mucor* sp., yeasts such as *Saccharomyces cerevisiae* and *B. subtilis*, and various lactic acid bacteria have been reported by many research groups [3]. *Aspergillus oryzae* isolated from *nuruk* was characterized [15, 21], and the useful fungus and *S. cerevisiae* isolated from traditional *nuruk* were also characterized and used to ferment rice wine or *yakju* [6, 11]. The microbiota in *nuruk* and their biochemical roles in the fermentation process of *makgeolli* have been documented [2, 7, 8], but these studies were restricted to the microbiota in one or several *nuruk* produced in specific areas in Korea. *Nuruk* is widely produced in almost all provinces in Korea and it is

presumed that the microbiotas in *nuruk* produced in different provinces are different from one another. Because the quality and organoleptic properties of *makgeolli* are clearly dependent on the *nuruk* used for fermentation, the microbiota in *nuruk* is very important in the quality and organoleptic properties of *makgeolli*. Despite the importance of the microbiota of the *nuruk*, there are poor studies on the distribution and difference of the microbiotas in *nuruk* produced in diverse areas.

In this study, a variety of *nuruk* were collected from various provinces in Korea, and their microflora profiles were analyzed.

Materials and Methods

Collection of *Nuruk*

Nuruk samples were purchased from local markets in various provinces accross Korea and grouped according to their regions collected.

Enumeration of Microorganisms in *Nuruk*

The viable cell number of total bacteria, fungi, yeasts, and lactic acid bacteria in *nuruk* were enumerated. Ten grams of *nuruk* was homogenized with 100 ml of sterile saline solution for 2 h and serially diluted. For the enumeration of total bacteria, the serially diluted sample solution was spread onto nutrient agar and the colonies formed were counted after 2 days of incubation at 30°C. For fungi and yeasts, potato dextrose agar and yeast mold agar containing 20 μg/ml of chloramphenicol were used, respectively, and plates were incubated at 25°C for 4 days. For the enumeration of lactic acid bacteria, MRS agar was used to grow colonies, with an anaerobic gas pak system (BBL, Becton Dickinson, Franklin Lakes, NJ, USA) used to make an anaerobic environment, and the cells were incubated at 37°C for 3 days.

Identification of the Isolates

To identify the isolates from the *nuruk* at the species level, colonies grown on agar plate were resuspended with 50 mM EDTA solution containing 50 mg/ml of lysozyme and incubated at 37°C for 16 h. Genomic DNAs of total bacteria, fungi, yeasts, and lactic acid bacteria were extracted and purified according to the manufacturer's manual using the Wizard genomic DNA purification kit (Promega, Madison, WI, USA).

For each individual genomic DNA, polymerase chain reaction (PCR) was performed to clone 16S rRNA or 18S rRNA genes. Reactions contained 20–80 ng of genomic DNA, 1× PCR reaction buffer, 2.5 mM dNTP mixture, 20 pmol of forward primer (27F for total bacteria and lactic acid bacteria, 5'-AGAGTTTGATCATGGCTCAG-3'; NS1 for yeasts, 5'-GTAGTCATATGCTTGTCTC-3'; and ITS 1 for fungi; 5'-TCCGTAGGTGAACCTGCGG-3'), 20 pmol of reverse primer (1492R for total bacteria and lactic acid bacteria, 5'-GGATAC CTTGTTACGACTT-3'; NS8 for yeasts, 5'-TCCGCAGGTTCACCT ACGGA-3'; ITS4 for fungi, 5'-TCCTCCGCTTATTGATATGC-3'), and 2 units of Ex-Taq polymerase (TaKaRa, Tokyo, Japan) in a final volume of 50 μl. The temperature profile consisted of 5 min initial denaturation at 95°C followed by 40 cycles of 95°C for 30 s, 60°C for 1 min, and 72°C for 45 s, followed by a final extension at 72°C for 7 min, and the reaction was performed using the MyCycler Thermal Cycler System (Bio-Rad, Hercules, CA, USA).

The amplified PCR products were electrophoresed using 0.8% (w/v) agarose gel and the DNA band that showed the estimated size was eluted from the gel and purified using the Wizard SV Gel and PCR clean-up system (Promega). The nucleotide sequences of purified 16S rRNA or 18S rRNA genes were determined using ABI PRISM BigDye Terminator Cycle Sequencing kits (Applied Biosystems Co., Carlsbad, CA, USA) with the ABI PRISM 3730XL Analyzer (Applied Biosystems Co.). Sequences were compared against those 16S rRNA sequences available in the GenBank database using the BLAST program (http:/www.ncbi.nlm.nih.gov./blast/). The nearly identical sequences were aligned using the CLUSTALX program [12] and the phylogenetic trees were constructed using the MEGA4 program by the neighbor-joining method [18].

Results and Discussion

Quantitative Analysis of Microorganisms in *Nuruk*

A total of 42 *nuruk* samples were collected from provinces all around Korea; 11 *nuruk* were collected from Chungcheong-do (CC, including Dangjin, Hapdeok, Buyeo, and Hongseong), 3 were from Gangwon-do (GW, including Sokcho and Yangyang), 5 were from Gyeonggi-do (GG, including Seongnam, Pocheon, and Gapyeong), 9 were from Gyeongsang-do (GS, including Andong, Munkeong, Yeochon, Kyeongju, Changwon, Haman, Jinju, Tongyoung, and Geoje), 4 were from Jeju-do (JJ, including Jeju-si, Seongeup, and Pyoseon), and 10 were from Jeolla-do (JL, including Jeonju, Imsil, Namwon, Sunchang, Gunsan, Gwangju, Mokpo, Boseong, Haenam, and Muan). When viable cell numbers in the *nuruk* were enumerated, the average cell numbers of bacteria, fungi, yeast, and lactic acid bacteria from all *nuruk* were 7.21, 7.91, 3.49, and 4.88 log CFU/10 g, respectively (Table 1). These viable cell numbers of bacteria, fungi, yeast, and lactic acid bacteria ranged from 4.73 (JL NR4) to 11.76 log CFU/10 g (GS NR3), from 1.79 (JL NR6) to 12.46 log CFU/10 g (GS NR4), from none to 7.77 log CFU/10 g (GS NR8), and from none to 11.42 log CFU/10 g (GW NR1), respectively. In this result, the viable cell number of fungi was the highest and that of bacteria was second highest. Some *nuruk* did not contain yeast or lactic acid bacteria; 13 and 6 out of the 42 *nuruk* did not have yeast and lactic acid bacteria, respectively.

The *nuruk* samples were grouped according to the provinces from which each *nuruk* was collected and the average viable cell numbers of bacteria, fungi, yeast, and lactic acid bacteria were calculated according to province of collection. As shown in Table 2, there were no statistically significant differences ($p > 0.05$) in average viable cell numbers of bacteria or fungi according to the province from which they were isolated. In contrast, the highest average viable cell number of yeast in *nuruk* was

Table 1. Viable cell number of total bacteria, fungi, yeast, and lactic acid bacteria in *nuruk* collected from various provinces in Korea.

(Unit: log CFU/10 g)

Source	Total bacteria	Fungi	Yeast	Lactic acid bacteria
CC[1] NR1	5.62 ± 0.11[2]	8.23 ± 0.17	ND[3]	3.16 ± 0.04
CC NR2	6.74 ± 0.14	9.11 ± 0.18	ND	3.99 ± 0.04
CC NR3	7.35 ± 0.12	9.10 ± 0.33	7.38 ± 0.31	4.10 ± 0.44
CC NR4	6.24 ± 0.22	8.54 ± 0.44	4.58 ± 0.11	ND
CC NR5	5.54 ± 0.27	7.11 ± 0.14	2.67 ± 0.45	3.49 ± 0.44
CC NR6	7.65 ± 0.23	9.24 ± 0.30	4.54 ± 0.33	3.00 ± 0.22
CC NR7	6.67 ± 0.24	8.69 ± 0.25	3.48 ± 0.36	5.26 ± 0.27
CC NR8	8.28 ± 0.30	6.61 ± 0.52	3.55 ± 0.44	3.45 ± 0.35
CC NR9	8.20 ± 0.32	8.99 ± 0.15	3.27 ± 0.31	3.55 ± 0.42
CC NR10	7.75 ± 0.22	8.29 ± 0.23	4.82 ± 0.17	5.46 ± 0.45
CC NR11	8.23 ± 0.31	8.48 ± 0.26	ND	7.31 ± 0.21
GW NR1	5.47 ± 0.14	8.33 ± 0.17	4.05 ± 0.09	11.42 ± 0.39
GW NR2	7.41 ± 0.17	9.24 ± 0.20	3.77 ± 0.10	5.36 ± 0.07
GW NR3	7.04 ± 0.14	8.75 ± 0.18	5.62 ± 0.15	7.16 ± 0.17
GG NR1	4.97 ± 0.08	6.47 ± 0.19	3.47 ± 0.06	5.93 ± 0.08
GG NR2	8.11 ± 0.16	8.17 ± 0.16	ND	6.24 ± 0.08
GG NR3	8.32 ± 0.13	8.08 ± 0.12	ND	ND
GG NR4	7.56 ± 0.11	6.06 ± 0.12	4.58 ± 0.11	5.63 ± 0.07
GG NR5	6.90 ± 0.12	3.80 ± 0.11	ND	3.63 ± 0.05
GS NR1	5.68 ± 0.09	6.57 ± 0.18	ND[2]	ND
GS NR2	5.70 ± 0.11	8.28 ± 0.17	3.29 ± 0.19	4.90 ± 0.06
GS NR3	11.76 ± 0.17	8.43 ± 0.18	ND	4.24 ± 0.08
GS NR4	6.53 ± 0.13	12.46 ± 0.25	5.76 ± 0.14	4.45 ± 0.06
GS NR5	6.73 ± 0.07	7.56 ± 1.02	3.97 ± 0.05	5.38 ± 0.14
GS NR6	9.32 ± 0.19	8.28 ± 0.17	5.88 ± 0.15	5.42 ± 0.07
GS NR7	6.54 ± 0.14	8.73 ± 0.20	5.54 ± 0.17	7.52 ± 0.30
GS NR8	8.93 ± 0.30	9.63 ± 0.19	7.77 ± 0.18	7.17 ± 0.15
GS NR9	7.69 ± 0.20	7.25 ± 0.19	7.36 ± 0.14	9.54 ± 0.29
JJ NR1	7.15 ± 0.19	8.64 ± 0.17	6.28 ± 0.18	8.39 ± 0.26
JJ NR2	9.04 ± 0.18	6.24 ± 0.32	6.00 ± 0.15	8.28 ± 0.16
JJ NR3	9.04 ± 0.18	8.57 ± 0.26	5.36 ± 0.14	8.20 ± 0.24
JJ NR4	7.62 ± 0.13	4.82 ± 0.26	5.19 ± 0.35	7.23 ± 0.33
JL NR1	5.65 ± 0.10[1]	10.33 ± 0.19	4.15 ± 0.03	5.29 ± 0.16
JL NR2	7.62 ± 0.20	8.20 ± 0.21	4.18 ± 0.06	5.24 ± 0.16
JL NR3	6.55 ± 0.13	6.22 ± 0.13	3.79 ± 0.09	5.76 ± 0.07
JL NR4	4.73 ± 0.09	6.17 ± 0.12	ND	ND
JL NR5	6.16 ± 0.09	8.38 ± 0.13	ND	ND
JL NR6	5.36 ± 0.11	1.79 ± 0.04	ND	ND
JL NR7	8.87 ± 0.18	7.81 ± 0.16	ND	6.30 ± 0.08
JL NR8	6.30 ± 0.20	8.81 ± 0.18	5.48 ± 0.14	3.22 ± 0.04
JL NR9	8.71 ± 0.18	9.11 ± 0.18	ND	4.25 ± 0.27
JL NR10	7.04 ± 0.14	8.65 ± 0.17	7.04 ± 0.18	6.10 ± 0.23
Mean ± SD	7.21 ± 1.41	7.91 ± 1/76	3.40 ± 2.57	4.88 ± 2.70

[1]CC: Chungcheong-do; GW: Gangwon-do; GG: Gyeonggi-do; GS: Gyeongsang-do; JJ: Jeju-do; JL: Jeolla-do.

[2]Means ± SD of triplicate experiments.

[3]ND: not detected.

Table 2. Average viable cell number of total bacteria, fungi, yeast, and lactic acid bacteria in *nuruk* collected from various provinces in Korea.

(Unit: log CFU/10 g)

Province	Bacteria	Fungi	Yeast	Lactobacilli
Chungcheong-do	7.12 ± 1.02[1)a2)]	8.40 ± 0.84[a]	3.12 ± 2.34[ab]	3.89 ± 1.82[b]
Gangwon-do	6.64 ± 1.03[a]	8.77 ± 0.46[a]	4.48 ± 1.00[ab]	7.98 ± 3.11[a]
Gyeonggi-do	7.12 ± 1.35[a]	6.52 ± 1.79[a]	1.61 ± 2.24[b]	4.29 ± 2.60[b]
Gyeongsang-do	7.65 ± 2.01[a]	8.58 ± 1.70[a]	4.40 ± 2.86[ab]	5.40 ± 2.66[ab]
Jeju-do	8.21 ± 0.97[a]	7.07 ± 1.87[a]	5.71 ± 0.52[a]	8.03 ± 0.54[a]
Jeolla-do	6.70 ± 1.37[a]	7.58 ± 2.38[a]	2.46 ± 2.75[ab]	3.62 ± 2.65[b]

[1)]Means ± SD of triplicate experiments.

[2)]The same superscripts in a column are not significantly different from each other at $p < 0.05$.

collected from Jeju-do (5.71 log CFU/10 g) and the average viable cell number of *nuruk* collected from Gyeonggi-do (1.61 log CFU/10 g) was the lowest. Similarly, the average viable cell number of lactic acid bacteria was highest in *nuruk* collected from Jeju-do (8.03 log CFU/10 g) and lowest in *nuruk* collected from Jeolla-do (3.62 log CFU/10 g). The reason why the average viable cell number of yeast or lactobacilli was different according to provinces collected is not clear, although there are some patterns that the viable cell numbers of yeast and lactic acid bacteria were highest in *nuruk* collected from Jeju-do and statistically lowest in *nuruk* collected from Gyeonggi-do.

Identification of Bacteria

All microorganisms isolated from each *nuruk* were identified at the species level using nucleotide sequence analysis of the 16S rRNA gene, followed by phylogenetic analysis, and the result is shown in Fig. 1. *Bacillus amyloliquefaciens* existed in most *nuruk* collected from Chungcheong-do and Gangwon-do and was the most predominant bacteria in *nuruk* JL NR7, of which the proportion was over 80%. *Bacillus subtilis* was detected in 27 out of the 42 *nuruk* and 20–29% of microorganisms were *B. subtilis* in several *nuruk* such as GG NR2, GG NR5, and GS NR6. A significant portion of *nuruk*, 13 out of the 42, contained foodborne pathogens such as *B. cereus* or *Cronobacter sakazakii,* and in some *nuruk* such as GG NR5 and JJ NR4, over 40% and over 30% of microorganisms were *C. sakazakii,* respectively. This result indicated that there are serious problems in sanitation during the manufacturing process of some *nuruk*.

When the lactic acid bacteria isolated from *nuruk* was identified, various species of lactic acid bacteria such as *Enterococcus faecium, Lactobacillus plantarum, Leuconostoc mesenteroides, Pediococcus acidilactici, P. pentosaceus, Weissella paramesenteroides,* and *W. cibaria* were detected. *E. faecium* and *P. pentosaceus* were widely distributed in 18 and 26 *nuruk,* respectively, and in GS NR9, over 30% of microorganisms were *P. pentosaceus.*

Lee and Yu [13] isolated three lactic acid bacteria from *nuruk*, identified as *Lactococcus lactis* subsp. *lactis* NR C-1, *Leuconostoc mesenteroides* subsp. *mesenteroides* NR K-3, and *Pediococcus pentosaceus* NR T-1 by morphological, physiological, and biochemical characterization. When their results were compared with this study, the predominant species of lactic acid bacteria in *nuruk* was different in both study, and furthermore much more species of lactic acid bacteria were detected in this study. Yu *et al.* [22] reviewed the research papers on the microorganisms of *nuruk* and summarized that bacteria in *nuruk* were probably not considered as important microorganisms in traditional Korean liquor fermentation, even though *Bacillus* and lactic acid bacteria were continually isolated from *nuruk*.

Identification of Fungi

Among the 42 *nuruk*, only 13 contained *Aspergillus oryzae*, and this result was unexpected because it has been known that *A. oryzae* is the representative saccharifying fungi in the fermentation of *makgeolli*, although other fungi such as *Mucor* spp. or *Rhizopus* spp. have also been used to ferment *makgeolli* [5, 9]. A variety of fungi species were isolated from *nuruk* including *Aspergillus flavus, A. fumigatus, A. niger, A. versicolor, Eurotium amstelodami, E. rubrum, Lichtheimia corymbifera, L. ramose, Mucor circinelloides, M. indicus, Penicillium chermesinum, P. chrysogenum, P. sumatrense, Rhizomucor pusillus, R. tauricus, R. variabilis,* and *Rhizopus oryzae.* A proportion of *A. oryzae* was over 50% in GS NR1 and JL NR5, and was below 50% in other *nuruk*. *Lichtheimia corymbifera* and *L. ramose* were widely distributed in 24 and 18 *nuruk,* respectively. In addition, the proportion of these fungi in *nuruk* was very high, and the proportion of *L. corymbifera* was over 50% in CC NR5, CC NR6, and JL NR9. The proportion of *L. ramose* was over 50% in CC NR7, CC NR9, GW NR2, GS NR2, JL NR1, and JL NR4.

The predominant fungi in *nuruk* collected from Chungcheong-do were *A. flavus* (CC NR1), *L. corymbifera* (CC NR1, CC NR5, CC NR6), *P. chermesinum* (CC NR1),

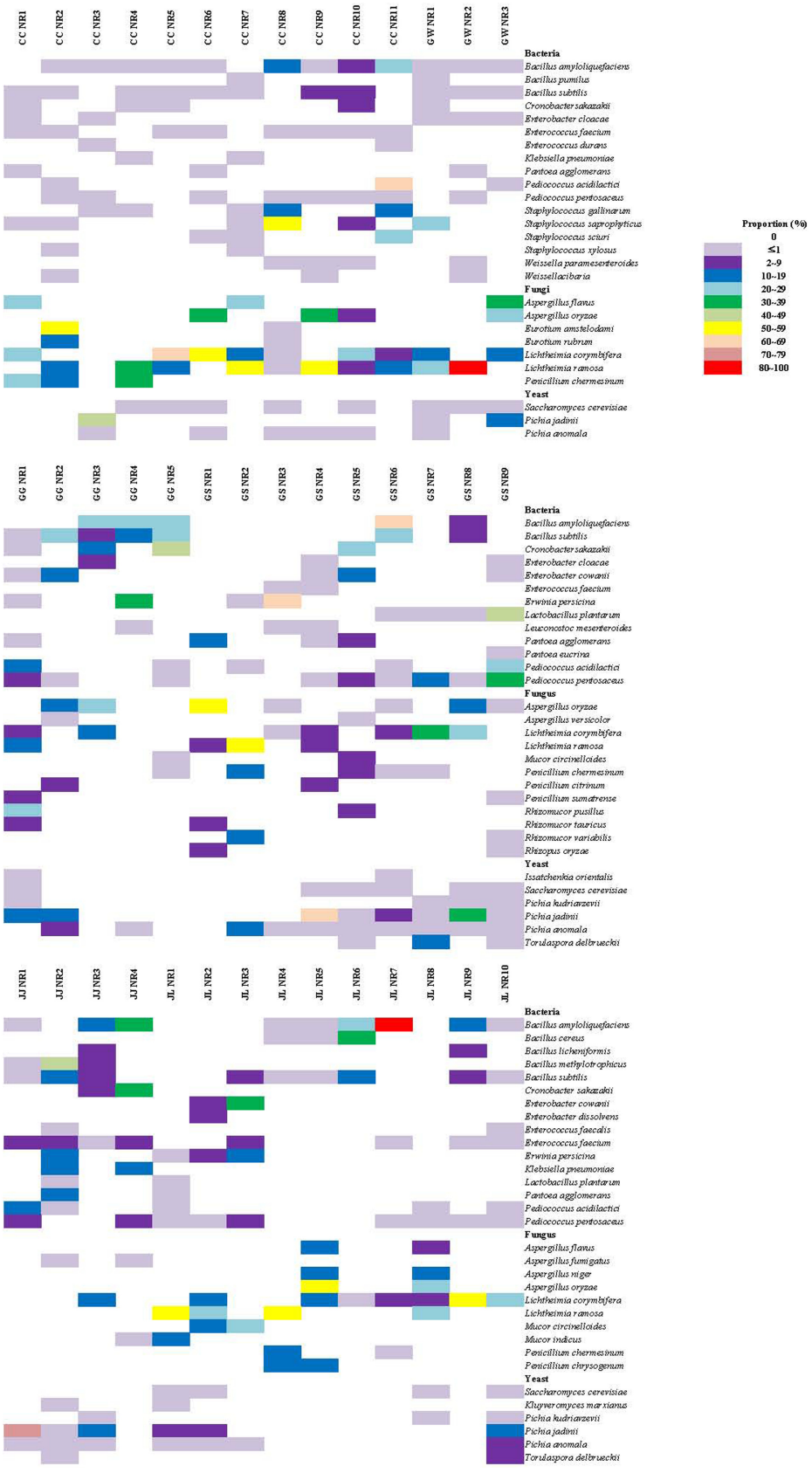

Fig. 1. Microflora profile of *nuruk*.
Microorganisms for which the proportion was over 0.1% are presented.

Eurotium amstelodami (CC NR2), *L. corymbifera* (CC NR4, CC NR10), and *L. ramose* (CC NR4, CC NR7, CC NR9, CC NR11). *L. ramose* (GW NR1, GW NR2) and *L. corymbifera* (GW NR3) were predominant in *nuruk* collected from Gangwon-do, and *Rhizomucor pusillus* (GG NR1) and *A. oryzae* (GG NR 2, GG NR3) were predominant in *nuruk* collected from Gyeonggi-do. Moreover, *A. oryzae* (GS NR1), *L. ramose* (GS NR2), and *L. corymbifera* (GS NR7, GS NR9) were the predominant fungi in *nuruk* collected from Gyeongsang-do, and *L. corymbifera* (JJ NR3) was predominant in *nuruk* collected from Jeju-do. In addition, *L. ramose* (JL NR1, JL NR2, JL NR4, JL NR8), *Mucor circinelloides* (JL NR3), *A. oryzae* (JL NR5, JL NR8), and *L. corymbifera* (JJ NR9, JL NR10) were the predominant fungi in *nuruk* collected from Jeolla-do. Park *et al.* [17] isolated and identified 159 strains of fungi from *nuruk* collected from several regions in Korea at the genus level, and there were many differences in numbers and distributions of fungi from each *nuruk* according to their collected region, of which results were similar to this study. In their study, *Absidia* spp. were the most frequently isolated from every *nuruk* sample, but *Penicillium* sp. or *Mucor* sp. were not detected, which was different from results of this study.

Kim *et al.* [10] isolated 10 strains of fungi and examined their saccharogenic enzyme activity, and reported that *Aspergillus* sp. and *Rhizopus* sp. were the predominant strains that showed high liquefying activity in *nuruk*.

Identification of Yeast

For yeast, *Pichia jadinii* was the predominant strain in most *nuruk*; that is, the proportions of *P. jadinii* in CC NR3, GS NR4, GS NR8, and JJ NR1 were over 40%, 60%, 30%, and 70%, respectively. *P. jadinii* was detected in 17 out of the 42 *nuruk*, and unexpectedly, *Saccharomyces cerevisiae,* the representative fermentation strain, was isolated from only 18 out of 42 *nuruk*.

Besides the strains mentioned above, minor strains of which distribution was less than 0.1% were found in *nuruk*; these minor bacterial strains were identified as *Bacillus circulans, B. flexus, B. infantis, B. licheniformis, B. megaterium, B. sonorensis, B. vallismortis, B. velezensis, B. vietnamensis, Citrobacter braakii, Cronobacter muytjensii, Cupriavidus gilardii, Enterobacter asburiae, E. cloacae, E. cancerogenus, E. hormaechei, E. ludwigii, E. pulveris, E. turicensis, Enterococcus durans, Erwinia soli, Erwinia tasmaniensis, Escherichia coli, Escherichia hermannii, Klebsiella variicola, Lactobacillus brevis, L. casei, L. coryniformis, L. rhamnosus, Leuconostoc citreum, Leuconostoc fallax, Pantoea calida, P. gaviniae, P. stewartii, Pseudomonas aeruginosa, Staphylococcus kloosii,* and *S. xylosus.* The minor fungi strains included *Aspergillus clavatus, A. tritici, Cladosporium cladosporioides, C. sphaerospermum, C. uredinicola, Eurotium chevalieri, E. intermedium, Irpex lacteus, Mucor racemosus, Penicillium cinnamopurpureum, P. commune, P. crustosum, P. fellutanum. P. funiculosum, P. phoeniceum, P. sumatrense, P. waksmanii, Rhizopus microsporus, Saccharomycopsis fibuligera,* and *S. racemosum.* The minor yeast strains isolated from *nuruk* were *Candida glabrata, C. tropicalis, Clavispora lusitaniae, Issatchenkia orientalis, Kluyveromyces lactis, Pichia fabianii, P. farinose,* and *P. guilliermondii.*

In this study, a total of 64 bacterial species, 39 fungal species, and 15 yeast species were isolated and identified from *nuruk*. Among these strains, 37 bacterial species, 20 fungal species, and 8 yeast species were distributed less than 0.1%. When Yu *et al.* [23] reviewed the research papers on the microorganisms from Korean traditional *nuruk*, they summarized that the total number of fungal species identified was 38 species among 12 different genera and the total number of yeast species was up to 18 species from 8 different genera. The total numbers of fungal and yeast species in their review were very similar to this study, but the species in their review were somewhat different from this study. Furthermore, the number of total bacterial species was 19, which was smaller than the total number in this study.

Some studies on the microorganisms isolated from *nuruk* have been undertaken, but these studies were mainly focused on the availability in the fermentation of *makgeolli,* and dealt with the saccharification activity of fungi or the ability of ahcohol fermentation of yeasts [1]. Studies of the changes of microflora of *makgeolli* or diversity of microorganisms in *makgeolli* have also been done by several research groups, and these microfloral changes or microflora profiles were examined mainly during the fermentation process of *makgeolli* [19, 20]. Although a variety of researches have been done for identification of microorganisms in *makgeolli*, no one has been reported on the microflora profiles in *nuruk*. In this study, the microorganisms isolated from *nuruk* were identified at the species level, based on the nucleotide sequence of the 16S rRNA gene, and the microflora profiles in *nuruk* collected from all around Korea were analyzed for the first time.

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