Microsatellite and RAPD Analysis of Six Local Wine Strains of *Saccharomyces cerevisiae*

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The development of fast and reliable molecular typing techniques that can identify genetic markers specific for industrially important strains of *Saccharomyces cerevisiae* are valuable for wine fermentation and biofuel production and can ensure the use of correct strain*s* and will pave the way for producing consistent quality of product. Preliminary experiments verified that five (WS-1, WS-3, WS-4, WS-5, WS-6) of six local wine strains of *Saccharomyces cerevisiae* used in this study exhibited better growth than the control non-wine strain in the presence of 5% ethanol, while five (WS-1, WS-2, WS-3, WS-5, WS-6) of six strains grew better than the control in 10% ethanol. Growth of four wine strains (WS-1, WS-2, WS-5, and WS-6) was comparable to the control while two strains (WS-3 and WS-4) exhibited significantly higher growth in 15% ethanol. All the yeast strains generated an identical DNA profile from PCR using the microsatellite primer $(GAC)_5$ except strain WS-4 and the control that generated unique profiles. Results of randomly amplified polymorphic DNA (RAPD) PCR on the yeast strains revealed that 8 of the 11 primers were able to distinguish all the wine strains from the control strain. RAPD PCR using the M13 primer and three 10-mer RAPD primers (OPA-11, OPY-02, and OPY-05) resulted in profiles unique to strain WS-4 and the control non-wine strain. RAPD primer 1283 generated unique profiles for WS-3 in addition to WS-4 and the control. Additional unique RAPD profiles were also observed for wine strain WS-3 with PCR primers OPY-3 and OPY-4, while WS-2 produced a unique profile with primer OPB-11. The significant difference in the ethanol tolerance of control versus wine strains, strain WS-4 versus other strains, as well as some unexpected ethanol tolerance results for strains WS-2 and WS-3, could be attributed to significant genetic variability of the strains that was also reflected as variations in DNA profiles generated through the microsatellite and several RAPD PCR. Results suggest that the molecular typing tools used in this study may be able to generate DNA profiles that could distinguish *S. cerevisiae* strains exhibiting different ethanol tolerance.

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INTRODUCTION

The yeast *Saccharomyces cerevisiae* is an industrially important yeast, as it is used mainly in winemaking, brewing, baking, and biofuel production. This yeast predominates over other yeast and bacterial species present in the wine during the spontaneous fermentation of grape must (Querol and Ramon 1996, Guillamon et al. 1998) due to its higher tolerance to ethanol compared to the other microorganisms (Takahashi et al.

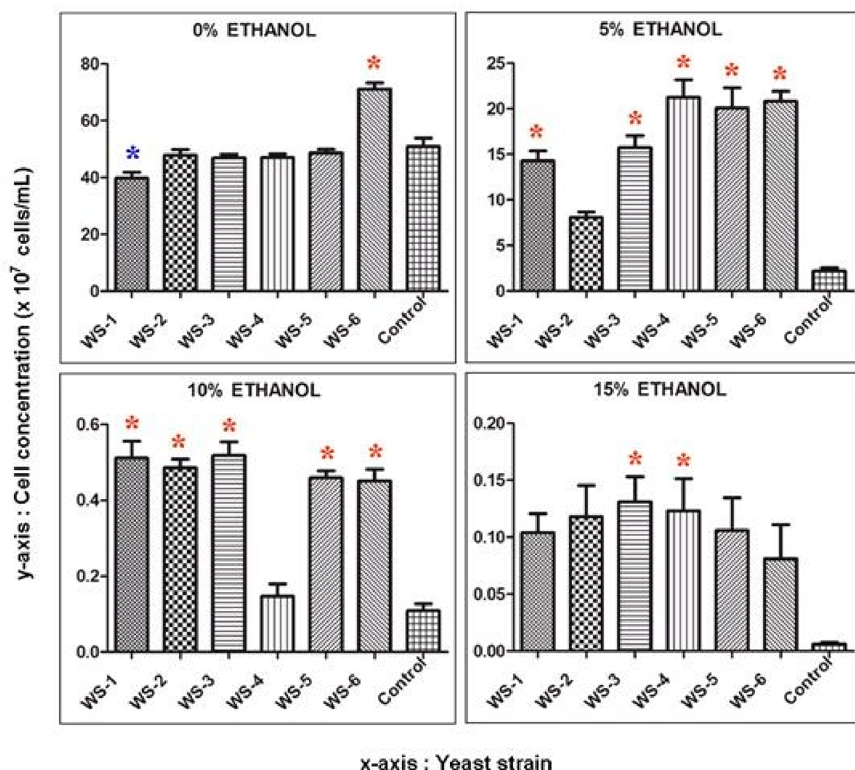

Figure 1. Growth of wine strains of *S. cerevisiae* (WS-1 to WS-6) in 5, 10 and 15% ethanol. The control is a non-wine strain of *S. cerevisiae*. Blue and red asterisks mark the growth of wine strains that were significantly lower and higher, respectively, compared to the control strain.

2001). High tolerance to ethanol is one of the distinguishing characteristics of *S. cerevisiae* and certain strains of this yeast have gained the name "wine yeasts" and have become the organisms of choice for initiating inoculated wine fermentations (Pretorius 2000). Six strains of *S. cerevisiae* (designated WS-1, WS-2, WS-3, WS-4, WS-5 and WS-6) used in large but non-commercial scale homemade local fruit wine fermentation (including bignay, *guyabano* and lipote wines) for several years are referred to as wine strains in this study.

The molecular mechanisms of ethanol tolerance have been continuously sought by identifying the genes involved in yeast response to alcohol stress. Over 250 genes have been implicated in ethanol tolerance, suggesting that the trait is under polygenic control (Hu et al. 2007). It is therefore hypothesized that gene markers which can distinguish yeast strains exhibiting high alcohol tolerance could be obtained and used to distinguish ethanol tolerant strains of *S. cerevisiae*. Moreover, in contrast to laboratory strains, the genomes of wine strains of *S. cerevisiae* are believed to undergo changes through recombination, crossing-over and gene conversion (Querol et al. 2003, Carreto et al. 2008) in order to facilitate faster adaptation of the wine yeast to environmental changes (Perez-Ortin et al. 2002). These events are expected to contribute to genomic differences between wine and laboratory strains and even among different wine yeasts, suggesting the possibility of obtaining genetic markers that can identify and distinguish wine strains from non-wine strains and wine strains from one another. Apart from being useful in screening for additional strains of wine yeast for possible use in wine production, genetic markers can also facilitate monitoring and maintenance of inoculated wine fermentations. Genetic markers provide an accurate means of strain characterization to ensure the use of correct strains of yeast in the production process and ultimately ensure consistency in the quality of the final product (Fernandez-Espinar et al. 2001).

Modern DNA-based techniques to obtain strain-specific genetic markers for yeast are preferred over the conventional methods for their simplicity, less laborious protocols, as well as rapid and highly reproducible results (Esteve-Zarzoso et al. 1999). Moreover, morphological, physiological or biochemical means of yeast characterization are often not able to differentiate between strains of the same species (Fernandez-Espinar et al. 2001, Lopez et al. 2003). DNA-based characterization methods can create "molecular signatures" to differentiate even closely related strains of yeasts. The main objective of this study was to perform microsatellite analysis and RAPD PCR to generate genetic markers that could differentiate six local wine strains of *Saccharomyces cerevisiae* from a control strain of the species (UPCC 2115) that is less alcohol tolerant. The difference in molecular typing profiles of the *S. cervisiae* strains was also correlated to the difference in their ability to survive and grow in the presence of 5, 10, and 15% ethanol.

MATERIALS AND METHODS

Yeast Strains

Six strains of *Saccharomyces cerevisiae* (designated WS-1, WS-2, WS-3, WS-4, WS-5 and WS-6) that are used in local fruit wine production were kindly provided by Dr. Priscilla C. Sanchez, a retired Professor and wine fermentation expert (Institute of Food Science and Technology, University of the Philippines Los Baños). A control non-wine strain of *Saccharomyces cerevisiae* var. *ellipsoideus* (UPCC 2115) was obtained from the Natural Sciences Research Institute, University of the Philippines Diliman. The yeast strains were grown at 30°C and maintained at room temperature in YEPD agar (1% yeast extract, 2% peptone, 2% dextrose, 2% agar) slants.

Assay for Ethanol Tolerance

Cultures of the yeast strains were grown overnight in 5 mL YEPD broth (1% yeast extract, 2% peptone, 2% dextrose) at 30°C with shaking at 220 rpm. Optical density of the cultures was determined via spectrophotometry (NanoDrop 2000c Spectrophotometer, Thermo Scientific, USA) to measure cell concentration. Absorbance was read at 600 nm wavelength (Abs), and the conversion factor 0.1 Abs = 3,000,000 cells/mL was used for absorbance values less than 1. Cell concentration of the cultures was adjusted to 100,000 cells/mL by serial dilution using YEPD broth, and 5 µL (approximately 500 cells) of the diluted cultures was inoculated in 20 mL YEPD broth without ethanol and with 5%, 10% and 15% (v/v) ethanol. Setups were prepared in triplicate. The cultures were then incubated for 15 hours at 30°C with shaking at 220 rpm. Cell concentration of the cultures was determined via spectrophotometry, as described above. Data were analyzed using one-way analysis of variance (ANOVA) with Tukey's Multiple Comparison Test at 0.05 level of significance using GraphPad Prism version 5.02 for Windows (GraphPad Software, Inc., USA).

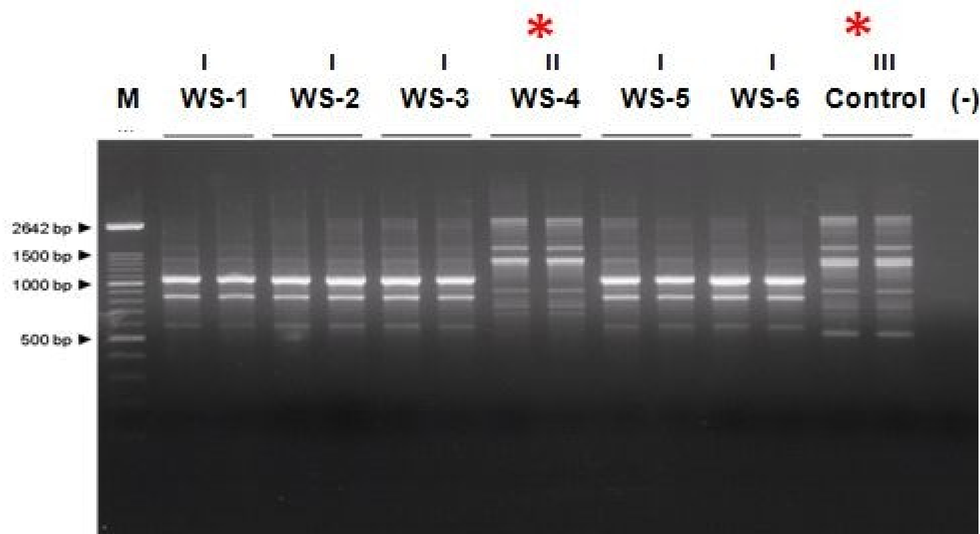

Figure 2. DNA profiles of the *S. cerevisiae* strains generated by microsatellite typing using $(GAC)_5$ primer. Types of profiles are marked by Roman numerals and profiles unique for a strain are marked by red asterisks. M is the Roche100 bp DNA ladder.

Genomic DNA Extraction

Genomic DNA from all yeast strains used in this study was isolated using ZR Fungal/Bacterial DNA Kit™ (Zymo Research, USA), following the protocol described by the manufacturer. The DNA extracts were stored at 4°C until further use.

Microsatellite Typing

Amplification of microsatellite regions was performed in 10-µL reactions containing 1X GoTaq PCR buffer (Promega, USA), 0.4 mM dNTPs (Takara Bio, Inc., Japan), 0.5 µM $(GAC)_5$ primer [GAC GAC GAC GAC GAC] (Baleiras Couto et al. 1996), 0.025 U/µL GoTaq *Taq* polymerase (Promega, USA) and 1 µL yeast genomic DNA extract. Reactions were prepared in replicate. Thermocycler conditions (as specified by Baleiras Couto et al. 1996) were as follows: initial denaturation at 94°C for 5 min; 40 cycles of denaturation at 94°C for 15 sec, annealing at 45°C for 45 sec and extension at 72°C for 1 min; and final extension at 72°C for 10 min (Multigene, Labnet International, Inc., USA).

RAPD PCR using M13 Primer

The PCR reaction mixture (10-µL) contained 1X PCR buffer (Roche, Germany), 0.4 mM dNTPs (Takara Bio, Inc., Japan), 0.5 µM M13 primer [GAG GGT GGC GGT TCT] (Cocolin et al. 2004, Araujo et al. 2007), 0.025 U/µL *Taq* polymerase (Roche, Germany) and 1 µL yeast genomic DNA extract. Reactions were prepared in replicate. Thermocycler conditions used (as specified by Cocolin et al. 2004) were as follows: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 38°C for 1 min and extension at 72°C for 2 min; and final extension at 72°C for 5 min (Multigene, Labnet International, Inc., USA).

RAPD PCR using 10-mer Primers

The PCR reaction mixture (10-µL) contained 1X GoTaq PCR buffer (Promega, USA), 0.4 mM dNTPs (Takara Bio, Inc., Japan), 0.5 µM 10-mer primer (Table 1), 0.025 U/µL GoTaq *Taq* polymerase (Promega, USA) and 1 µL yeast genomic DNA extract. Reactions were prepared in replicate. Thermocycler conditions (as specified by Echeverrigaray et al. 2000) were as follows: initial denaturation at 92°C for 4 min; 40 cycles of denaturation at 92°C for 45 sec, annealing at 37°C for 1.5 min and extension at 72°C for 2 min; and final extension at 72°C for 4 min (Multigene, Labnet International, Inc., USA).

Primers

All primers used in the study (Table 1) were synthesized by AITBiotech Pte. Ltd. (Singapore).

RESULTS

Growth measurements (in terms of cell concentration) in YEPD broth containing 0, 5, 10 and 15% ethanol for six wine strains and the control non-wine strain of *S. cerevisiae* are summarized in Table 2 and Figure 1. DNA profiles generated through PCR using a microsatellite and eleven RAPD primers (Fig. 2 to 5) were evaluated to identify primers that can produce profiles specific for each strain. Similar types of profiles

generated in PCR are marked by the same Roman numerals and profiles unique for a strain are marked by red asterisks.

Growth of the *S. cerevisiae* Strains in Different Concentrations of Ethanol

Growth of strain WS-6 in YEPD broth without ethanol was the highest among all the strains studied and growth was significantly higher than the control strain. While growth of the control yeast strain without ethanol was comparable to the growth of wine strains WS-2, WS-3, WS-4 and WS-5, strain WS-1 exhibited significantly lower growth than the control (Fig. 1). In YEPD broth containing 5% ethanol, growth of the control non-wine strain was significantly lower compared to the growth of the wine strains, except for strain WS-2 that exhibited comparable growth with the control. Growth of the control strain in YEPD broth with 10% ethanol was also significantly lower than the wine strains, except WS-4. With 15% ethanol, only two strains (WS-3 and WS-4) exhibited significantly higher growth than the control and the rest exhibited comparable growth. The toxic effect of alcohol to yeast cells was evident because cell growth of each strain was observed to decrease with increased ethanol concentration except for WS-4 which grew better than the control in 15% compared to 10% ethanol. The wine strains, in general, exhibited greater alcohol tolerance than the control non-wine strain of *S. cerevisiae*.

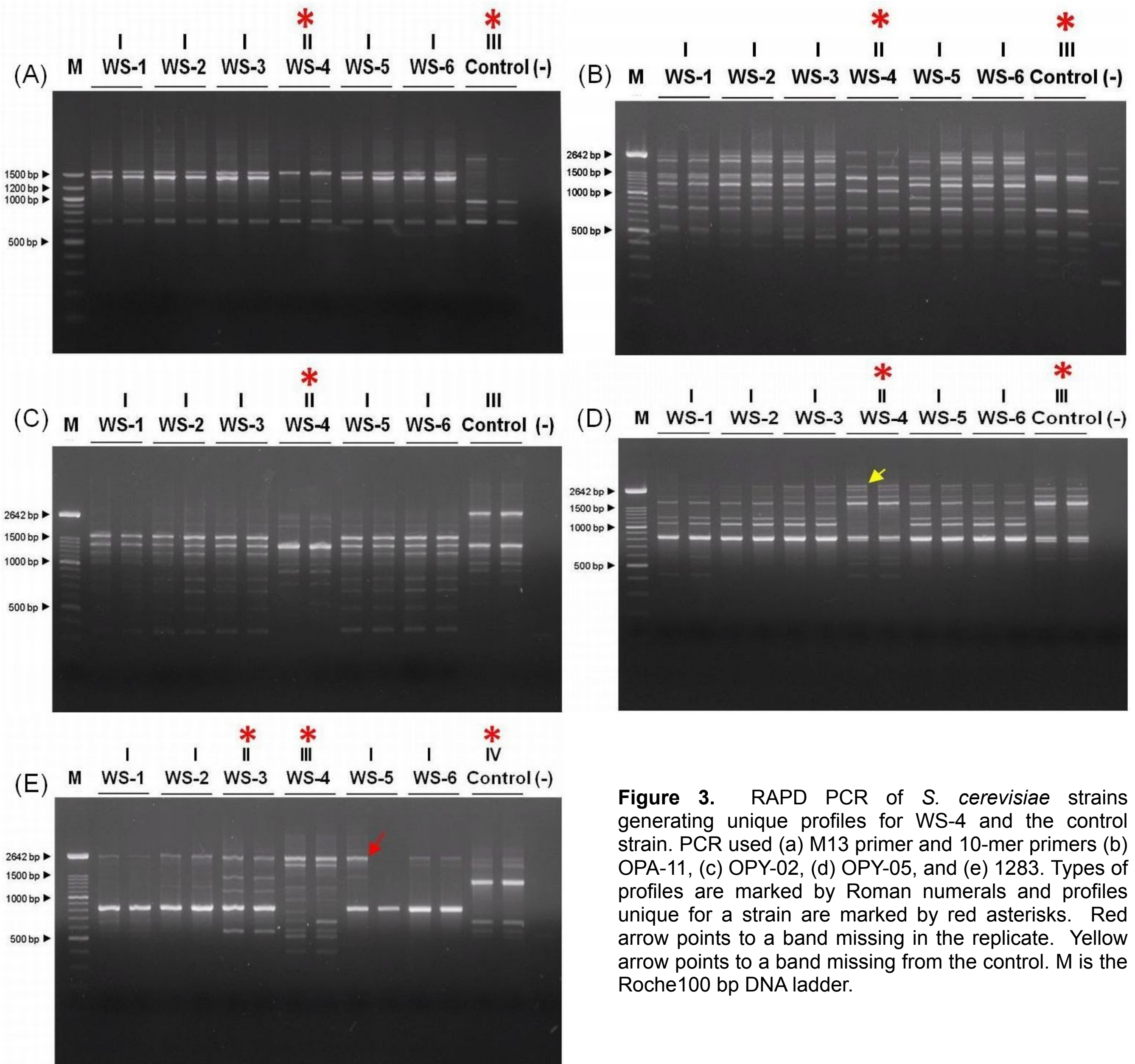

Figure 3. RAPD PCR of *S. cerevisiae* strains generating unique profiles for WS-4 and the control strain. PCR used (a) M13 primer and 10-mer primers (b) OPA-11, (c) OPY-02, (d) OPY-05, and (e) 1283. Types of profiles are marked by Roman numerals and profiles unique for a strain are marked by red asterisks. Red arrow points to a band missing in the replicate. Yellow arrow points to a band missing from the control. M is the Roche100 bp DNA ladder.

Microsatellite Typing and RAPD PCR Analysis

Microsatellite typing using the $(GAC)_5$ primer generated an identical profile for all strains except strain WS-4 and the control *S. cerevisiae* both of which generated unique profiles (Fig. 2). Use of additional microsatellite primers is expected to produce additional markers that could distinguish the wine strains. Results of the RAPD PCR revealed that eight of eleven primers used generated DNA profiles that could clearly distinguish the control non-wine strain from the six wine strains (Fig. 3 to 5). Unique profiles were generated from RAPD PCR only from the control strain and wine strains WS-2, WS-3, and WS-4 while WS-1, WS-5, and WS-6 generated almost identical profiles for several primers used.

RAPD PCR using the M13 primer (Fig. 3a) and three 10-mer RAPD primers OPA-11, OPY-02, and OPY-05 (Fig. 3b, 3c, and 3d) resulted in profiles unique for strain WS-4 and the control non-wine strain. RAPD primer 1283 that generated unique profiles in WS-4 and the control (Fig. 3e) also produced a unique profile in strain WS-3. Unique RAPD profiles were also observed for WS-3 using two more primers, OPY-03 and OPY-04 (Fig. 4b and 4c) and for WS-2 using primer OPB-11 (Fig. 4a). The RAPD primer OPX-03 generated a unique DNA profile for the control strain that could distinguish it from all six wine strains (Fig. 5a). Primers OPY-01 (Fig. 5c) and RF2 (Fig. 5b) did not produce a unique profile for any of the wine strains used. Primer OPY-01 produced the same profile for all strains and primer RF2 generated three types of profiles, an identical profile for strain WS-1, WS-3, and WS-5, another profile common to strains WS-2, WS-4, and WS-6 (Fig. 5b), and the control generated a unique profile with one additional faint band (Fig. 5b, with yellow arrow.).

Reproducible results were obtained for all PCR experiments except for the PCR that used the primers 1283, OPX-03 and RF2, which resulted in a missing band (red arrow) for the replicate of the WS-5, WS-3 and WS-4 strains, respectively (Fig. 3e, 5a and 5b). Unexpected non-specific bands that did not correspond to any of the bands generated from PCR of yeast strains were observed in 4 of 12 agarose gels for the no-template negative control (Fig.3b, 4c, 5a, and 5b).

DISCUSSION

It has long been established that the yeast *Saccharomyces cerevisiae* plays a principal role in the fermentation of wines. In spontaneous wine fermentation and as the ethanol level in the wine rises, strains of *S. cerevisiae* begin to predominate over the other microorganisms to become solely responsible for ethanol fermentation (Querol and Ramon 1996, Guillamon et al. 1998). Because of its high tolerance for ethanol, *S. cerevisiae* has become the preferred organism for initiating inoculated wine fermentations and certain strains have gained the name “wine yeasts”. At present, a wide variety of dehydrated cultures of selected wine yeast strains of *S. cerevisiae* with known properties is manufactured (Pretorius 2000). However, even with the use of starter yeast cultures, studies show that indigenous yeasts are still present to affect wine fermentation and are not eliminated by the competitive effect of the addition of a high-density starter culture (Heard and Fleet 1985, Querol et al. 1992, Schutz and Gafner 1993). There is the need, therefore, to constantly monitor the growth of the starter yeast strains and check for contamination by other yeasts. This requires a rapid and reliable method to verify the identity and purity of starter yeast cultures that would be valuable for manufacturers of wine yeast starter cultures as well as for wine producers maintaining

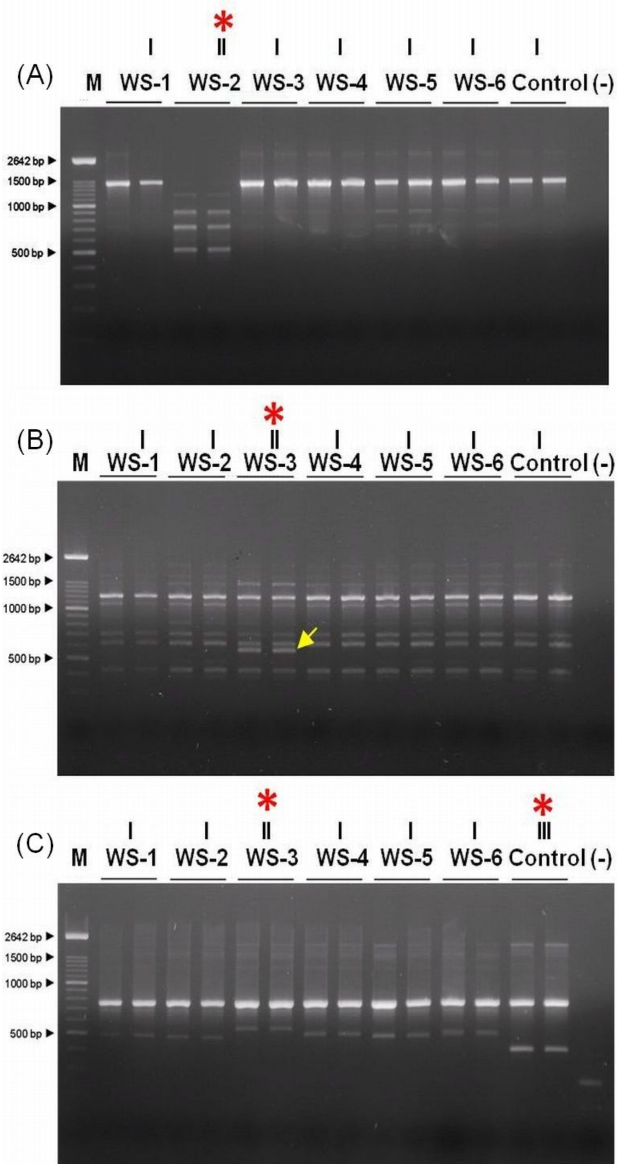

Figure 4. RAPD PCR of *S. cerevisiae* strains generating unique profiles for WS-2, WS-3, and the control strain. PCR used 10-mer primers (a) OPB-11, (b) OPY-03 and (c) OPY-04. Yellow arrow points to a band only present in strain WS-3. M is the Roche100 bp DNA ladder.

their own wine yeast strains. Results of this study illustrate the ability of microsatellite typing and RAPD PCR (Fig. 2 to 5) to generate DNA profiles that distinguish six wine strains (WS-1 to WS-6) from one another and from the control non-wine strain of *S. cerevisiae* (UPCC 2115).

Preliminary experiments assessed alcohol tolerance, measured in terms of the inhibitory effect of adding 5, 10 and 15% ethanol to the culture medium, on the growth of the yeast strains (Table 2 and Fig. 1). This is important in wine fermentation and biofuel production because for the yeasts to continue producing ethanol even after initial production of the alcohol and until the desired alcohol concentration is produced, the strain should exhibit ethanol tolerance. Tolerance to ethanol could be attributed to several factors, including the presence in the strain of higher levels of survival factors such as certain unsaturated, long-chain fatty acids, sterols and heat shock proteins (Pretorius 2000). The results of this study showed that without ethanol, the growth of 4 wine strains was comparable with the control yeast strain. Growth of wine strains WS-6 and WS-1 was significantly higher and lower, respectively, than the control strain. As expected, the wine strains were observed to exhibit higher cell concentrations (interpreted as higher cell growth) than the control strain (Table 2 and Fig. 1) upon the addition of ethanol, with a few exceptions. For example, with 5% ethanol, all other wine strains grew better than the control, but growth of strain WS-2 was just comparable with the non-wine control. Another exception is wine strain WS-4 that exhibited comparable growth with the control in the presence of 10% ethanol, while the rest exhibited significantly higher growth. In 15% ethanol, however, strain WS-4 (and strain WS-3) grew significantly better than the control while the rest of the wine strains exhibited only comparable growth with the control. Strains of *S. cerevisiae* that could grow in 10 and 15% alcohol could be valuable for inoculation in a culture medium that is already undergoing fermentation or that already contains 10 to 15% alcohol, in order to produce even higher concentrations of ethanol.

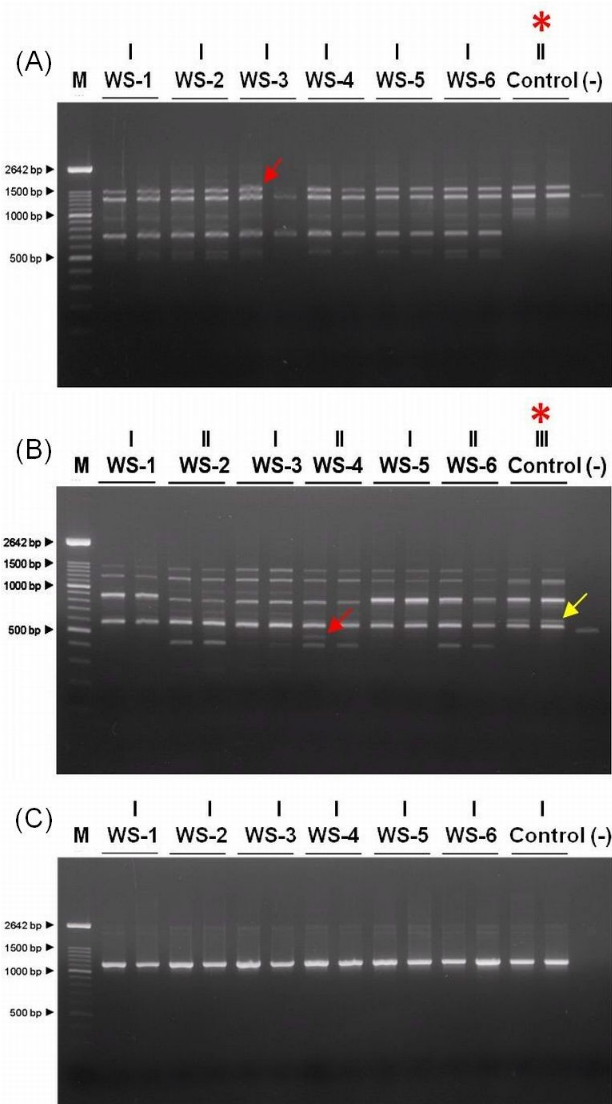

Figure 5. RAPD PCR of *S. cerevisiae* strains that did not generate a unique profile for any of the wine strains. PCR used 10-mer primers (a) OPX-03, (b) RF2 and (c) OPY-01. Types of profiles are marked by Roman numerals. Red arrow points to a band missing in the replicate. Yellow arrow points to a band only present in the control. M is the Roche100 bp DNA ladder.

The main focus of this study was the use of two molecular typing tools to generate DNA profiles that could help distinguish the six wine strains of *S. cerevisiae* from a control non-wine strain of the species and to differentiate the wine strains from one another. One approach used was microsatellite typing which involves the amplification of satellite sequences, short (usually less than 10 bp) tandem repetitive DNA sequences dispersed throughout the genome (Perez et al. 2001). The method relies on the significant level of polymorphism in the lengths of the microsatellite loci, and has been previously reported to generate distinguishing profiles in yeast (Baleiras Couto et al. 1996, Perez et al. 2001, Schuller et al. 2004). The single microsatellite primer $(GAC)_5$ produced an identical profile for all strains except wine strain WS-4 and the control which generated unique profiles (Fig. 2), providing a means to differentiate WS-4 and the control strain from the rest of the wine strains.

The other approach, RAPD PCR analysis, was also used with eleven primers of arbitrary sequence (NCBI, 2011) for the random amplification of DNA segments. The difference in RAPD profiles generated from different species, or from various strains of the same species, depends on the difference in DNA sequences within the genome. RAPD analysis has been widely used in yeast strain characterization to study genetic variability among *S. cerevisiae* isolates (Capece et al. 2004, Cocolin et al. 2004, Giusto et al. 2006, Araujo et al. 2007). Just like the microsatellite primer, RAPD primers M13, which is based on the

core sequence of the M13 phage (Graser et al. 1993), and 10-mer RAPD primers OPA-11, OPY-02, OPY-05 and 1283, also yielded DNA profiles that could distinguish strain WS-4 and the control non-wine strain from the rest of the wine strains (Fig. 3a to 3e). The unique DNA profiles from strain WS-4 and the control strain using the microsatellite and six 10-mer RAPD primers suggest significant DNA sequence variation between the control and all wine strains as well as the significant DNA variation of strain WS-4 from the rest of the wine strains. Specific primers also generated PCR profiles that could distinguish wine strains WS-2 (Fig. 4a) and WS-3 (Fig. 3e, 4b and 4c) from the rest of the wines strains and the control. RAPD primer OPX-03 (Fig. 5a) generated a unique profile for the control and an identical profile for the rest of the wine strains. Primer OPY-01 could not differentiate the yeast strains because an identical profile was obtained for all (Fig. 5c). Another RAPD primer, RF2, generated three types of profiles including a unique profile for the control (Fig. 5b). This paper reports that unique DNA profiles that could identify strains WS-2, WS-3, WS-4 and the control non-wine strain were obtained in PCR using one microsatellite and eleven RAPD PCR primers.

PCR using nine of 12 primers was able to generate profiles unique to the control strain, indicating the significant genetic variation of the control from the rest of the wine strains. This is consistent with the observation that the control exhibited significantly different (lower) alcohol tolerance than most wine strains used in the study. Significant genetic variation is expected to result in significant differences in certain phenotypes, which may have also contributed to significant differences in ethanol tolerance. Several unique profiles generated for strain WS-4 could also be correlated with the observation that alcohol tolerance of WS-4 is different from the rest. While most of the wine strains exhibited better growth than the control in 5 and 10% alcohol, WS-4 exhibited higher growth than the control only in 5% but not in 10% ethanol. Moreover, while four wine strains did not exhibit significant growth difference with the control in 15% ethanol, growth of WS-4 was significantly higher than the control. Production of a unique profile in PCR using strains WS-2 (Fig. 4a) and WS-3 (Fig. 3e, 4b and 4c) could be correlated with the observation that strain WS-2 did not exhibit significantly higher growth than the control in 5% ethanol, unlike the rest of the wine strains, while strain WS-3 exhibited significantly higher growth in 15% ethanol compared to the control.

The validity and usefulness of data from RAPD PCR rely on the reproducibility of profiles generated. Reproducibility of three 10-mer primers, 1283, OPX-03 and RF2, generated slightly variable profiles for strains WS-5, WS-3 and WS-4, respectively (Fig. 3e, 5a and 5b). This is one limitation of RAPD PCR analysis (Penner et al. 1993, Jones et al. 1997, Perez et al. 1998). As reported by Meunier and Grimont (1993), RAPD profiles are heavily dependent on the materials used, from the reaction components to the thermocycler used. Moreover, different

Table 1. Sequences of the ten 10-mer primers used in RAPD PCR

PRIMER NAME	SEQUENCE	REFERENCES
OPY-01	GTG GCA TCT C	Eurofins MWG Operon (2010)
OPY-02	CAT CGC CGC A	
OPY-03	CAT CGC CGC A	
OPY-04	GGC TGC AAT G	
OPY-05	GGC TGC GAC A	
OPA-11	CAA TCG CCG T	Sebastiani et al. (2004)
1283	GCG ATC CCC A	
RF2	CGG CCC CTG T	
OPB-11	GTA GAC CCG T	Echiverrigaray et al. (2000)
OPX-03	TGG CGC AGT G	

Table 2. Growth of the wine strains and the control non-wine strain of *S. cerevisiae* in YEPD broth with 5, 10 and 15% ethanol

STRAIN	AVERAGE CELL CONCENTRATION (x 10^7 cells/mL)			
	0% ETHANOL	5% ETHANOL	10% ETHANOL	15% ETHANOL
WS-1	39.8	14.3	0.511	0.104
WS-2	47.9	8.10	0.485	0.118
WS-3	46.9	15.7	0.518	0.131
WS-4	47.1	21.2	0.147	0.123
WS-5	48.7	20.1	0.459	0.106
WS-6	71.1	20.8	0.450	0.0810
Control	51.0	2.20	0.109	0.006

profiles generated by two different thermocyclers may result from temperatures inside the tubes that are significantly different from the reported temperatures in the thermocyclers (Penner et al. 1993). Most of the RAPD profiles also produced faint bands in the negative control reaction that are different from the bands in the sample amplification reactions. This observation is similar to that observed by Williams et al. (1990), Meunier and Grimont (1993), and Haig et al. (1994) in their RAPD profiles, and could probably be attributed to inherent contaminations in the materials that were exclusively used in preparing the negative control setup, such as the PCR tube, or the pipette tips used to dispense the master mix or the 1 µL water into the tube. However, the absence of these kinds of bands when template DNA is included in the reaction was noted, suggesting that their occurrence is likely to be seen only in the negative-control reactions.

CONCLUSION

Preliminary experiments confirmed that six wine strains of *Saccharomyces cerevisiae* used in the study, exhibited greater tolerance to ethanol than the non-wine control strain (Table 2 and Fig.1). Five of six local wine strains exhibited better growth than the control strain in 5% ethanol, while five of six wine strains grew better than the control in 10% ethanol. Two wine strains exhibited significantly higher growth in 15% ethanol than the control.

The yeast strains were subjected to microsatellite and RAPD PCR, generating DNA profiles that could differentiate all six wine strains of *S. cerevisiae* from the control non-wine strain. Significant differences in DNA profiles were observed between the wine strains and the control as well as strain WS-4 from the control and other wine strains. Unique profiles were also generated from wine strains WS-2 and WS-3. The study revealed that microsatellite and RAPD PCR could provide fast and reliable ways to ensure that commercially important *S. cerevisiae* strains are identified and distinguished from other strains of the same species.

Significant difference in alcohol tolerance between two strains of yeast is the consequence of DNA variation that led to the phenotypic difference and DNA variation, which translates to the generation of different DNA profiles from molecular typing analysis. In this study, the significant differences in alcohol tolerance between the control and the wine strains, as well as between strain WS-4 and the rest of the wine strains, are consistent with significant differences in DNA profiles obtained from them. Results also suggest that molecular typing tools such as microsatellite and RAPD PCR may be used to generate DNA profiles that could distinguish strains of *S. cerevisiae* exhibiting different ethanol tolerance.

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NO CONFLICT OF INTEREST STATEMENT

There is no conflict of interest among authors, institutions, and individuals mentioned above in the conduct of this study and the preparation and submission of this manuscript.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Ms. Zahara Joy A. Guiamal conducted the experiments under the guidance of Dr. Cynthia T. Hedreyda, who mainly conceptualized the study. Both worked together to write and revise the manuscript.

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