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Diversity analysis of *Saccharomyces cerevisiae* isolated from natural sources by multilocus sequence typing (MLST)

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Abstract We used multilocus sequence typing (MLST) to analyze the diversity of natural isolates of *Saccharomyces cerevisiae*, the most important microorganism in alcoholic fermentation. Six loci, ADP1, RPN2, GLN4, ACC1, MET4, and NUP116, in *S. cerevisiae* genome were selected as MLST markers. To investigate genetic diversity within *S. cerevisiae*, 42 *S. cerevisiae* isolated from natural sources in Korea as well as six *S. cerevisiae* obtained from Genbank and four industrial *S. cerevisiae* were examined using MLST. Twenty-six polymorphic sites were found in the six loci. Among them, ACC1 had the most genetic variation with eight polymorphic sites. MLST differentiated the 52 strains into three clades. Alcohol fermentation results revealed that *S. cerevisiae* in Clade III produced less alcohol than those in Clades I and II. These results suggested that MLST is a powerful tool to differentiate *S. cerevisiae* and can potentially be used to select *S. cerevisiae* suitable for industrial use.

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Introduction

Saccharomyces cerevisiae is a unicellular budding yeast that has been used to make bread, wine, beer, and various other fermented foods since ancient times. *S. cerevisiae* is ubiquitous in nature and frequently isolated from sugary foods and alcoholic beverages. This yeast is capable of fermentative and oxidative metabolism and can reversibly switch between these two metabolism types depending on environmental conditions. Under anaerobic conditions, this microorganism can ferment sugars and produce ethanol and carbon dioxide [1]. However, under aerobic conditions, it propagates rapidly by assimilating sugar, resulting in high cell yields. Because both characteristics are utilized industrially, it is very important to select suitable strains for production of breads, beers, wines, and nutritional yeast biomass [2].

Numerous DNA and protein-based methods have been developed to identify and classify various microorganisms such as bacteria and yeast [3]. Different *S. cerevisiae* wine strains have been discriminated by amplified fragment length polymorphism (AFLP) [4], multilocus sequence typing (MLST) [5], pulsed-field gel electrophoresis (PFGE) [6], random amplified polymorphic DNA (RAPD) [7], and restriction fragment length polymorphism (RFLP) analyses [8]. Recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been shown to be a rapid and reliable tool for identification of *S. cerevisiae* strains [9].

MLST is a molecular biological tool that can be used to differentiate among isolates of microbial species. The

procedure of MLST involves PCR amplification of about 450–500 bp internal fragments of multiple housekeeping genes, followed by determination of their sequences using an automated DNA sequencing tool. Final, multilocus sequence analysis is used to differentiate among microbial strains [10, 11]. Muñoz et al. [5] successfully employed MLST analysis for molecular discrimination at the strain level of Spanish wine yeast. This study used five housekeeping genes (*ADP1*, *ACC1*, *RPN2*, *GLN4*, and *ALA1*) for the MLST analysis and reported 10 polymorphic sites in the amplified fragments of these five genes.

Makgeolli is a popular traditional Korean alcoholic beverage made mainly from rice and *Nuruk* (a Korean fermentation starter). Because fine-filtering is avoided during its preparation, *Makgeolli* contains numerous nutritional compounds originating from rice, yeast, and even lactic acid bacteria [12]. Those nutritional compounds include vitamins, essential amino acids, organic acids, oligosaccharides, and dietary fiber [13]. *Makgeolli* has been demonstrated to have health-promoting biological activities including antioxidant [14], anti-hypertensive [15], anti-diabetic, [16] and anti-cancer [17] activities.

In Korea, various alcoholic beverages, including *Makgeolli*, are traditionally made in home-based facilities containing various natural microorganisms. Even though many microorganisms are involved in *Makgeolli* fermentation, *S. cerevisiae* strains are considered the main determinants of the quality of the final product and to contribute to the diversity of Korean traditional alcoholic beverages [18, 19]. Hence, it is very important to identify and classify natural *S. cerevisiae* strains for their industrial application [20]. Many studies have selected *S. cerevisiae* strains from natural sources, mainly *Nuruk*, based on the alcoholic fermentation characteristics of the strains. However, molecular typing of selected *S. cerevisiae* strains from natural sources has not been performed. Therefore, we selected and examined various MLST markers from the *S. cerevisiae* genome to evaluate genetic diversity among natural *S. cerevisiae* strains in Korea.

Materials and methods

Yeast strains

Yeast strains used in this work are listed in Table 1. These strains are from our laboratory collection that includes previous isolates from natural sources such as *Nuruk* and flowers. Information about reference *S. cerevisiae* strains (ref 1, ref 2, ref 3, ref 4, ref 5, and ref 6) was obtained from the literature [21–24] and the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). We also analyzed four commercial *S. cerevisiae* strains (com 1, com 2, com 3, and

Table 1 List of *S. cerevisiae* strains analyzed by MLST

Isolate no.	Date isolated	Source	
		Geographical location	Sample
95	2016	Korea	*Nuruk*
97	2016	Korea	*Nuruk*
98	2016	Korea	*Nuruk*
99	2016	Korea	*Nuruk*
100	2016	Korea	*Nuruk*
101	2016	Korea	*Nuruk*
102	2016	Korea	*Nuruk*
103	2016	Korea	*Nuruk*
104	2016	Korea	*Nuruk*
105	2016	Korea	*Nuruk*
106	2016	Korea	*Nuruk*
107	2016	Korea	*Nuruk*
W	2016	Korea	*Nuruk*
22S	2016	Korea	*Nuruk*
3-1	2017	Korea	*Nuruk*
S1-1	2017	Korea	*Cornus officinalis*
S1-2	2017	Korea	*Cornus officinalis*
S1-5	2017	Korea	*Cornus officinalis*
S2-6	2017	Korea	*Cornus officinalis*
S2-7	2017	Korea	*Cornus officinalis*
S2-8	2017	Korea	*Cornus officinalis*
S2-9	2017	Korea	*Cornus officinalis*
S2-10	2017	Korea	*Cornus officinalis*
S3-1	2017	Korea	*Cornus officinalis*
S3-3	2017	Korea	*Cornus officinalis*
S3-4	2017	Korea	*Cornus officinalis*
S3-5	2017	Korea	*Cornus officinalis*
3O1	2017	Korea	*Prunus serrulata*
3O2	2017	Korea	*Prunus serrulata*
3O3	2017	Korea	*Prunus serrulata*
3O4	2017	Korea	*Prunus serrulata*
3O5	2017	Korea	*Prunus serrulata*
3X1	2017	Korea	*Prunus serrulata*
3X2	2017	Korea	*Prunus serrulata*
3X3	2017	Korea	*Prunus serrulata*
3X4	2017	Korea	*Prunus serrulata*
3X5	2017	Korea	*Prunus serrulata*
5O1	2017	Korea	*Prunus serrulata*
5O2	2017	Korea	*Prunus serrulata*
5O3	2017	Korea	*Prunus serrulata*
5O4	2017	Korea	*Prunus serrulata*
5O5	2017	Korea	*Prunus serrulata*
Ref 1[a]		USA	Wine barrel
Ref 2[a]		Japan	Sake
Ref 3[a]		Chile	Wine
Ref 4[a]		South Africa	White wine

Table 1 continued

Isolate no.	Date isolated	Source	
		Geographical location	Sample
Ref 5[a]		Singapore	Bakery
Ref 6[a]			Laboratory strain [21]
Com 1[b]		Korea	Beer
Com 2[b]		Korea	Bakery
Com 3[b]		Korea	Bakery
Com 4[b]		Korea	Beer

[a]Reference strains isolated from various countries. Genetic information for these strains was obtained from Genbank

[b]Commercial strains used for industrial-scale baking and alcohol fermentation in Korea

com 4) used in the bakery and alcohol fermentation industries in Korea.

Genomic DNA extraction

Genomic DNA extraction was performed as described by Tavanti et al. [25] with slight modifications. Yeast cells were grown overnight at 30 °C in 10 mL YPD medium containing 1% yeast extract, 2% peptone, and 2% dextrose (Difco, Detroit, MI, USA). Yeast cells were centrifuged at 13,000 rpm for 5 min and the supernatant was discarded. Liquid nitrogen was used to break the yeast cell walls. Cells were then resuspended in 500 µl lysis buffer (200 mM Tris–HCl pH 8.5, 250 mM NaCl, 25 mM EDTA, and 0.5% SDS) and 500 µl phenol/chloroform/iso-amyl alcohol (25:24:1) was added. Cell lysate were pelleted at 13,000 rpm for 30 min in a microcentrifuge. Sodium acetate (3 M) and isopropyl alcohol were added to the supernatant at 1/10 and 6/10 of the supernatant volume, respectively. Following centrifugation at 13,000 rpm for 5 min, the supernatant was discarded and the pellet was washed with 1 mL ethanol. The pellet was then completely dried and 30 µl of TE-RNase [100 mM Tris–HCl (pH 8), 10 mM EDTA, and 20 µg of RNase/mL] was added to resuspend the genomic DNA.

Amplification of 5.8S-ITS region and identification

Amplification was performed using an ASTEC PC-320 thermal cycler (Astec Inc., Fukuoka, Japan). The ITS1 forward primer and ITS4 reverse primer were used to amplify the conserved 5.8S-ITS region [26]. Amplifications were performed in 30 µl reaction mixtures containing DNA polymerase (TaKaRa Taq^{TM}, 5 U/µl), 10X PCR buffer, dNTPs (2.5 mM each), forward primer (0.2–1.0 µM, final conc.), reverse primer (0.2–1.0 µM, final conc.), template (< 500 ng), and sterilized distilled water. After denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min were performed. This was followed by a final extension step of 5 min at 72 °C. The presence of amplified product was monitored on agarose gels. Sequencing of the amplified fragments was performed by Macrogen (Seoul, Korea). Isolates were identified using LaserGene (http://www.dnastar.com) and BLAST software (https://www.ncbi.nlm.nih.gov/BLAST/).

MLST analysis

Even though 10 genes were examined for the MLST analysis, only six genes were selected for the final analysis. The six genes encoded the following proteins: acetyl-CoA carboxylase (ACC1), ATP-dependent permease (ADP1), glutamine tRNA synthetase (GLN4), leucine-zipper transcriptional activator (MET4), the FG-nucleoporin component of central core of the nuclear pore complex (NUP116), and a subunit of the 26S proteasome (RPN2) [5, 27]. Internal fragments of each gene were amplified by PCR from genomic DNA of the sample *S. cerevisiae* strains. Primer sequences used for MLST analysis in this study are listed in Table 2. PCR was implemented in reaction volumes of 25 µl containing DNA polymerase (TaKaRa Ex Taq^{TM}, 5 U/µl), 10X Ex Taq buffer, dNTPs (2.5 mM each), forward primer (0.2–1.0 µM, final conc.), reverse primer (0.2–1.0 µM, final conc.), template (< 500 ng), and sterilized distilled water. One cycle of denaturation for 10 min at 95 °C was followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at various temperatures for 30 s, and elongation at 72 °C for 1 min, and then a final post-extension step of 10 min at 72 °C [27].

PCR fragments obtained for each locus were sequenced and compared with each other and/or the reference *S. cerevisiae* strains using Lagergene software (http://www.dnastar.com) and Clustal omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) [28]. Heterozygosity was defined as the occurrence of a mixed peak. Different sequences at each locus were assigned an arbitrary number. Concatenation of sequences from the six markers was performed and edited sequences were saved in FASTA file format using the MLSTest program (https://mlstest.codeplex.com/) [29]. A neighbor-joining (NJ) phylogenetic tree was generated using MEGA 6 software (www.megasoftware.net/). Confidence in nodes was determined using the bootstrap procedure with 1000 randomizations. A neighbor-joining tree shows the relationships among strains, and the branch lengths of the tree are proportional to the evolutionary distances among strains [30]. Because NJ trees are unrooted, common ancestry cannot be inferred.

Table 2 Primer sequences used for MLST analysis of *S. cerevisiae* strains

ORF	Gene	Primers	Sequence 5′ → 3′	Amplicon size (bp)
YNR016R	ACC1	ACC1 F	GCAAGAGAAATTTTGATTCAAGG	492
		ACC1 R	TTCATCAACATCATCTAAATG	
YOR168W	GLN4	GLN4 F	GAGATTGTCAAGAATAAAAAGGT	489
		GLN4 R	GTCTCTCTCATCCTTTGGACC	
YCR011C	ADP1	ADP1 F	GAGCCTTCTATGAATGATTTG	585
		ADP1 R	TTGATCGACGAACCCGATTAT	
YIL075C	RPN2	RPN2 F	TTTATGCACGCTGGTACTAC	450
		RPN2 R	GAGACCCATACCTAATGCAG	
YNL103W	MET4	MET4 F	CGAGGATAAGCCGAGCAA	395
		MET4 R	GCGCATCCACTCCATTGT	
YMR047C	NUP116	NUP116 F	AAGCAACTGTCACCAACACG	501
		NUP116 R	CTTCCCCATCGTTCTTTGAG	

Makgeolli brewing

The process used to brew *Makgeolli* is illustrated in Fig. 1. Raw rice was washed and soaked in water for 1 h. After soaking, the rice was drained for 30 min and ground in water using a blender. The ground rice was added to a bottle along with *Nuruk* (Kooksoondang Brewery CO., Ltd, Korea), yeast, and distilled water followed by fermentation at 25 °C for 24 h. After fermentation, more ground rice and distilled water were added to the first brew followed by fermentation at 25 °C for 6 days.

Alcohol content analysis

The alcohol content of *Makgeolli* was measured using a method described previously with slight modifications [31]. Briefly, 100 mL of sample was distilled using a distiller until around 80 mL was collected. The collected sample was adjusted to 100 mL with distilled water. The alcohol content (%) was measured using a density meter (DMA 4500, Anton-Paar, Ashland, VA, USA).

Alcohol production capacity of *Saccharomyces cerevisiae* strains

To measure alcohol production capacity, 100 mL of GY medium (20% glucose, and 3% yeast extract) was placed in a flask, and *Saccharomyces cerevisiae* was inoculated at a 0.1% concentration followed by incubation without shaking at 30 °C for 7 days. Culture medium was centrifuged at 10,000 rpm for 10 min and the supernatant was used to measure the alcohol content using the method described above [31].

Results and discussion

Selection of housekeeping genes for MLST analysis

The main objective of this research was to examine the suitability of MLST to differentiate 42 *S. cerevisiae* strains from natural sources as well as four Korean commercial strains (com 1, com 2, com 3, and com 4). In addition, the genomic diversity of those strains was compared with each other and those isolated from other countries (ref 1, ref 2, ref 3, ref 4, ref 5, and ref 6).

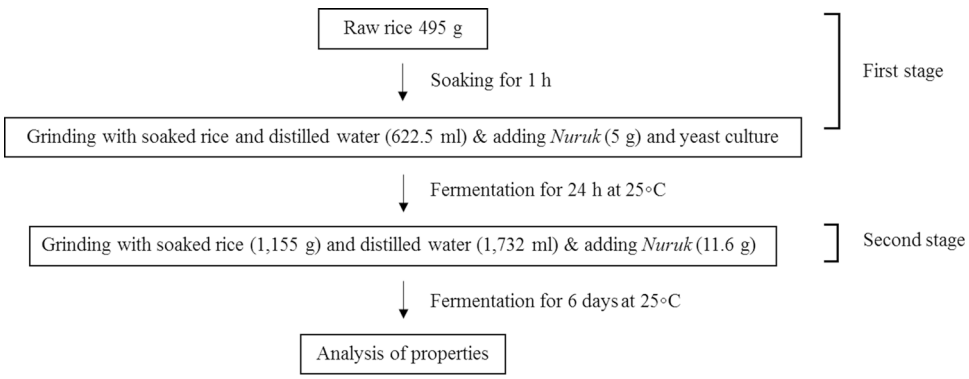

Fig. 1 Process used to brew *Makgeolli*

The first step in MLST analysis is the selection of adequate housekeeping genes. Previously, there were only two published reports in which MLST was applied to study the diversity of *S. cerevisiae* wine yeasts [5, 27]. Ayoub et al. [27] adopted seven loci (ATF1, MET4, RPN2, NUP116, STE50, YBL081W, and IntAY) to analyze the biodiversity of indigenous *S. cerevisiae* wine yeasts from Lebanon whereas Munoz et al. [5] used six different loci (ACC1, ADP1, GLN4, MET4, NUP116, and RPN2) to discriminate Spanish wine yeast strains at the strain level. Among those 12 loci, YBL081W and IntAY were not housekeeping genes [27]. YBL081W was the hypothetical ORF while IntAY was an intergenic locus between ORFs APP1 and YPT53). Therefore, we examined 10 loci (ACC1, ADP1, ALA1, ATF1, GLN4, MET4, NUP116, RPN2, STE50 and VPS13) to assess the degree of genetic variability in *S. cerevisiae* strains isolated from *Nuruk* and flowers in Korea.

Munoz et al. [5] examined 14 nuclear genes as potential MLST target genes, but found only a few single nucleotide polymorphisms (SNPs) (0–3) in the subset of strains analyzed depending on the particular locus. Therefore, they used six loci (ACC1, ADP1, ALA1, GLN4, RPN2, and VPS13) previously used to characterize clinical isolates of *Candida albicans* for MLST analysis and found 10 polymorphic sites and 13 different genotypes based on these five loci.

When we examined 10 loci, the sequences of the ALA1 and ATF1 fragments were conserved among all tested *S. cerevisiae* strains (data not shown), indicating that these genes had low discriminatory power. Therefore, ALA1 and ATF1 were eliminated from the list of target housekeeping genes for MLST analysis. MET4 and NUP116 genes were chosen because these genes have shown high discriminatory power in MLST analysis of wine yeast strains [27]. STE50 and VPS 13 were also considered target genes, but were excluded because they also displayed relatively low discriminatory power (data not shown). As a conclusion, six of the 10 loci (ACC1, ADP1, GLN4, MET4, NUP116, and RPN2) were finally selected. The PCR primers used for MLST analysis are listed in Table 2.

Effectiveness of MLST analysis and genetic diversity

Sequencing results for the 42 *S. cerevisiae* strains for the six loci revealed 26 polymorphic sites among 2617 bp, which corresponds to a sequence diversity of 0.99%. The proportion of polymorphic sites per locus varied between 0.74% (GLN4) and 1.73% (ACC1) with an average of 1.09%. All mutations were base substitutions. Approximately 23% (6 of 26) of the sites were heterozygous. ACC1 contained eight polymorphic sites that resulted into a classification of the 42 *S. cerevisiae* strains into five genotypes, while the MET4 gene yielded four genotypes among the 42 strains based on three polymorphic sites (Table 3). These results indicated that MLST can be used to type *S. cerevisiae* strains.

The major advantage of MLST is that simple PCR and sequencing of several house-keeping genes can generate sequence data that can be easily compared to data in a database; thus, it is fast and unambiguous. This technique has been successfully employed to discriminate among clinically important bacterial strains such as *Neisseria meningitides*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Campylobacter jejuni*, and *Listeria monocytogenes* [10, 32–35]. Recently, Wu et al. [36] used MLST to study the epidemiology and evolution of *C. albicans* by typing 62 *C. albicans* isolates. MLST has also been shown to be a useful tool for typing *S. cerevisiae* isolated from wine [5, 27]. Until now, however, it has not been employed to study the biodiversity of wild-type *S. cerevisiae* found in Korea.

MLST analysis using six housekeeping genes allowed construction of a reliable phylogenetic tree (Fig. 2). To verify the effectiveness of MLST, the sequence information of six loci (ACC1, ADP1, GLN4, MET4, NUP116, and RPN2) from ten more *S. cerevisiae* strains including six reference and four commercial strains (Table 1) were included in the phylogenetic tree.

Genetic information for four wine *S. cerevisiae* strains (ref 1–ref 4 in Table 1) from various countries (USA, Japan, Chile, and South Africa), a baking yeast (ref 5, Singapore), and a laboratory strain (ref 6, USA) were obtained from Genbank. The sequence information of six loci (ACC1, ADP1, ALA1, GLN4, MET4, NUP112, and RPN2) of these strains were extracted from their whole genome sequences. As shown in Fig. 2, three of the reference strains (ref 1, ref 3, and ref 4, all of which are wine-making strains) were clustered together. However, ref 2, a strain used in sake fermentation, which is different from typical wine fermentation but close to *Makgeolli* fermentation, was closely related to the *S. cerevisiae* strains isolated in Korea. In addition, ref 6, which is the most common laboratory strain used in academia, and ref 5, which is a baker's yeast from Singapore, were isolated from other strains.

Furthermore, we examined four commercial *S. cerevisiae* strains (com 1–com 4 in Table 1), used in the baking and alcohol fermentation industries in Korea. They all clustered together except com 1. In fact, all baking yeasts including com 2, com 3, and ref 5 were closely grouped together in the MLST analysis (Fig. 2). This suggests that there is little variation among commercial *S. cerevisiae* strains. Also, it is obvious that the wild *S. cerevisiae* strains isolated in Korea are somewhat different from commercial *S. cerevisiae* strains isolated in other countries. It was

Table 3 Polymorphic sites in the fragments of the six housekeeping genes tested

GLN4	1	1	4	*NUP116*	2	2	2	2	2	2
	3	9	5		3	3	4	4	4	6
	6	5	0		3	8	2	4	5	0
					5	2	2	2	4	4
Genotype 1 (38)	C	G	T	Genotype 1 (36)	A	A	G	G	C	C
Genotype 2 (1)	G	G	T	Genotype 2 (3)	G	G	C	T	A	T
Genotype 3 (3)	C	A	C	Genotype 3 (3)	R	A	G	G	C	C
MET4	6	7	7	*ADP1*	9	1	1			
	3	1	5		6	0	1			
	0	6	9		9	5	0			
						0	9			
Genotype 1 (29)	T	G	Y	Genotype 1 (34)	G	G	G			
Genotype 2 (10)	T	G	T	Genotype 2 (1)	R	R	G			
Genotype 3 (1)	C	T	T	Genotype 3 (4)	A	R	G			
Genotype 4 (2)	C	K	T	Genotype 4 (3)	G	A	A			

ACC1	3	3	3	3	3	3	3	3	*RPN2*	3	3	3
	3	6	6	6	6	7	7	7		0	1	1
	6	0	4	6	6	0	4	5		1	0	4
	3	3	1	3	9	2	4	4		8	4	7
Genotype 1 (33)	C	T	A	C	C	A	A	T	Genotype 1 (39)	T	A	C
Genotype 2 (5)	C	C	T	T	C	A	A	C	Genotype 2 (3)	C	C	T
Genotype 3 (1)	C	T	W	C	C	A	A	C				
Genotype 4 (2)	T	C	A	C	T	G	G	T				
Genotype 5 (1)	T	T	A	C	C	A	A	T				

The number of the polymorphic sites (vertical format) is the order from the first nucleotide of each housekeeping gene. All polymorphic sequences are shown. (Y = T or C, K = G or T, R = G or A, and W = A or T)

interesting that two commercial *S. cerevisiae* (com 1 and com 4) used in beer fermentation in Korea were not tightly linked together. Especially, com 4 was grouped with baker's yeast, suggesting that it was originally used in baking industry.

A total of 42 *S. cerevisiae* strains isolated from natural sources, such as *Nuruk* and flowers, were broadly classified into three clades based on MLST analysis and their applications as shown Fig. 2. Clade I contained 39 of the 42 *S. cerevisiae* strains (93%). No *S. cerevisiae* strains isolated from natural sources were members of Clade II, while Clade III contained the remaining three isolated *S. cerevisiae* strains (7%). None of the clades showed any correlation with the original sample sources from which the strains were isolated.

Alcohol fermentation characteristics of selected *S. cerevisiae* strains

To investigate if there is any difference between *S. cerevisiae* strains belong to each Clade groups in MLST analysis, *Makgeolli* fermentation characteristics of each *S. cerevisiae* strains were examined. In fact, there was not much difference in *Makgeolli* fermentation characteristics in Clade I and Clade II groups by showing typical *Makgeolli* fermentation. However, *S. cerevisiae* strains belonged to Clade III showed rather different properties in *Makgeolli* fermentation. The final alcohol contents of *S. cerevisiae* strains belonged to Clade III (14.7–17.5%) was lower than other *S. cerevisiae* strains in Clade I (19.7–20.7%) and Clade II (18.5%) groups (Table 4).

The ability of the *S. cerevisiae* strains to produce alcohol when the GY medium was supplemented with 20% glucose was examined. While there was little difference between strains from Clades I and II, there was distinct difference between strains in these clades and the alcohol production capacity of *S. cerevisiae* strains in Clade III. *S. cerevisiae* strains in Clade III produced less alcohol (9.2–9.7%) than strains in Clades I and II (10.4–11.6%) (Table 4). These results indicate that classification of *S. cerevisiae* based on MLST analysis is somewhat related to the alcohol fermentation properties of the *S. cerevisiae* strains.

In conclusion, MLST analysis was performed to differentiate among *S. cerevisiae* strains isolated from natural

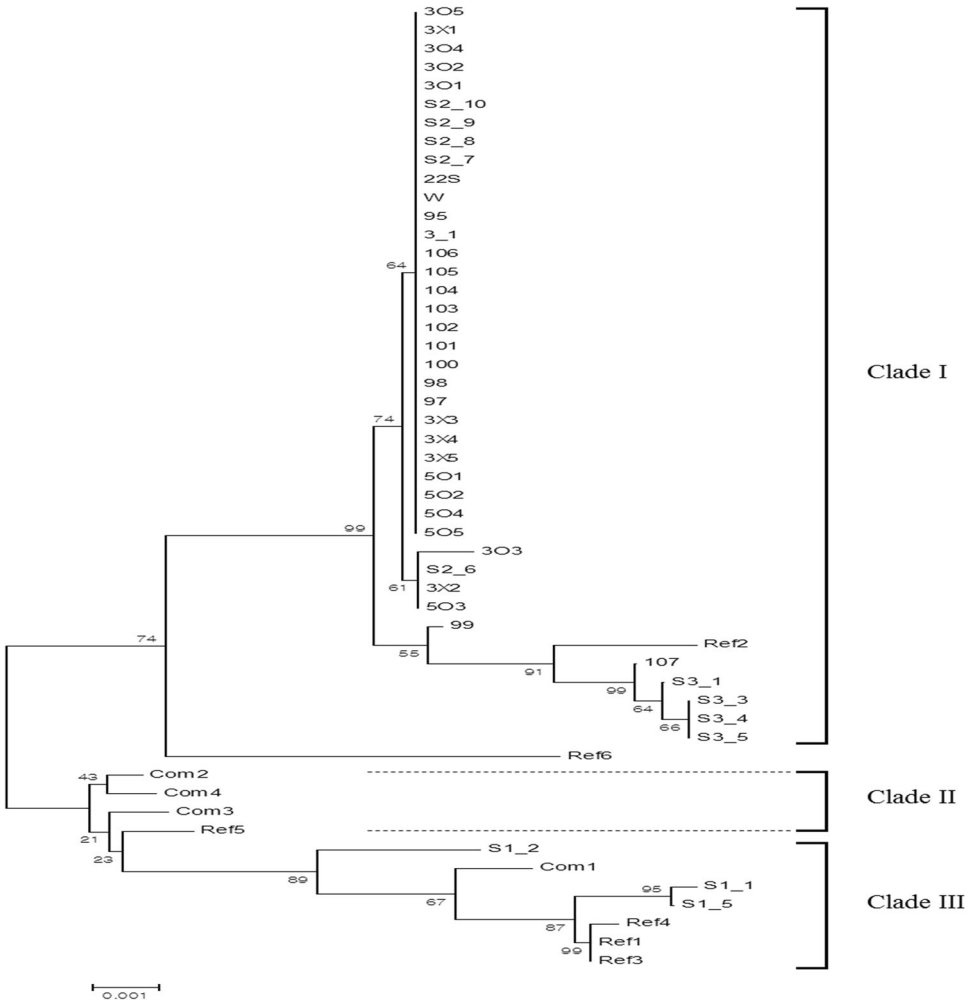

Fig. 2 Unrooted neighbor-joining tree of the genetic relationships among 52 *S. cerevisiae* strains with branch lengths proportional to the *p*-distance. Numbers within the tree indicate the bootstrap values for nodes

Table 4 Alcohol fermentation characteristics of representative *Saccharomyces cerevisiae* strains isolated from natural sources

Number	Alcohol content of *Makgeolli* (%)	Alcohol production capacity (%)	Clade
S2-6	20.7	11.0	Clade I
S2-7	20.5	10.6	
S2-8	20.5	11.0	
S2-9	20.6	10.5	
S2-10	19.9	10.4	
3O1	20.7	11.1	
3O3	19.7	11.4	
3X1	20.2	11.2	
3X2	20.2	10.9	
5O1	20.0	10.6	
5O3	20.0	11.4	
Com2	18.5	11.2	Clade II
S1-1	14.7	9.5	Clade III
S1-2	16.0	9.2	
S1-5	15.4	9.5	
Com1	17.5	9.7	

sources. MLST analysis can be used to assess genetic variation. Clustering of allele profiles differentiated the 52 *S. cerevisiae* strains examined into three groups (Clade I, Clade II, and Clade III). Among those groups, strains belonging to Clade III exhibited lower alcohol fermentation capacity than strains belonging to Clades I and II. This result indicates that MLST analysis could be a highly reliable method to study the genetic diversity of *S. cerevisiae* strains and a useful tool to classify *S. cerevisiae*, an important baking and brewing yeast.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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