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Genetic Analysis of Alcohol Yeasts Isolated from Korean Traditional Liquor by Polymerase Chain Reaction

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Abstract Forty alcohol yeast strains were isolated from the main mashes (10 strains from each mash) for brewing of 4 different kinds of Korean traditional liquor (3 different types of *Yakju* and 1 *Takju*). Thirty-eight out of 40 strains were identified to be the same strain, *Saccharomyces boulardii*, by the Automated Bacteria, Yeast, and Fungi Identification System (Biolog Co., U.S.A.) based on the metabolic fingerprints. One strain that showed the highest ethanol production among the 38 strains in YPD medium, designated SHY 111, was selected and used for differentiating from other yeast type strains using the polymerase chain reaction (PCR). Amplified DNA, from transcribed internal spacers of SHY 111 chromosomal DNA, was found to be the same in both size and sequence as those of *S. cerevisiae* KCCM 11215 (formerly *S. coreanus*) and *S. boulardii* along with that of *S. cerevisiae* AB 972, which was used as a type strain for the yeast genome project. However, when PCR was carried out with the intron splice site primer, it resulted in the amplification of the SHY 111-specific DNA fragment which was about 200 bp in size. When PCR was carried out using the primer to test diversity of 40 isolated yeast strains, it was found that the PCR patterns were similar to each other except for the 200 bp bands derived from all the 10 strains from one *Yakju*, and 2 strains from another *Yakju*. These results suggest the strain identified as *S. boulardii* by the Automated Identification System to be a dominant strain for the fermentation of Korean traditional liquors.

Key words: Alcohol yeasts, *S. boulardii*, Korean traditional liquor, differentiation, PCR analysis, internal transcribed spacer (ITS), intron splice site

Although brewers' yeasts had been classified as a variety of species based on the type of alcoholic beverages, the majority of the strains are classified as a single species, *Saccharomyces cerevisiae* [3, 16, 24]. It includes formerly *S. cerevisiae*, *S. ellipsoideus*, *S. bayanus*, *S. sake*, *S. uvarum*, *S. coreanus*, *S. vini*, etc [3, 24]. Most modern brewers use a pure culture of selected yeast strains that are necessary for reliable fermentation to be carried out [23], but, on the other hand, it has been shown that an indigenous yeast can still be a part of the fermentation process [6, 20, 27]. Specifically, there are many brewers who do not inoculate a pure yeast culture but use wild-type yeasts from *Nuruk*, which is a unique koji for the brewing of Korean traditional liquors. However, little is known about the genetic characteristics of the Korean wild yeast strains, mostly because of the fact that microbiological studies conducted on Korean traditional liquors have been largely focussed on microflora in Korean *Nuruk* and main mashes [7, 15, 18, 25, 30]. In addition, much attention has been paid to the improvement of the yeast strain for ethanol production from biomass as an alternative energy [12, 13, 17, 28]. There have only been a few reports on the subject of morphological and physiological characterization of alcohol yeasts participating in the fermentation of Korean traditional liquors [11, 15, 25]. Because of the fact that *S. coreanus* from *Nuruk* [25] and *S. cerevisiae* from the main mash of *Takju* [11, 15] were isolated, it is commonly believed that the two yeast strains are dominant species participating in the fermentation process of Korean traditional liquors.

In recent years, genetic diversity of yeast strains has become apparent and thus led to a number of DNA sequence-based identifications [4, 5, 9, 14, 21, 22]. The variability in the chromosomal composition of yeast strains made chromosome karyotyping a useful method [9, 20]. A development of the polymerase chain reaction (PCR) technique has contributed tremendously towards the yeast strain identification, as well as differentiation between yeast strains by using amplification of random [5, 22] or specific [2, 14] target sequences.

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Our objective was to isolate the dominant yeast strain in Korean traditional liquors, and to develop feasible methods of discriminating the yeast from other alcohol yeast strains. In this study, alcohol yeast strains were isolated from main mashes of various Korean traditional liquors during fermentation. They were then analyzed by the Automated Yeast Identification System based on the metabolic fingerprint and the PCR amplification of DNA fragments containing yeast internal transcribed spacer sequences [8] or intron splice sites [5].

MATERIALS AND METHODS

Strains and Media

Saccharomyces cerevisiae KCCM 11215 (formerly *S. coreanus*) was obtained from the Korean Culture Center of Microorganisms (Seoul, Korea). *S. boulardii* was purchased from the Allergy Research Group (San Leandro, U.S.A.). Other strains including *S. cerevisiae* AB 972 and *Escherichia coli* HB 101 were from the stock in our laboratory. Yeast cells were grown at 30°C in a YPD medium (1% yeast extract, 2% bacto-peptone, and 2% glucose). *E. coli* HB 101 used for transformation and plasmid DNA isolation was grown at 37°C in an LB medium (1% bacto-tryptone, 0.5% yeast extract, and 0.5% NaCl) supplemented with 200 μg/ml of ampicillin as needed.

Isolation of Alcohol Yeasts

Yeast strains were isolated using YPD-agar plates from main mashes for brewing of 4 different kinds of Korean traditional liquors (3 different kinds of *Yakju* and 1 *Takju*) during fermentation. All strains were tested for their alcohol production in the YPD liquid medium. Ten strains from each mash, which showed a high alcohol production, were selected and stored at -70°C in the presence of 15% glycerol.

Identification of Yeast by the Metabolic Fingerprint

Yeast strains were mostly identified for their grouping using microplates coated with various carbon sources by the method described by Automated Bacteria, Yeast, and Fungi Identification System (Biolog Co., U.S.A.).

Synthesis of Oligonucleotide Primers for Polymerase Chain Reaction (PCR)

Oligonucleotide primers for PCR were synthesized by a commercial company (Bioneer Co., Chongwon, Korea). They were designed to amplify the DNA fragments containing the yeast internal transcribed spacer (ITS) sequences (primers ITS1 and ITS2) or random intron splice site regions (primers EI1, EI2, LA1, and LA2). The sequences of the primers ITS1 and ITS2 were derived from those of *S. cerevisiae* AB 972 18S and 25S rDNA, respectively [9], and were designed to produce a *Bam*HI restriction site in both ends of the PCR fragments. The other 4 primers, derived from yeast intron splice sites, have been described in detail by de Miguel *et al.* [5]. Their sequences and target sites are shown in Table 1.

Yeast Chromosomal DNA Isolation

Chromosomal DNA, for the PCR template, was isolated from yeast cells grown in a YPD medium for 24 h by the method described by Kaiser [10] or Philippsen [19]. Cells treated with 0.25 mg/ml zymolyase (100T, ICN Biomedicals, U.S.A.) and 0.28 M β-mercaptoethanol at 37°C for 1 h in 0.9 M sorbitol-0.1 M EDTA (pH 7.5) were lyzed with 1% SDS at 65°C for 20 min in 50 mM Tris · HCl-20 mM EDTA (pH 7.4). Supernatant fluid was collected by centrifugation after being treated with 0.4 volume of 5 M potassium acetate at 0°C for 30 min. Two volumes of 95% ethanol were added to the supernatant fluid to precipitate yeast chromosomal DNA, which was then spooled out and resuspended in 10 mM Tris · HCl-1 mM EDTA (pH 7.4). The DNA was spooled out again in 50% isopropanol after RNase treatment at 37°C for 1 h, and resuspended in 10 mM Tris · HCl-1 mM EDTA (pH 7.4).

Polymerase Chain Reaction (PCR)

PCR was performed in 20 or 100 μl using the TaKaRa Taq DNA polymerase (Takara Shuzo Co., Otsu, Japan) with a GENE cycler (BioRad Co., Richmond, U.S.A.). The PCR mixture consisted of 1 μg of template DNA, 100 pmol of each primer, 1 U of *Taq* DNA polymerase, 0.25 mM of each dNTP, 10 mM Tris · HCl (pH 8.3), 50 mM KCl, and 2.5 mM $MgCl_2$. The PCR cycle program was comprised of one cycle of 94°C (3 min); 31 cycles of 94°C (1 min), 45°C

Table 1. Sequences of synthetic oligonucleotide primers for PCR used in this study.

Primer	Sequence (5'→3')	Target site	Reference
ITS1	CGCGGATCCGTAGGTGAACCTGCGG	ITS I and II	This study
ITS2	CGCGGATCCCTCCGCTTATTGATATG		
EI1	CTGGCTTGGTGTATGT	Random intron splice sites	5
EI2	CTGGCTTGCTACATAC		
LA1	GCGACGGTGTACTAAC		
LA2	CGTGCAGGTGTTAGTA		

(2 min), 74°C (1.5 min); and, finally one cycle of 74°C (10 min).

Recombinant DNA Techniques

DNA manipulation, agarose or polyacrylamide gel electrophoresis, and *E. coli* transformation were carried out as described by Sambrook *et al.* [26].

DNA Sequencing

PCR products containing yeast ITS regions were cloned into the *Bam*HI site of vector plasmid pUC119, which was then used for a DNA sequencing reaction. The reaction, with a forward (ALFred M13-40) or a reverse primer (ALFred M13), was carried out according to the AutoCycle Sequencing Kit provided by Amersham Pharmacia Biotech Co. (Uppsala, Sweden). The PCR cycle program for a DNA sequencing was comprised of one cycle of 95°C (36 sec); 25 cycles of 95°C (36 sec), 50°C (36 sec), 72°C (84 sec); and one cycle of 4°C for cooling. The reaction products were resolved on a 7 M urea-5.5% polyacrylamide gel, which was read with an ALFexpress DNA sequencer supplied by the same company.

RESULTS AND DISCUSSION

Isolation and Identification of Alcohol Yeasts from Korean Traditional Liquors

Forty alcohol yeast strains, which were able to produce ethanol in a YPD medium, were isolated from main mashes (10 strains from each mash) for the brewing of 4 different Korean traditional liquors (3 different kinds of *Yakju* and 1 *Takju*). They were analyzed by the Automated Bacteria, Yeast, and Fungi Identification System (Biolog Co., U.S.A.) which was based on the metabolic fingerprint (Table 2). All ten strains from *Takju* and 27 out of 30 strains from 3 different kinds of *Yakju* were identified as *S. boulardii*. Their similarity to *S. boulardii* was found to be in the range of 64.7% and 98.3%. It is interesting to note that only 2 strains isolated from each mash of 2 kinds of *Yakju* did not belong to *S. boulardii*. These results suggest that the strain identified as *S. boulardii* in this study to be a dominant strain, which participates in the fermentation process of Korean traditional liquors. Although a strain of *S. cerevisiae* (formerly *S. coreanus*) has been thought to play an important role in the brewing of Korean traditional liquors [11, 15, 25], none of the strains were identified as *S. cerevisiae* by using this system. It is not well known as to whether the Automated Identification System is accurate enough to confirm yeast strains, but it is thought to be a useful method for the grouping of a large number of microorganism strains. Morphological and physiological characteristics of the strains are now being investigated for their identification by using the conventional method.

Table 2. Grouping of alcohol yeast strains isolated from main mashes of various Korean traditional liquors.

Sample	Identification[1]	No. of strain	Similarity (%)
Yakju 1	*Saccharomyces boulardii* *Candida multisgummis*	91	83.5-89.9 77.2
Yakju 2	*Saccharomyces boulardii* *Zygosaccharomyces cidri*	91	64.7-97.8 93.0
Yakju 3	*Saccharomyces boulardii*	10	74.1-90.8
Takju 1	*Saccharomyces boulardii*	10	89.4-98.3

[1]Forty alcohol yeast strains isolated from main mashes (10 strains from each mash) for brewing of various Korean traditional liquors were identified by the Automated Bacteria, Yeast, and Fungi Identification System (Biolog Co., U.S.A.). Strain SHY 111 showing the highest ethanol production in YPD medium among 40 strains was selected for further study.

Table 3. Similarity of alcohol yeast strain SHY 111 isolated from *Yakju* main mash by Automated Identification System based on metabolic fingerprint.

Closest species	SIM[1]	DIST[2]	AVG[3]	MAX[3]
Saccharomyces boulardii	0.890	1.241	0.063	0.119
Geotricuhum terrestre	0.015	2.584	1.250	7.338
Kluyneromyces lactis	0.003	3.091	0.375	1.337

[1]Similarity=P(in)×P(db).
P(in)=probability of the identification being correct, given the entered data that belongs to a species in the database.
P(db)=probability that entered data belongs to a species in the database.
[2]Distance (DIST): between the entered data and each searched record in the database is calculated.
[3]Average (AVG) and maximum (MAX) distance values that are obtained when all of the strains Biolog run for that species are compared against the data record for the species.

One *Yakju* yeast strain, designated as SHY 111, which showed the highest ethanol production in a YPD liquid medium, was selected for further study (data not shown). The results from an Automated Identification System are shown in Table 3. Its similarity to *S. boulardii* was as high as 89% and those to other strains were less than 1.5%. Although the database of the identification system includes most strains of *S. cerevisiae*, the SHY 111 strain was found to be different from *S. cerevisiae* in the system.

Analysis of Yeast Internal Transcribed Spacer (ITS) Sequences

Although there have been a great number of reports on the subject of *S. cerevisiae*, unfortunately, little is known about *S. boulardii*. The identification results of the strain SHY 111 prompted us to analyze its ITS regions in order to confirm whether it is genetically close to *S. boulardii*. The ITS I (located between 18S and 5.8S rDNA) and ITS II (located between 5.8S and 25S rDNA) regions on the chromosomal DNA have been known to be variable, and

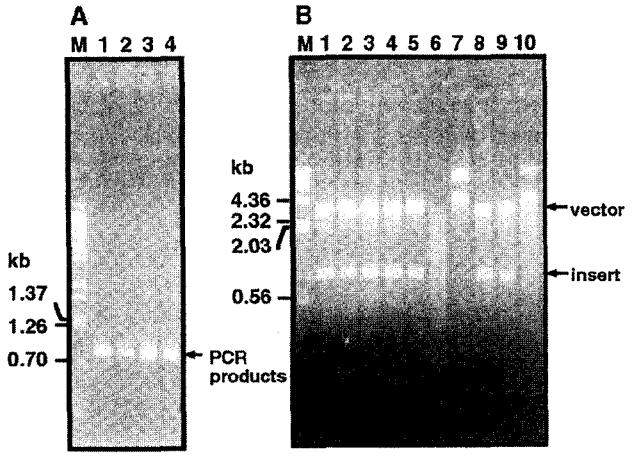

Fig. 1. Analysis and cloning of PCR products containing yeast internal transcribed spacer (ITS) I and II regions amplified from yeast chromosomal DNA.

A: Agarose gel electrophoresis of PCR products amplified from the chromosomal DNA of the strain SHY 111 (lane 1), *S. boulardii* (lane 2), *S. cerevisiae* AB 972 (lane 3), and *S. cerevisiae* KCCM 11215 (lane 4). B: *Bam*HI analysis of recombinant plasmids to confirm the PCR fragments derived from the strain SHY 111 (lanes 1 to 3), *S. cerevisiae* KCCM 11215 (lanes 4 to 6), and *S. boulardii* (lanes 7 to 10). PCR was carried out using synthetic oligonucleotides as primers which were derived from the sequences of yeast 18S and 25S rDNA and were designed to produce *Bam*HI restriction sites in both ends of the PCR products. The PCR products were cloned into the *Bam*HI site of vector plasmid pUC119. Recombinant plasmids were analyzed on a 0.8% agarose gel after digestion with *Bam*HI to confirm the 800 bp insert. Mark Ms indicate DNA size markers, λ DNA digested with *Bst*EII in Fig. A, and *Hin*dIII in Fig. B.

thus they are useful in classifying the fungi [1, 2, 29]. PCR was performed to amplify the DNA fragments covering yeast ITS I and ITS II regions with synthetic oligonucleotide primers (ITS1 and ITS2), shown in Table 1. The amplified DNA fragments, from the strain SHY 111 chromosomal DNA, were resolved on a 0.8% agarose gel, and compared with those from various yeast type strains including *S. cerevisiae* AB 972, *S. cerevisiae* KCCM 11215, and *S. boulardii*. It produced exactly the same size of about 800 bp DNA fragments (Fig. 1A). Therefore, we have cloned and sequenced the DNA fragments. After digesting with *Bam*HI, the PCR products derived from the strains SHY 111, *S. cerevisiae* KCCM 11215, and *S. boulardii* were cloned into the *Bam*HI site of the pUC119 vector plasmid. Figure 1B shows the result of *Bam*HI analysis of the recombinant plasmids. Lanes 1 to 3, 4 to 6, and 7 to 10 were derived from the strains of SHY 111, KCCM 11215, and *S. boulardii*, respectively. Most of the plasmids were confirmed to have the 800 bp insert. Some of them showed unexplainable DNA patterns, which were thought to have come from abnormal ligations (lanes 6, 7, and 10). A total of three plasmids (one each derived from strains SHY 111, *S. boulardii*, and *S. cerevisiae* KCCM 11215), which have the 800 bp inserts, were selected and used for the sequencing. The sequences of the insert fragments from

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ITS1
SHY111      AAAGAAATTT AATAATTTTG AAAATGGATT TTTTTGTTTT GGCAAGAGCA  50
S.boulardii AAAGAAATTT AATAATTTTG AAAATGGATT TTTTTGTTTT GGCAAGAGCA
KCCM11215   AAAGAAATTT AATAATTTTG AAAATGGATT TTTTTGTTTT GGCAAGAGCA
Genome      AAAGAAATTT AATAATTTTG AAAATGGATT TTTTTGTTTT GGCAAGAGCA

SHY111      TGAGAGCTTT TACTGGGCAA GAAGACAAGA GATGGAGAGT CCAGCCGGGC 100
S.boulardii TGAGAGCTTT TACTGGGCAA GAAGACAAGA GATGGAGAGT CCAGCCGGGC
KCCM11215   TGAGAGCTTT TACTGGGCAA GAAGACAAGA GATGGAGAGT CCAGCCGGGC
Genome      TGAGAGCTTT TACTGGGCAA GAAGACAAGA GATGGAGAGT CCAGCCGGGC

SHY111      CTGCGCTTAA GTGCGCGGTC TACTGGGCAA GAAGACAAGA GATGGAGAGT 150
S.boulardii CTGCGCTTAA GTGCGCGGTC TACTGGGCAA GAAGACAAGA GATGGAGAGT
KCCM11215   CTGCGCTTAA GTGCGCGGTC TACTGGGCAA GAAGACAAGA GATGGAGAGT
Genome      CTGCGCTTAA GTGCGCGGTC TACTGGGCAA GAAGACAAGA GATGGAGAGT

SHY111      CCAGCCGGGC CTGCGCTTAA GTGCGCGGTC TACTGGGCAA GAAGACAAGA 200
S.boulardii CCAGCCGGGC CTGCGCTTAA GTGCGCGGTC TACTGGGCAA GAAGACAAGA
KCCM11215   CCAGCCGGGC CTGCGCTTAA GTGCGCGGTC TACTGGGCAA GAAGACAAGA
Genome      CCAGCCGGGC CTGCGCTTAA GTGCGCGGTC TACTGGGCAA GAAGACAAGA

SHY111      GATGGAGAGT CCAGCCGGGC CTGCGCTTAA GTGCGCGGTC ACTTTTTCTT 250
S.boulardii GATGGAGAGT CCAGCCGGGC CTGCGCTTAA GTGCGCGGTC ACTTTTTCTT
KCCM11215   GATGGAGAGT CCAGCCGGGC CTGCGCTTAA GTGCGCGGTC ACTTTTTCTT
Genome      GATGGAGAGT CCAGCCGGGC CTGCGCTTAA GTGCGCGGTC ACTTTTTCTT

SHY111      TGGGCATTCG AGCAATCGGG GCCCAGAGGT AACAAACACA AACAATTTTA 300
S.boulardii TGGGCATTCG AGCAATCGGG GCCCAGAGGT AACAAACACA AACAATTTTA
KCCM11215   TGGGCATTCG AGCAATCGGG GCCCAGAGGT AACAAACACA AACAATTTTA
Genome      TGGGCATTCG AGCAATCGGG GCCCAGAGGT AACAAACACA AACAATTTTA

SHY111      TCTATTCATT AAATTTTTGT CAAAAACAAG AATTTTCGTA ACTGGAAATT 350
S.boulardii TCTATTCATT AAATTTTTGT CAAAAACAAG AATTTTCGTA ACTGGAAATT
KCCM11215   TCTATTCATT AAATTTTTGT CAAAAACAAG AATTTTCGTA ACTGGAAATT
Genome      TCTATTCATT AAATTTTTGT CAAAAACAAG AATTTTCGTA ACTGGAAATT

SHY111      TTAAAATATT AA 362
S.boulardii TTAAAATATT AA
KCCM11215   TTAAAATATT AA
Genome      TTAAAATATT AA

ITS2
SHY111      CCTTCTCAAA CATTCTGTTT GGTAGTGAGT GATACTCTTT GGAGTTAACT  50
S.boulardii CCTTCTCAAA CATTCTGTTT GGTAGTGAGT GATACTCTTT GGAGTTAACT
KCCM11215   CCTTCTCAAA CATTCTGTTT GGTAGTGAGT GATACTCTTT GGAGTTAACT
Genome      CCTTCTCAAA CATTCTGTTT GGTAGTGAGT GATACTCTTT GGAGTTAACT

SHY111      TGAAATTGCT GGCCTTTTCA TTGGATGTTT TTTTTCCAAA GAGAGGTTTC 100
S.boulardii TGAAATTGCT GGCCTTTTCA TTGGATGTTT TTTTTCCAAA GAGAGGTTTC
KCCM11215   TGAAATTGCT GGCCTTTTCA TTGGATGTTT TTTTTCCAAA GAGAGGTTTC
Genome      TGAAATTGCT GGCCTTTTCA TTGGATGTTT TTTTTCCAAA GAGAGGTTTC

SHY111      TCTGCGTGCT TGAGGTATAA TGCAAGTACG GTCGTTTTAG GTTTTACCAA 150
S.boulardii TCTGCGTGCT TGAGGTATAA TGCAAGTACG GTCGTTTTAG GTTTTACCAA
KCCM11215   TCTGCGTGCT TGAGGTATAA TGCAAGTACG GTCGTTTTAG GTTTTACCAA
Genome      TCTGCGTGCT TGAGGTATAA TGCAAGTACG GTCGTTTTAG GTTTTACCAA

SHY111      CTGCGGCTAA TCTTTTTTTA TACTGAGCGT ATTGGAACGT TATCGATAAG 200
S.boulardii CTGCGGCTAA TCTTTTTTTA TACTGAGCGT ATTGGAACGT TATCGATAAG
KCCM11215   CTGCGGCTAA TCTTTTTTTA TACTGAGCGT ATTGGAACGT TATCGATAAG
Genome      CTGCGGCTAA TCTTTTTTTA TACTGAGCGT ATTGGAACGT TATCGATAAG

SHY111      AAGAGAGCGT CTAGGCGAAC AATGTTCTTA AA 232
S.boulardii AAGAGAGCGT CTAGGCGAAC AATGTTCTTA AA
KCCM11215   AAGAGAGCGT CTAGGCGAAC AATGTTCTTA AA
Genome      AAGAGAGCGT CTAGGCGAAC AATGTTCTTA AA
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Fig. 2. Comparison of the sequences of ITS I and II regions derived from strain SHY 111, *S. boulardii*, and *S. cerevisiae* KCCM 11215 with the sequences acquired from the results of the Yeast Genome Project, which has used *S. cerevisiae* AB 972 as a type strain. The upper and lower boxes indicate yeast ITS I and ITS II regions, respectively.

the 3 strains were aligned with the sequences which were acquired from the results of the Genome Project that has used *S. cerevisiae* AB 972 as a type strain [8]. All the sequences within the ITS I and ITS II regions were found to be identical, suggesting that all three strains used in this study are genetically very close to *S. cerevisiae* AB 972 (Fig. 2).

Differentiation of Korean Traditional Liquor Yeasts Using Intron Splice Site Primers

Differentiation of the strain SHY 111 from various yeast strains were investigated using the PCR primers which

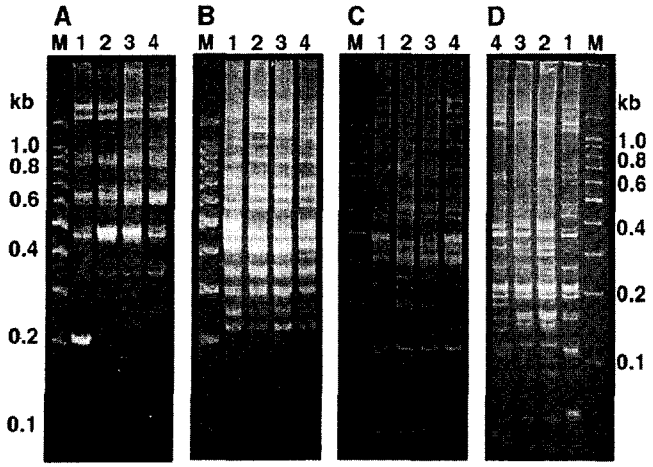

Fig. 3. PCR differentiation of the strain SHY 111, isolated from *Yakju*, from other yeast strains using synthetic oligonucleotide primers derived from the yeast intron splice site.

PCR primers EI2, LA1 described in detail by de Miguel [5] were used alone (EI2 in Fig. 3A, LA2 in Fig. 3B) or in combination (EI2+LA1 in Fig. 3C, EI2+LA2 in Fig. 3D). Chromosomal DNA used for a PCR template was isolated from strain SHY 111 (lane 1), *S. boulardii* (lane 2), *S. cerevisiae* KCCM 11215 (lane 3), or *S. cerevisiae* AB 972 (lane 4). The PCR products were resolved on a 4% polyacrylamide gel. Mark M represents 100 bp DNA ladder as a DNA size marker.

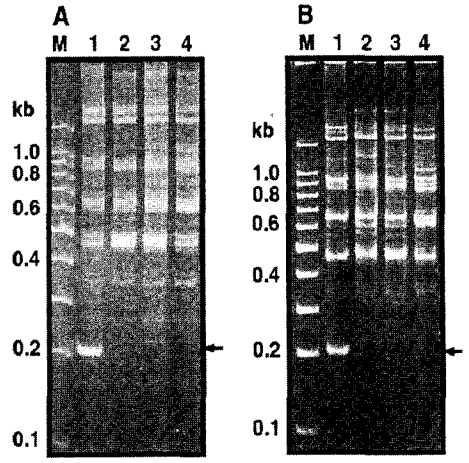

Fig. 4. Reproducibility of PCR patterns using EI2 primer derived from intron splice site sequences.

PCR patterns which were repeated using another batch of yeast chromosomal DNA preparations (B) as templates were compared with the original (A). All other designations were the same as those used in Fig. 3.

were derived from the yeast intron splice site (described in Table 1). These strains have been reported to be very useful for differentiating between commercial wine-making yeast strains [5]. The amplified DNA patterns from yeast chromosomal DNA are shown in Fig. 3. When the primer EI2 was used for the PCR, a 200 bp DNA band was observed only from the strain SHY 111 (Fig. 3A, lane 1). No significant differences were observed between *S. boulardii*, *S. cerevisiae* strains KCCM 11215 and AB 972 with the exception of the 200 bp DNA band (Fig. 3A, lanes 2 to 4). The PCR analyses with the primers LA1 alone (Fig. 3B), EI2+LA1 (Fig. 3C), and EI2+LA2 (Fig. 3D) failed to discriminate between the strains. Although there were some differences in its DNA patterns, it was very difficult to discriminate one strain from another. PCR amplification with the primer EI2 with the use of another batch of 4 yeast chromosomal DNA preparations as DNA templates was repeated in order to confirm the observation of the 200 bp DNA fragment (Fig. 4). The two reactions resulted in the same DNA patterns, suggesting the primer EI2 is very useful in differentiating the *Yakju* yeast strain SHY 111 from other strains.

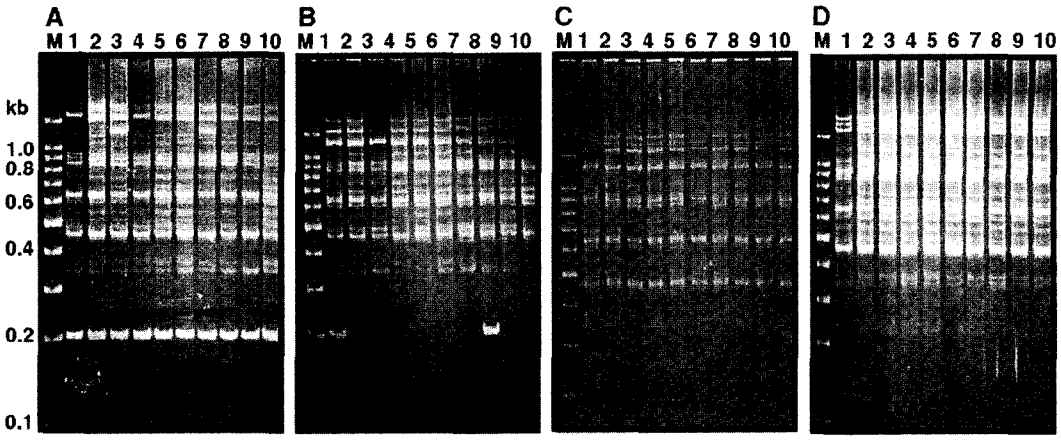

Fig. 5. PCR patterns of yeast strains isolated from various Korean traditional liquors using EI2 primer derived from intron splice site sequences.

Forty alcohol yeast strains isolated from the mashes (10 strains from each mash) of 3 different kinds of *Yakju* (A to C) and 1 *Takju* (D) were used as sources of chromosomal DNA for the PCR template. Lanes 1 to 10 represent different yeast strains isolated from the same mashes for brewing of each liquor. The PCR products were resolved on a 4% polyacrylamide gel. Mark M represents the 100 bp DNA ladder as a DNA size marker.

Genetic Relationship of Korean Traditional Liquor Yeast Strains

Forty alcohol yeast strains, isolated from main mashes (10 strains from each mash) for the brewing of 3 different kinds of *Yakju* (Fig. 5, A to C) and 1 *Takju* (Fig. 5D), were tested for their diversity using PCR with the EI2 primer containing intron splice site sequences. All the ten strains from one *Yakju* and 2 out of 10 strains from another *Yakju* resulted in the production of the specific 200 bp PCR fragments. This might be due to the fact that the geographical location for brewing the 2 kinds of *Yakju* are close to each other. Therefore, the 200 bp PCR fragments were considered to be useful as a potential marker for those yeast strains. It is interesting to note that although the two strains were shown to be different from *S. boulardii* (Fig. 5A, lane 8; Fig. 5B, lane 1) by the Automated Identification System, they produced the same fragments. However, it is not known whether the identification system used in the present study to group yeast strains is reliable enough for the identification of microbial strains. All the ten yeast strains, isolated from the *Yakju* which has a long history of brewing, produced 200 bp PCR products. However, it is not clear yet whether the 200 bp DNA fragments have something to do with the period of brewing history. Cloning of 200 bp fragments is in progress to elucidate the specific gene(s) involved. Except for the 200 bp PCR products, the yeast strains showed similar PCR patterns. Specifically, no significant differences were observed in each strain isolated from the same mashes. These results suggest that the yeast strains catalyzing fermentation of Korean traditional liquors are genetically close to each another.

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