Food Microbiology 34 (2013) 207–214

Contents lists available at SciVerse ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Role of non-*Saccharomyces* yeasts in Korean wines produced from Campbell Early grapes: Potential use of *Hanseniaspora uvarum* as a starter culture

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ARTICLE INFO

Article history:
Received 13 October 2011
Received in revised form
9 March 2012
Accepted 27 December 2012
Available online 5 January 2013

Keywords:
Campbell Early
Grape
Hanseniaspora uvarum
Indigenous yeast
Wine

ABSTRACT

Several yeasts were isolated from Campbell Early grapes (*Vitis labrusca* cultivar Campbell Early), the major grape cultivar in Korea, grown in two different regions. PCR-RFLP analysis of the ITS I-5.8S-ITS II region showed that 34 isolates out of a total of 40 were in the same group. Phylogenetic analysis revealed that the major strain belonged to one species, *Hanseniaspora uvarum*, although they displayed some nucleotide mismatches between them. During spontaneous alcohol fermentation at 20 °C, the two grape musts containing 24 °Brix sugar exhibited similar fermentation patterns with differences in final alcohol production and yeast viable counts. PCR analysis of the yeasts randomly isolated during the fermentation using an intron splice site primer showed changes in yeast flora between 8 and 10 days of fermentation. We found that the dominant yeasts displaying various PCR patterns using the primer remained the same throughout the early stages of fermentation, as determined by molecular typing of their ITS regions using PCR-RFLP, and these yeasts were identical to those isolated from grape berries. Among the isolates, the strain designated SS6 was selected based on its potassium metabisulfite resistance, alcohol production (distillation method), and flavor (by sniffing test) of grape juice. When Campbell Early grape must was inoculated with *H. uvarum* SS6 cells, no differences in fermentation pattern were observed compared with that inoculated with cells of *Saccharomyces cerevisiae* W-3, an industrial wine yeast strain. However, SS6 wine showed higher levels of organic acid (especially lactic acid), aldehydes, and minor alcohols (except *n*-propyl alcohol), as well as a higher score in sensory evaluation, compared to those of W-3 wine.

1. Introduction

Wine, in general, is made from one or more varieties of the European species *Vitis vinifera*, among which are there Cabernet Sauvignon, Merlot, Pinot Noir, Chardonnay, etc. It can be also made from other grape species such as *Vitis labrusca*, a native North American grape variety, or from its hybrids (Jackson, 1994). Most Korean domestic wines are fermented using Campbell Early grapes (*V. labrusca* cultivar Campbell Early) since the variety constitutes more than 70% of the total grape production in Korea (Kim, 2005; Yook et al., 2007). Campbell Early grape has been reported to contain a low level of sugar (13–15%) and high level of malic acid (4–16 mg/mL) (Kim et al., 1999; Lee and Park, 1980; Park et al., 2004). However, Campbell Early grape berries contain a significantly high foxy aroma content that negatively affects wine quality (Hong et al., 2010). These features are believed to be the main barriers to making a good wine using Campbell Early grapes.

Although there have been several attempts to grow other grape varieties suitable for winemaking in grape farmlands in Korea, none has been successful thus far. Therefore, several studies have recently attempted to solve the problems associated with winemaking using Campbell Early grapes, which has adapted to the Korean environment (Lee et al., 2004; Park et al., 2002). Among them, there have been studies on the fermentation of a mixture of Korean grapes with other fruits (Yook et al., 2007), the effects of sugar addition on wine fermentation (Kim et al., 1999), application of carbonic maceration or reduction of malic acid content (Park et al., 2004), the application of malo-lactic acid fermentation (Lee and Park, 1980), and malic acid degradation yeast (Hong et al., 2010; Kim et al., 2008), etc.

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http://dx.doi.org/10.1016/j.fm.2012.12.011

It is generally assumed that indigenous yeasts are suppressed competition with starter monocultures inoculated at high-density due to the general use of pure yeast cultures (Pretorius, 2000; Rankine, 1977). However, several studies have shown that indigenous yeasts can still participate in fermentation, resulting in a unique style of wine depending on the grape cultivar and the regions where the grapes were grown (Heard and Fleet, 1985; Mercado et al., 2007; Querol et al., 1992; Schütz and Gafner, 1993). Therefore, the efficacy of non-*Saccharomyces* yeasts in wine fermentation has received increased attention by wine microbiologists (Ciani et al., 2010; Fleet, 2008; Jolly et al., 2006). Several attempts have been made to perform mixed fermentation with non-*Saccharomyces* yeasts and *Saccharomyces cerevisiae* for the purpose of enhancing particular and specific characteristics of wines (Bely et al., 2008; Ciani et al., 2006, 2010; Kim et al., 2008; Mendoza et al., 2007). To produce a unique wine using indigenous yeasts, it is helpful to emphasize their beneficial functions as well as inhibit their harmful effects (Ciani et al., 2010). This requires methods for identifying yeasts and differentiating between yeast isolates based on raw grapes in order to prevent the isolation of certain strains sharing the same genetic background.

Despite a short history, molecular biology methods have been successfully applied to the differentiation as well as identification of yeasts. Among such methods, DNA-based methods have the advantage of being able to handle many strains at once as well as being independent of gene expression (de Barros-Lopes et al., 1996; Ness et al., 1993). Especially, methods based on polymerase chain reaction (PCR) have been shown to be appropriate tools for the rapid differentiation and identification of yeast. Differences in internal transcribed spacer (ITS) and 5.8S rDNA sequences can be used to identify yeast species based on PCR-RFLP (Granchi et al., 1999; Torija et al., 2001) and phylogenetic analyses (Montrocher et al., 1998; Oda et al., 1997; Seo et al., 2007). Although intron splice site primers were originally used for the differentiation of commercial yeast strains (de Barros-Lopes et al., 1996), use of these primes in PCR amplification has been applied to the differentiation of yeast strains isolated from other sources (Park et al., 1999).

The aims of this work were to analyze yeast diversity in Korean Campbell Early grape berry during spontaneous wine fermentation as well as to isolate indigenous yeasts capable of improving wine quality. To minimize the problems associated with multiple isolation of certain yeast strains, all of the isolates were analyzed for their PCR amplification patterns using intron splice primers and PCR-RFLP of the ITS 1-5.8S-ITS II region. Characteristics of the yeast isolates were also investigated, such as the tolerance to potassium metabisulfite as well as their alcohol and flavor production from Korean grape must.

2. Materials and methods

2.1. Strain, media, and culture conditions

S. cerevisiae W-3, an industrial wine yeast strain, was used as a control for the wine fermentation. Yeast cells were generally grown at 30 °C with shaking (150 rpm) in YPD media composed of 1% yeast extract, 2% bacto-peptone, 2% glucose, and/or Difco malt extract media to harvest cells for wine fermentation. All isolated yeast cells were stored at −70 °C in the presence of 15% glycerol for the next experiments.

2.2. Wine fermentation

Spontaneous wine fermentation was carried out using Campbell Early grapes grown in two different regions of Korea about 200 km away from each other. Grape must (10 kg) obtained by destemming and crushing of grapes was fermented at 20 °C without metabisulfite treatment and starter yeast cells.

To test alcohol production, yeast cells were obtained by centrifugation of their culture broth in 1 L of Difco malt extract media. The yeast cells were then inoculated into 10 kg of Campbell Early grape must to a final concentration of $\sim 10^6$ cfu/ml containing $\sim 10^4$ cfu/ml of wild yeasts in the presence of 200 μg/ml of potassium metabisulfite. The inoculated must was incubated at 20 °C for 8 days to perform alcohol fermentation.

2.3. Yeast isolation

Yeasts were randomly isolated from Campbell Early grape berry and grape must during spontaneous fermentation. For isolation of grape indigenous yeasts during fermentation, alcohol fermentation of grape must was carried out at 20 °C for 18 days without inoculation with starter yeast cells. Grape berry and must samples were collected and sequentially diluted with sterilized 0.9% NaCl solution. The diluted samples were then spread onto YPD agar plates, which were incubated at 30 °C for 2 days. After incubation, colonies were randomly picked and grown on YPD plates again for isolation of single colonies. All colonies were observed on a microscope to confirm their yeast-like shapes and sizes, followed by PCR analysis to determine their diversity.

2.4. Triphenyl tetrazolium chloride (TTC) staining

Yeast TTC color counts were directly determined based on their different colony colors on YPD plates using 2,3,5-triphenyl tetrazolium chloride (TTC) staining according to the method of Bochneri and Savageau (1977). Diluted wine samples were spread onto YPD plates, which were incubated at 30 °C for 24 h to allow the yeast cells to form colonies. When small colonies formed, 10 ml of a warm solution containing 0.05% (w/v) 2,3,5-triphenyl-2H-tetrazolium chloride (separately sterilized), 0.5% glucose, and 1.5% agar was poured on top of the YPD agar plates. The plates were then incubated at 30 °C overnight to stain the colonies. Colonies with a red or white color were counted directly on the plates. Yeast viable counts are expressed as the total number of colonies. Relative ratio of TTC red cells to white cells was calculated as the percentage of TTC red and white colonies per their respective total viable count.

2.5. PCR and PCR-RFLP

Chromosomal DNA for the PCR template was isolated from yeast cells grown in YPD media for 24 h by the method described by Kaiser et al. (1994) and Philippsen et al. (1991). Oligonucleotide primers for PCR were synthesized by a commercial company (Bioneer Co., Chongwon, Korea). Yeast intron splice site primer set (5′- CTGGCTTGGTGTATGT and 5′- CTGGCTTGCTACATAC) (de Barros-Lopes et al., 1996) and ITS primer set (ITS1, 5′-CATTTAGAGGAACTAAAAGTCG-3′ and ITS4, 5′-CCTCCGCTTATTGATATGC-3′) were used for PCR and PCR-RFLP analysis. PCR was performed in a 20 μl reaction volume using TaKaRa Taq DNA polymerase (Takara Shuzo Co., Otsu, Japan) with a GENE cycler (BioRad Co., Richmond, USA). The PCR mixture consisted of 1 μg of yeast genomic DNA, 100 pmol of each primer, 1 U of Taq DNA polymerase, 0.25 mM each dNTPs, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, and 2.5 mM $MgCl_2$. The PCR cycle program for DNA amplification consisted of one cycle of 94 °C for 3 min, 35 cycles of 94 °C for 45 s, 55 °C for 60 s, 72 °C for 60 s, and finally one cycle of 72 °C for 10 min. For PCR-RFLP, excess dNTPs and primers were removed from the PCR products using a PCR purification kit (Solgent, Daejeon, Korea). Appropriate amounts of amplicons were digested at 37 °C for 1 h

with 0.5 μl of *Hinf* I and *Hae* III endonucleases (Takara Shuzo Co., Otsu, Japan). DNA fragments were resolved on 5% polyacrylamide gel or 1.5% agarose gel based on size according to general methods (Sambrook and Russel, 2001). One kilobase or 100 bp ladders were used as a marker for checking DNA size.

2.6. Phylogenetic analysis

The nucleotide sequences of the ITS I-5.8S rDNA-ITS II region were compared with those available in the GenBank database using the BLAST method in order to investigate their approximate phylogenetic affiliation, and their sequence similarities were determined at the National Center for Biotechnology Information, USA (Altschul et al., 1997; http://ncbi.nlm.nih.gov/BLAST). The sequences of the related taxa were acquired from the same website. Nucleotide sequences were initially aligned using the CLUSTAL X program (Thompson et al., 1997) and then manually adjusted. Distance matrices were calculated, and a phylogenetic tree for the data set was created according to the Kimura two-parameter model (Kimura, 1980) and neighbor-joining method (Saitou and Nei, 1987) using the Mega 4 (version 4.02) software package obtained from the website (www.megasoftware.net).

2.7. Analytical methods

For analysis of alcohol content during wine fermentation, samples were collected and filtered through Whatman No. 1 paper. The filtrates were distilled, cooled down to 15 °C, and then diluted with distilled water to the same volume of the filtrate before the distillation. Alcohol content was assayed using a hydrometer based on the specific gravity of the wine distillates and is expressed as % (v/v) at 15 °C according to the AOAC method (Caputi, 1995). Contents of soluble solid and reducing sugar were determined by using a refractometer according to the AOAC method. The total acid content was assayed by titration of the filtrates with 0.1 N NaOH and is expressed as % tartaric acid (Caputi, 1995). Organic acid content was determined by HPLC with a Shodex RSpak KC-811 column (diam. 8 × 300 mm, Showa Denko KK, Kawasaki, Japan). The column chromatography conditions were as follows: flow rate 1 ml/min, temperature 40 °C, and mobile phase 0.1% phosphoric acid. Organic acids were detected using a refractive index detector. Contents of acetaldehyde and minor alcohols were determined using a GC equipped with an HP-FFAP column (diam. 0.25 mm × 30 m) with temperature programming. An FID was used for detection. Hue and intensity values of the wine were obtained based on OD_{420}/OD_{520} and $OD_{420} + OD_{520}$, respectively. Hunter's value was determined using a vertical type spectrophotometer (Konica Minolta Holdings, Inc. CM-3600d, Tokyo, Japan).

2.8. Sensory evaluation

Sensory evaluation of wine was conducted according to a 5-point hedonic scale. The panel was composed of 10 judges (five females and five males) from the Department of Food Science and Technology, Kyungpook National University, Korea who were deemed sensitive at taste discrimination. Sensory scores were 5 (excellent), 3 (fair), and 1 (very poor). All data were analyzed with the SPSS (Statistics Package for the Social Science, version 12.0 for Windows) package to obtain averages and standard deviations. ANOVA was conducted to compare physicochemical characteristics and sensory properties according to each wine as well as to test the significance between average values of each measurement at $P < 0.05$ using Duncan's multiple range test.

3. Results and discussion

3.1. Identification of major wild yeasts associated with Campbell Early grapes

Several yeast strains were isolated from Campbell Early grape berries grown in two different regions in Korea in order to identify major wild yeasts. Analysis of the 20 yeast isolates from each of the two grape types by PCR-RFLP of the ITS I-5.8S-ITS II region found that all isolates from each grape type showed the same patterns. Among the 20 isolates of each type, 14 showed the same patterns while six isolates showed different patterns (Fig. 1). Phylogenetic analysis of the 20 strains isolated from one region revealed that all isolates were closest to *Hanseniaspora uvarm* type strain CBS 2584, although they could be divided into five groups due to minor nucleotide mismatches in the ITS I-5.8S-ITS II region (Fig. 2). Sequence identities of the isolates were between 99 and 100% compared with the type strain. The significance of the mismatches has not yet been elucidated.

The two non-*Saccharomyces* yeasts, *Hanseniaspora uvarum* and *Hanseniaspora guilliermondii*, are reportedly found at high cell densities, up to 10^6–10^8 cells/ml in *V. vinifera* grape must. Therefore, *H. uvarum* (its anamorph *Kloeckera apiculata*) is the major indigenous yeast strain in grape must together with *H. guilliermondii* (Rojas et al., 2003). However, *H. guilliermondii* was not found among the 20 randomly yeasts isolated from Campbell Early (*V. labrusca*) grape berries in this study. This could be attributed to differences in the physicochemical properties of the grape varieties that affect the adaptation of wild yeasts. Another possible reason could be the different environmental conditions such as the soil, climate, etc. based on the regions where the grapes were grown. In order to explore these possible differences, the diversity of yeast flora, is being studied using two different Korean major grape varieties grown in several different farm regions.

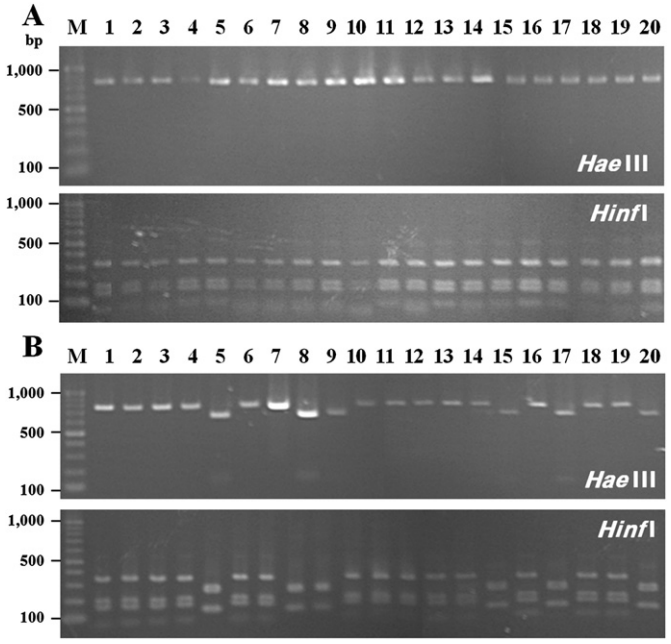

Fig. 1. PCR-RFLP of ITS I-5.8S-ITS II fragments amplified from several yeasts isolated from Campbell Early grape berries. Yeasts (20 isolates each) were randomly isolated from grape berries grown in two different regions (A and B) about 200 km away from each other. DNAs were digested with *Hae* III or *Hinf* I and resolved on 1.5% agarose gel. Lane M represents a 100 bp DNA ladder.

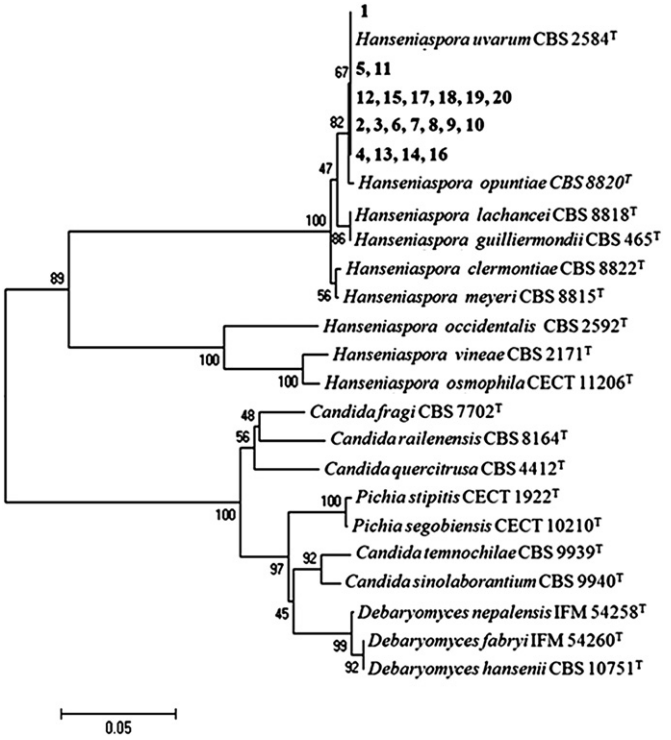

Fig. 2. Phylogenetic tree of yeast isolates based on ITS I-5.8S-ITS II sequences. Other related sequences were obtained by BLAST search at the NCBI website (www.ncbi.nlm.nih.gov) and were aligned using Clustal X software. The tree was constructed using MEGA 4 software with the neighbor-joining method and the Kimura two-parameter calculation model (ref. the Materials and methods section).

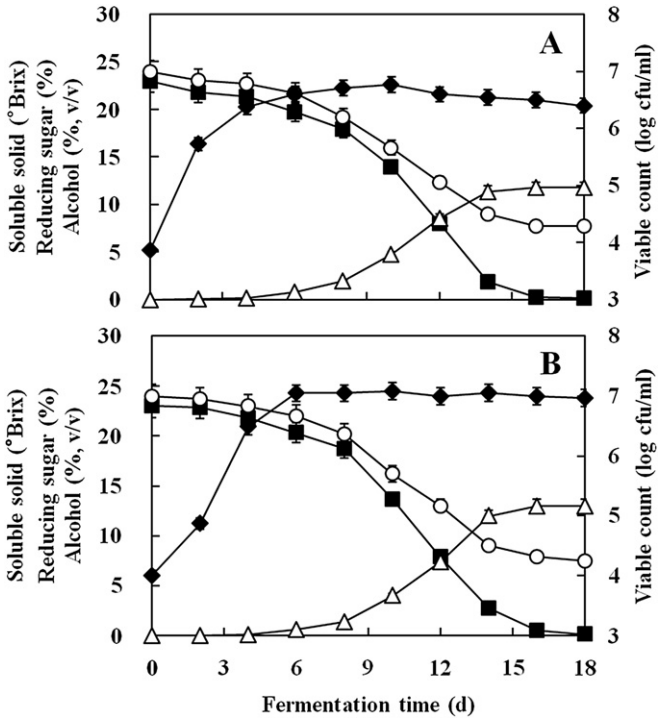

Fig. 3. Characteristics of spontaneous alcohol fermentation of Campbell Early grape must. Grapes grown in two different regions (A and B) were fermented without starter inoculation at 20 °C for 18 days. During fermentation, changes in the contents of soluble solids (○), reducing sugar (■), and alcohol (△), as well as the viable count (◆), were monitored every 2 days.

3.2. Yeast population dynamics of spontaneous fermentation of Campbell Early grape must

During the spontaneous fermentation of Campbell Early grape must, patterns of alcohol production and sugar consumption were similar to each other among the grapes grown in the two different regions. During the first 4 days of fermentation, neither sugar consumption nor alcohol production was observed. High level of alcohol production and a rapid decrease in sugar content were observed between 8 and 14 days of fermentation. The highest alcohol contents of the two grapes were 11.0 and 13.0% (v/v), respectively, dependent on the region where the grapes were grown. Yeast viable counts were higher in the must showing higher final alcohol production (Fig. 3).

In general wine fermentation, yeast starter is prepared using an industrial wine yeast strain in order to reduce the lag phase period and to accelerate alcohol production. This can also help prevent contamination by undesirable microorganisms during fermentation. When yeast starter is used, the lag phase decreases according to the type of yeast starter and the amount used (Fleet and Heard, 1993). It has been demonstrated that inoculation of grape must with a pure culture of a selected yeast strain results in rapid, reliable, and predictable fermentation (Rankine, 1968). In this study, alcohol production was delayed until 4 days of fermentation since no yeast starter was used. Since then, alcohol content increased rapidly along with an increase in the viable yeast count. Although fermentation took a long time, the maximal level of alcohol production was similar to that typically obtained with Campbell Early grapes using wine yeast starters.

During the spontaneous alcohol fermentation of Campbell Early grapes grown in the two different regions, several yeast strains were randomly isolated based on fermentation time. PCR analysis of the isolates using intron splice site primer revealed that there were two different typical yeasts, one at the early stage of fermentation and the other at the late stage. A significant change in the yeast flora occurred between 8 and 10 days of fermentation. Following this, yeasts showing the same PCR patterns were dominant until 18 days of fermentation (Fig. 4). Although PCR analysis found that there were two major yeast groups, the early stage yeast group showed strong diversity depending on fermentation time. Several early stage yeasts showing different PCR patterns were randomly selected (renamed SS1 to SS14) and their amplified DNA patterns analyzed again. Most of the yeasts showed different patterns in their amplified DNA fragment profiles using intron splice primer (Fig. 5A). However, they showed the same patterns in PCR-RFLP analysis of the ITS I-5.8S-ITS II region (Fig. 5B). The yeasts isolated during the late stage of fermentation were also analyzed by PCR-RFLP of the ITS I-5.8S-ITS II region (Fig. 6). All of the isolates showed the same patterns as that of *S. cerevisiae* W-3, which was used as a control strain (Fig. 6, lane W).

In order to select an early stage yeast strain capable of being applied to wine fermentation, all of the strains were tested for their resistance to potassium metabisulfite and a high concentration of glucose in YPD liquid media. They were also tested for their alcohol content (by the distillation method) and flavor production (by the sniffing test) in wine after fermentation of filter-sterilized grape juice on a small scale. One strain, designated SS6, was selected based on its resistance to fermentation conditions as well as it alcohol content and flavor production in wine. It was found that strain SS6 was highly resistant to up to potassium metabisulfite up to a concentration of 500 μg/ml. It also showed relatively strong sugar tolerance with a significantly slow growth rate in YPD liquid media containing 30% and 50% glucose compared with that obtained in YPD media. However, its cell density after culture in the

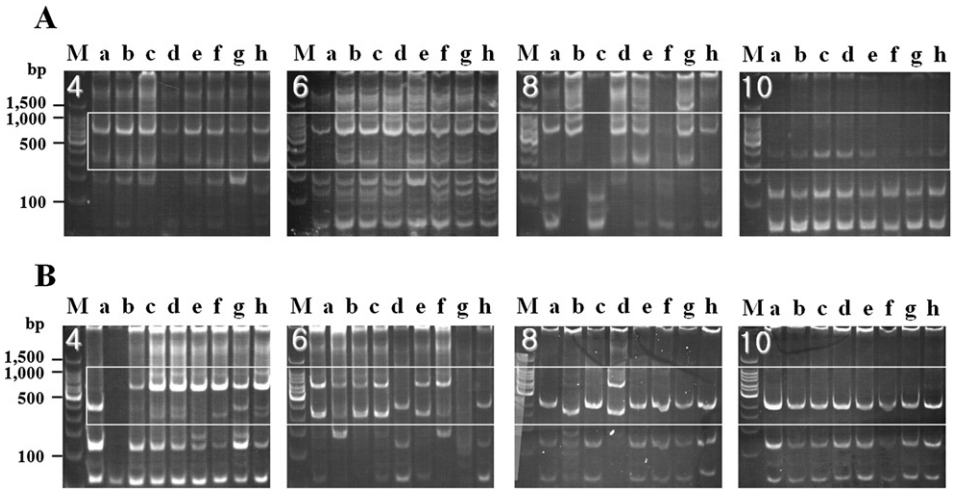

Fig. 4. Changes in PCR patterns of yeasts randomly isolated during alcohol fermentation of Campbell Early grape must. Yeasts randomly isolated during fermentation of grapes grown in two different regions (A and B) were used as PCR template DNA sources. DNA fragments were amplified using yeast intron splice primers and directly resolved on 5% polyacrylamide gel. Numbers inside each figure indicate fermentation day. Lane M represents a 100 bp DNA ladder.

presence of 500 μg/ml of potassium metabisulfite or 50% glucose was almost the same as that in YPD media, it was even higher in the presence of 30% glucose (Fig. 7). DNA sequences of the ITS I-5.8S-ITS II region of strain SS6 were 100% identical to those of *H. uvarum* type strain MH 501. Further, many of the physiological and biochemical characteristics tested in this study were the same as those described by Kurtzman and Fell (1998) (data not shown). Therefore, strain SS6 was identified as a strain of *H. uvarum*.

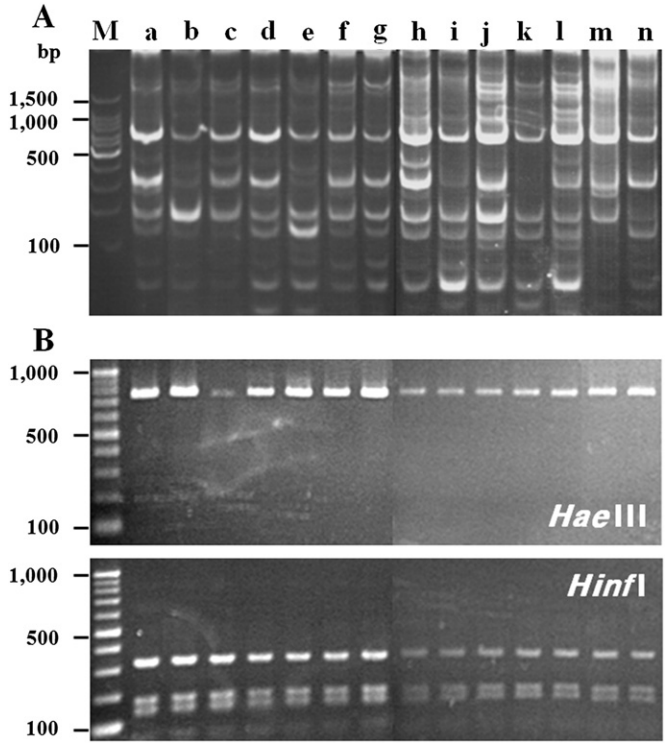

Fig. 5. PCR patterns of yeast isolates dominant at the early stage of fermentation of Campbell Early grape must. Yeasts were randomly isolated after 6 days of fermentation in which the dominant microflora did not shift. Panel A, Polyacrylamide gel electrophoretic patterns of the amplified with yeast intron splice primers. Panel B, Agarose gel electrophoretic patterns of amplified ITS I-5.8S-ITS II DNA fragments digested with *Hae* III or *Hinf* I.

3.3. Effects of *H. uvarum* SS6 on the chemical and sensorial properties of wine

Experimental wine fermentation was carried out using Campbell Early grape must in the presence of 200 μg/ml of potassium metabisulfite. Grape must was inoculated with *H. uvarum* SS6 cells or *S. cerevisiae* W-3, an industrial wine yeast strain, as a control. During fermentation, changes in the contents of alcohol and soluble solids as well as the viable counts of TTC white and red cells were analyzed (Fig. 8). W-3 cell-inoculated must showed a much more rapid decrease in soluble solid content along with an increase in alcohol content compared to SS6 cell-inoculated must. However, the maximal alcohol content reached the same level after fermentation for 8 days (Fig. 8A). Lower yeast viable counts were observed in SS6 cell-inoculated must compared to W-3 cell-inoculated must throughout all fermentation periods, except fermentation for 2 days. During the early stage of fermentation with inoculated SS6 cells for 3 days, almost all yeasts were stained as

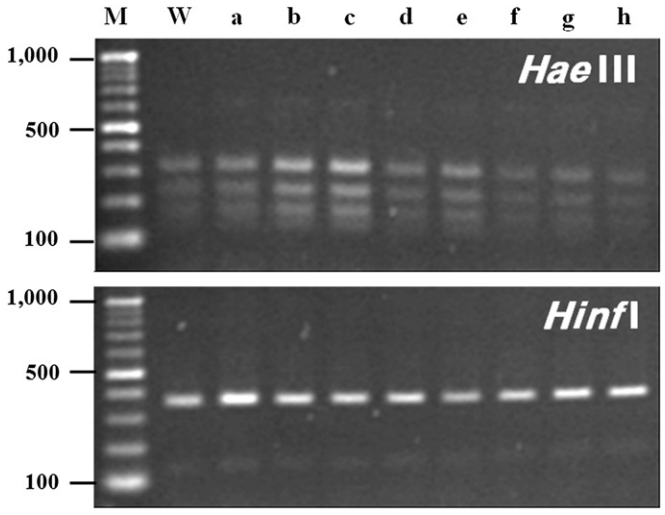

Fig. 6. PCR-RFLP of ITS I-5.8S-ITS II fragments amplified from several yeasts isolated during the late stage of fermentation. DNAs were digested with *Hae* III or *Hinf* I and resolved on 1.5% agarose gel. Lane M represents a 100 bp DNA ladder and lane W indicates *S. cerevisiae* W-3. Lanes a to h represent the yeast isolates.

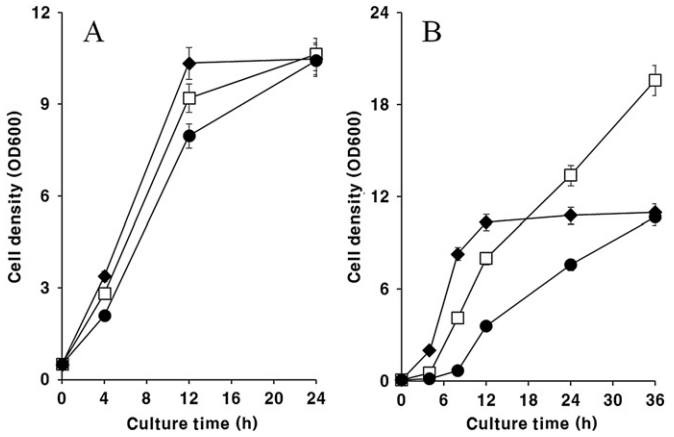

Fig. 7. Resistance of *H. uvarum* SS6 to potassium metabisulfite (A) and high concentrations of glucose (B). Panel A, Changes in cell density during culture in YPD liquid media in the absence (◆) or presence of 200 μg/ml (□) and 500 μg/ml (●) of potassium metabisulfite. Panel B, Changes in cell density during culture in YPD liquid media supplemented with 2% (◆), 30% (□) and 50% glucose (●).

white or pink. Further, the TTC white cell ratio rapidly decreased along with an increase in the TTC red cell ratio. After 6 days of fermentation, almost all of the yeast cells were stained as TTC red. However, no TTC white cells were observed during the entire fermentation when W-3 cells were inoculated (Fig. 8B). TTC staining was developed for the detection of biological agents (Gunz, 1949) as well as to determine the growth activity of yeast phase *Histoplasma capsulatum* (Reca, 1968). Since then, it has been applied to several types of research, including yeast species differentiation (Sobczak, 1985). *H. uvarum* and *S. cerevisiae* cells were stained as white or pink (designated TTC white) and red (TTC red), respectively, on the TTC agar plates (data not shown).

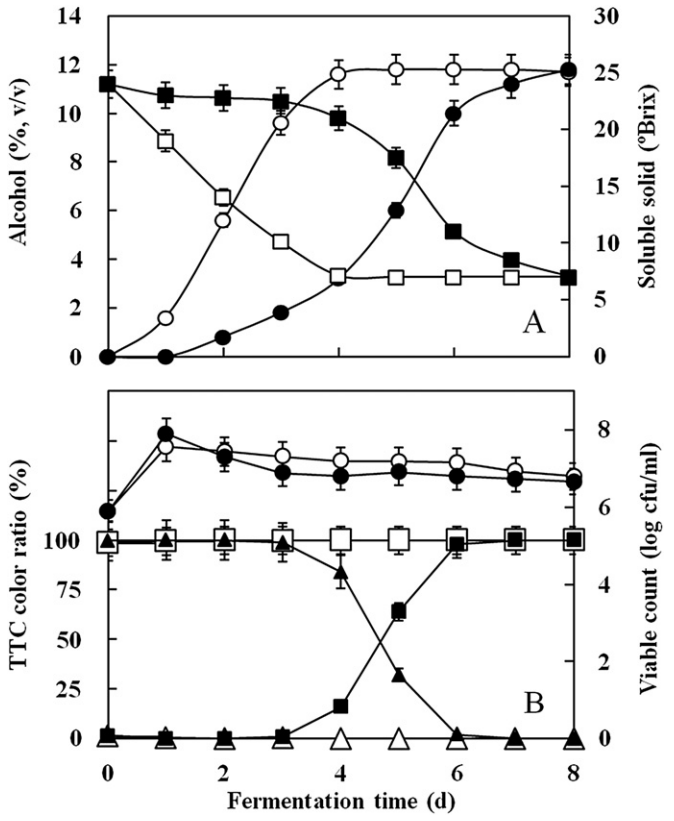

Fig. 8. Characteristics of alcohol fermentation of Campbell Early grape must upon inoculation with *H. uvarum* SS6 and *S. cerevisiae* W-3 cells. Panel A, Changes in the contents of alcohol (○,●) and soluble solids (□,■). Panle B, Changes in viable count (○,●) as well as TTC (2,3,5-triphenyl-2H-tetrazolium chloride) red (□,■) and white cell ratios (△,▲). Fermentation was carried out at 20 °C for 8 days after inoculation with starter cells to ~10^6 viable counts/ml. TTC color relative ratio is expressed as the percentage of TTC red and white colonies per total viable counts obtained for the TTC staining. Open and closed symbols represent inoculation with W-3 and SS6 cells, respectively.

After fermentation, the physicochemical and sensorial properties of SS6 wine were analyzed and compared with those of W-3 wine (Table 1). No significant differences between the two strains were observed in terms of alcohol, sugar, and total acid contents during fermentation (data not shown) as well as in the wine. Hunter color values were also similar to each other. However, significant differences between the two wines were observed in the contents of organic acids, aldehydes, and minor alcohols. Contents of all detected organic acids, such as malic, tartaric, citric, and lactic acids, were higher in SS6 wine than those in W-3 wine. Especially, SS6 wine contained 4.0- and 1.9-fold higher lactic and citric acid contents compared to W-3 wine. SS6 wine also showed higher content of aldehydes and minor alcohols, except n-propyl alcohol, than W-3 wine. In sensory evaluation, SS6 wine obtained higher scores in terms of color, flavor, taste, and overall preference than W-3 wine. Although the correlation between the physicochemical properties of SS6 wine and its sensory scores is not yet clear, it can be presumed that inoculation of Campbell Early grapes with *H. uvarum* SS6 cells into improves wine quality. Co-fermentation of grape must by strains SS6 and W-3 as well as fermentation of filter-sterilized grape juice by strain SS6 is currently being performed to elucidate the beneficial effects of the strains on fermentation of Campbell Early grapes.

Consumption of wine in Korea has greatly increased in the past 20 years in line with economic growth, but the Korean domestic wine industry is still very young. The majority of wines consumed

Table 1
Physicochemical properties and sensory score of the Campbell Early wine fermented with inoculation of *H. uvarum* SS6 starter and *S. cerevisiae* W-3 used as a control wine yeast.

Property		Must	SS6 wine	W-3 wine
Alcohol (%, v/v)			11.8 ± 0.2	11.7 ± 0.3
Soluble solid (°Brix)		24.2 ± 0.2	7.4 ± 0.3	7.0 ± 0.3
pH		3.54 ± 0.02	3.40 ± 0.02	3.42 ± 0.02
Total acid (%)		0.45 ± 0.05	0.70 ± 0.05	0.70 ± 0.06
Hunter color value	*L*		29.3 ± 1.8	29.8 ± 1.1
	a		55.3 ± 5.0	53.4 ± 4.5
	b		15.4 ± 1.0	16.7 ± 1.1
Organic acid (μg/ml)	Malic acid	4904 ± 198	154.2 ± 9.6	106.9 ± 6.5
	Tartaric acid	3747 ± 150	50.2 ± 4.1	44.9 ± 3.8
	Citric acid	283 ± 19	134.7 ± 11.0	70.4 ± 6.4
	Lactic acid	–	825.7 ± 89.1	204.9 ± 14.0
Aldehyde (mg/ml)			4.62 ± 0.42	2.37 ± 0.34
Alcohols (μg/ml)	Methyl alcohol		170.9 ± 11.2	155.3 ± 10.3
	n-Propyl alcohol		47.9 ± 4.5	53.0 ± 5.0
	io-Butyl alcohol		209.0 ± 13.8	123.8 ± 11.2
	io-Amyl alcohol		534.6 ± 34.5	585.8 ± 40.2
Sensory score	Color		4.17 ± 0.36	3.08 ± 0.30
	Flavor		3.83 ± 0.27	2.92 ± 0.21
	Taste		3.25 ± 0.26	2.67 ± 0.22
	Overall preference		3.58 ± 0.28	3.00 ± 0.24

The fermentation was carried out in the presence of 200 μg/ml potassium metabisulfite with the inoculation of *H. uvarum* SS6 or *S. cerevisiae* W-3 cells to a final concentration of ~10^6 cfu/ml.

in Korea are imported from other countries. It was previously found in 2006 that Korean domestic wine is limited to only 19.6% of the national wine consumption (Korean National Tax Service, 2007). Campbell Early grape type, which occupies over 70% of the total grape production in Korea, have several disadvantages for winemaking, including low sugar content (13–15%), high malic acid content (4–16 mg/ml) (Kim et al., 1999; Lee and Park, 1980; Park et al., 2004), and a significantly high foxy aroma content (Wang and de Luca, 2005). These are believed to be the main barriers to making a good wine using Campbell Early grapes. Several reports have attempted to overcome these weaknesses. Specifically, increasing the concentration of grape juice can increase the sugar content, resulting in a wine without capitalization, but this also increases the amounts of other constituents such as acids and flavors that affect wine quality. To prevent a high level of acids, malic acid-degrading yeast has been isolated from Campbell Early grape wine pomace (Seo et al., 2007). Its application to co-fermentation with industrial wine yeast in an immobilized form has resulted in a significant reduction in malic acid content in wine (Hong et al., 2010; Kim et al., 2008). Foxy aroma, known as a negative factor affecting wine quality, in *V. labrusca* grapes is reportedly caused by a high level of methyl anthranilate synthesized by the action of alcohol acyltransferase in grape plants (Wang and de Luca, 2005). Methyl anthranilate can also be synthesized by *Bacillus megaterium* (Taupp et al., 2005). However, not much research has reported decreases in foxy aroma levels in wines due to the fact that most wines are made from the *V. vinifera* grape cultivar, which is known to not contain foxy aroma (Jackson, 1994). Our recent studies have largely focused on finding microbiological solutions for the above-mentioned problems associated with winemaking using Campbell Early grapes, such as reduction of acid content (Hong et al., 2010; Kim et al., 2008; Seo et al., 2007), isolation and characterization of wine yeasts, and reduction of foxy aroma level in wine in order to improve the quality of Korean domestic wine.

Molecular biology techniques provide a simple and rapid method to differentiate and identify yeasts based on their genetic background (de Barros-Lopes et al., 1996; Granchi et al., 1999; Montrocher et al., 1998; Oda et al., 1997; Park et al., 1999; Seo et al., 2007; Torija et al., 2001). There are a good number of papers on wine yeast diversity featuring molecular biology methods, especially based on PCR analysis using intron splice site primer (de Barros-Lopes et al., 1996), microsatellite markers (Masneuf-Pomarede et al., 2007), 5.8S-ITS region (Agnolucci et al., 2007; Clemente-Jimenez et al., 2004), D1-D2 region of 26S rDNA (Mills et al., 2002), and mtDNA (Agnolucci et al., 2007; Blanco et al., 2008; Gonzalez et al., 2007; Lopes et al., 2007). In this study, we isolated various yeast strains participating in the fermentation of wine from Campbell Early grapes and characterized them using molecular tools. *H. uvarum* was found to be the major non-*Saccharomyces* yeast in both Campbell Early grape berries and must at the early fermentation stage. It has been reported that non-*Saccharomyces* yeast are present at a high cell density in the must of *V. vinifera* grapes during the first 4–6 days of fermentation until the ethanol content reaches 4–7% (v/v). However, much higher ethanol contents have been obtained in wines produced by inoculation with *H. uvarum* cells (Rojas et al., 2003). Yeasts grown during the early fermentation stage have also been shown to enhance the production of desirable compounds such as esters in wine without any negative effect on the production of higher alcohols and undesirable heavy sulfur compounds (Moreira et al., 2008). The results obtained in this study suggest that *H. uvarum* SS6 might be helpful for improving the quality of wines produced using Campbell Early grapes, which is a unique and major grape variety in Korea.

Acknowledgments

This study was carried out with the support of the Cooperative Research Program for Agricultural Science & Technology Development (200901AFT154192334), RDA, The Republic of Korea.

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