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ARTICLE

Culture-independent analysis of yeast diversity in Korean traditional fermented soybean foods (*doenjang* and *kanjang*) based on 26S rRNA sequence

Md. Azizul Haque · Weon Taek Seo · Chung Eun Hwang · Hee Yul Lee · Min Ju Ahn · Kye Man Cho

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Abstract The yeast-26S rRNA libraries were constructed from two different fermented soybean foods, *doenjang* and *kanjang*. A total of 42 clones, containing the partial 26S rRNA sequences, 0.6 kb in length, were sequenced and subjected to an online similarity search. All *doenjang* yeast (DY) clones only appeared in the *Saccharomycotina* class. The 21 clones from the *doenjang* library were classified into five groups: *Debaryomyces hansenii* (DY I, 76.0 %), *Zygosaccharomyces pseudorouxii* (DY II, 9.6 %), *Candida versatilis* (DY III, 4.8 %), *Candida etchellsii* (DY IV, 4.8 %), and *Debaryomyces castellii* (DY V, 4.8 %). The 21 *kanjang* yeast (KY) clones were affiliated with the *Saccharomycotina* (52.4 %), *Urediniomycetes* (19.0 %), *Ustilaginomycetes* (23.8 %), and *Hymenomycetes* (4.8 %) classes and divided into six groups: *D. hansenii* (KY I, 38.0 %), *Sterigmatomyces halophilus* (KY II, 19.0 %), *Malassezia restricta* (KY III, 23.8 %), *Cryptococcus magnus* (KY V, 4.8 %), and *Pichia triangularis* (KY VI, 9.6 %). Yeast belonging to the *Saccharomycotina* class was predominant (76.2 %) in fermented soybean foods, *doenjang* and *kanjang*. These findings are of fundamental value for understanding the complexity of two different fermented soybean foods.

Keywords Culture-independent · Phylogenetic analysis · Soybean-fermented foods · Yeast diversity · 26S rRNA gene

Md. A. Haque · W. T. Seo · C. E. Hwang · H. Y. Lee · M. J. Ahn · K. M. Cho (✉)
Department of Food Science, Gyeongnam National University of Science and Technology, Jinju, Gyeongnam 660-758, Republic of Korea
e-mail: kmcho@gntech.ac.kr

Introduction

Soybeans (*Glycine max*) and common edible beans are the world's first and second most important food legumes. Soybeans have been consumed as important protein source to complement grain protein in Asian countries for a long time (Lee et al. 2013). Common edible beans are a basic food in Africa, India, and Latin America (Xu and Chang 2011). Korean traditional fermented soybean foods, such as *doenjang* (soybean paste) and *kanjang* (soybean sauce), have served as side dishes that have been one of the major protein sources in Korean diet for thousands of years (Cho and Seo 2007). Fermented soybean foods have attracted considerable interest due to their excellent nutritional value (Choi et al. 2005). They are also well known as functional foods due to their anticancer properties (Jung et al. 2006), antioxidant activity (Kim et al. 2008), and anti-mutagenicity (Lim et al. 2004). Thus, they are expected to become important for human health.

Yeasts are unicellular fungi and represent diversified microorganisms in the phyla of *Ascomycota* and *Basidiomycota* of the kingdom fungi (Wang et al. 2008). They occur in a wide range of fermented foods of plant- or animal-originated raw materials (Aidoo et al. 2006). In particular, unique flavor and taste of fermented soy foods are mainly due to degradation of soybean proteins by microorganisms during fermentation, which play an important role in fermented soybean foods. The decomposed products of soybean protein are created by the actions of microorganisms during fermentation (Cho and Seo 2007; Kim et al. 1996, 2009). Lee and Lee (1970) observed yeasts in fermented soybean foods. Lee et al. (1970) isolated yeast belonging to *Saccharomyces* (including corresponding *Zygosaccharomyces*), *Pichia*, *Hansenula*, *Nadsonia*, *Debaryomyces*, *Torulopsis*, and *Candida*. Recently, Kim et al.

(2009) reported that *Debaryomyces hansenii* was found to be the predominant yeast in several *doenjang* samples using a culture-independent method of PCR-denaturing gradient gel electrophoresis (DGGE).

Several molecular biological methods have been used to identify, quantify, and visualize microorganism populations (Cho et al. 2009; Shin et al. 2004). They include DNA probe hybridization (Coignard et al. 2004), restriction fragment length polymorphism (RFLP) (Petersen et al. 2001), DGGE (Kim et al. 2009; Maro et al. 2007), intergenic transcribed spacers (ITS) region (de Llanos Frutos et al. 2004), randomly amplified polymorphic DNA (RAPD) (Bovo et al. 2009; Maro et al. 2007), temperature gradient gel electrophoresis (TGGE) (Hernan-Gomez et al. 2000), mitochondrial DNA restriction analysis (Bovo et al. 2009; Maqueda et al. 2010), single-strand conformation polymorphism (SSCP) analysis (Callon et al. 2006; Wang et al. 2008), and 26S ribosomal DNA (rDNA) sequences. In particular, the D1/D2 domain of 26S rRNA, a fragment of approximately 600–650 bp, is used for the molecular classification and identification of yeasts (Fell et al. 2000; Kurtzman and Robnett 1998). Taxonomic studies based on the molecular characters have resulted in the discovery of an unparalleled number of new yeast species in recent years and have greatly improved our understanding of yeast biodiversity (Boekhout 2005; Wang et al. 2008).

In this study, the diversity of yeast community in Korean traditional fermented soyfoods (doenjang and kanjang) was investigated by a culture-independent method involving DNA extraction, amplification of D1/D2 domain, and sequencing study attempts to investigate the microbial diversity of the yeast community in Korean traditional soybean-based fermented food (doenjang and kanjang) using a culture-independent approach that utilizes DNA extraction and ribosomal RNA (26S rRNA) gene amplification and sequencing.

Materials and methods

Sampling and DNA extraction

Doenjang and *kanjang* were obtained from several households in Jinju, Gyeongnam, Korea. Homemade *Doenjang* and *Kanjang* (five samples) were used in this study. Each sample was diluted and filtered to collect the fluid portion, and the salinity was measured with a salinity meter (Atago Co., Tokyo, Japan). Each sample (approximately 1 g or 1 ml) was mixed with 20 ml of phosphate buffered saline (pH 7.2) and vortexed for 30 min. For DNA extraction, the samples were collected through four layers of cheesecloth and centrifuged at 14,000×*g* for 5 min at 4 °C. The pellets were then subjected to DNA extraction using a Soil DNA Extraction Kit (Mo Bio, Solana Beach, CA, USA). The extracted DNA was then used as a template for PCR to amplify 26S rRNA.

PCR amplification

The extracted DNA was used as a template for PCR to amplify 26S rRNA. The PCR primers used to amplify 26S rRNA fragments were the yeast-specific primers 5′-ACCC GCTGAAYTTAAGCATAT-3′ (3YF/21 mer, forward primer based on the position number of *Saccharomyces cerevisiae* LSU rRNA) and 5′-CTCCTTGGTCGTGTTT CAAGACGG-3′ (3YR/25 mer, reverse primer) (Shin et al. 2004). Subsequently, rRNAs were amplified by PCR using the metagenomic DNA and Super-Therm DNA polymerase (JMR, Side Cup, Kent, UK). Based on the manufacturer's instructions, the PCR reaction mixture (50 µl) contained 1 µl of *Taq* polymerase (2.5 unit), 3 µl of each primer set, NLR184-NLR818 (10 *p*mol), 5 µl of reaction buffer, 15 mM $MgCl_2$, 5 µl of 2 mM dNTP, 5 µl of template DNA, and 28 µl of sterile water. Thirty cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 90 s were followed by a final incubation at 72 °C for 10 min. The anticipated product of approximately 0.6 kb was isolated by agarose gel electrophoresis. The yeast 26S rRNA gene amplicons were purified with a PCR purification kit (Intron Biotechnology, Suwon, Korea).

26S rRNA library construction, DNA sequencing, and sequence analysis

Amplified yeast 26S rRNA genes were inserted in the pGEM-T easy vector (Promega, Madison, WI, USA) and transformed into *Escherichia coli* DH5α. Recombinant clones and the insert size in purified plasmids were confirmed as described previously (Cho et al. 2009). The nucleotide sequences of the purified plasmids were analyzed by the dideoxy-chain termination method using the PRISM Ready Reaction Dye terminator/primer cycle sequencing kit (Perkin-Elmer Corp., Norwalk, CN, USA). Nucleotide sequences of the full-length inserted PCR product were analyzed using an automated DNA sequencer (Applied Biosystems, Foster City, CA). All reference sequences were obtained from the National Center for Biotechnology Information (NCBI) and Ribosomal Database Project databases (http://rdp8.cme.msu.edu). Sequences were analyzed using the CHIMERA program (Maidak et al. 2000) to identify and exclude sequences arising from chimeric rRNA clones. The 26S rRNA sequence identity searches were performed using the BLSATN and PSI-BLAST tools on the NCBI website (McGinnis and Madden 2004). Sequences were aligned using the multiple sequence alignment program CLSTAL W

(Thompson et al. 1994). Phylogenetic analysis was performed using the neighbor-joining method (Saitou and Nei 1997). Gaps and positions with ambiguities were excluded from the phylogenetic analysis. Bootstrap analysis was performed on data resampled 1,000 times using the DNAMAN analysis system (Lynnon Biosoft, Quebec, Canada).

Nucleotide sequence accession numbers and nomenclature

Nucleotide sequences have been deposited in the GenBank database under the accession numbers JN868076-JN868117. Clones names in the *doenjang* yeast library begin with the letters DY (e.g., DY01), and clone names in the *kanjang* yeast library have KY prefixes (e.g., KY01).

Results

Salinity and cloning of yeast rRNA

The diversity of the yeast populations in *doenjang* and *kanjang* was studied by analyzing PCR-amplified yeast 26S rRNA molecules. Ten fermented soybean food samples (five *doenjang* and five *kanjang*) were randomly collected from homemade products. The salinity varied from 12.4 to 13.8 % and from 18.6 to 22.3 % in *doenjang* and *kanjang*, respectively (data not shown). When the metagenomes isolated from each sample were used as the templates for 26S rRNA gene amplification, approximately 0.6 kb of DNA fragment was amplified. The product was isolated from an agarose gel and cloned into *E. coli* DH5α using pGEM-T Easy vector. A total of 42 clones were obtained, and 21 clones from the three libraries were sequenced (Tables 1, 2).

Similarity with database sequences

A total of 42 clones were obtained from two libraries, the first library from *doenjang* and the second from *kanjang*. All of the clones from the two libraries were subjected to sequence analysis, followed by online homology searches using two databases: the NCB website, which implements the BLAST algorithm (McGinnis and Madden 2004) and the RDP database, which implements the SIMILARITY_RANK program (Maidak et al. 2000) (Tables 1, 2). In the *doenjang* library, the clones were classified into five operational taxonomic units (OTUs) following the 26S rRNA sequence. Most of our sequences had the following similarities with cultured isolates: *D. hansenii* (DY01-DY02, DY04-DY06, DY08-DY09, DY12-DY16, DY18-DY19, and DY21), *Zygosaccharomyces pseudorouxii* (DY03 and DY17), *Candida versatilis* (DY07), *Debaryomyces castellii* (DY10), and *Candida etchellsii* (DY11). The twenty-one clones were all affiliated with the *Saccharomycotina* class. Most of the rRNA showed a sequence identity of 98–100 % with those of the species listed in the database (Table 1). A total of 21 clones were analyzed from *kanjang* library. The clones were divided into six groups based on their similarity with different species of yeast as follows: *D. hansenii* (KY01, KY04, KY06-KY07, KY09, KY11, KY15 and KY17), *Sterigmatomyces halophilus* (KY02, KY10, KY14, and KY16), *Malassezia restricta* (KY03, KY08, KY18, KY20, and KY21), *Z. pseudorouxii* (KY05), *Crytococcus magnus* (KY12), and *Pichia triangularis* (KY13 and KY19). The twenty-one

Table 1 Similarity values of 26S rRNA sequences retrieved from *doenjang*

Group	Clone (accession no.)	Class	Species	Similarity (%)[a]
I	DY01 (JN868076), DY02 (JN868077), DY04 (JN868079), DY05 (JN868080), DY06 (JN868081), DY08 (JN868083), DY09 (JN868084), DY12 (JN868087), DY13 (JN868088), DY14 (JN868089), DY15 (JN868090), DY16 (JN868091), DY18 (JN868093), DY19 (JN868094), DY20 (JN868095), DY21 (JN868096)	*Saccharomycotina*	*Debaryomyces hansenii*	97–100
II	DY03 (JN868078), DY17 (JN868092)	*Saccharomycotina*	*Zygosaccharomyces pseudorouxii*	98–100
III	DY07 (JN868082)	*Saccharomycotina*	*Candida versatilis*	99
IV	DY10 (JN868085)	*Saccharomycotina*	*Debaryomyces castellii*	97
V	DY11 (JN868086)	*Saccharomycotina*	*Candida etchellsii*	100

[a] Range of 26S rRNA genes sequence is similarity values between *kannjang* yeast clones and type strain. *DY doenjang* yeast

Table 2 Similarity values of 26S rRNA sequences retrieved from *kanjang*

Group	Clone (accession no.)	Class	Species	Similarity (%)[a]
I	KY01 (JN868097), KY04 (JN868100), KY06 (JN868102), KY07 (JN868103), KY09 (JN868105), KY11 (JN868107), KY15 (JN868111), KY17 (JN868113)	*Saccharomycotina*	*Debaryomyces hansenii*	99
II	KY02 (JN868098), KY10 (JN868106), KY14 (JN868110), KY16 (JN868112)	*Urediniomycetes*	*Sterigmatomyces halophilus*	99
III	KY03 (JN868099), KY08 (JN868104), KY18 (JN868114), KY20 (JN868116), KY21 (JN868117)	*Ustilaginomycetes*	*Malassezia restricta*	98
IV	KY05 (JN868101)	*Saccharomycotina*	*Zygosaccharomyces pseudorouxii*	100
V	KY12 (JN868108)	*Hymenomycetes*	*Cryptococcus magnus*	100
VI	KY13 (JN868109), KY19 (JN868115)	*Saccharomycotina*	*Pichia triangularis*	98

[a] Range of 26S rRNA genes sequence is similarity values between *kanjang* yeast clones and type strain. *KY kanjang* yeast

clones were affiliated with the *Saccharomycotina, Urediniomycetes, Ustilaginomycetes,* and *Hymenomycetes* classes. All of the rRNA sequences showed 98–100 % identity with those of the species listed in the database (Table 2).

Phylogenetic placement of sequences from the two libraries

The phylogenetic relationships among the affiliated fermented soybean yeasts were analyzed by determining the yeast having a reasonable degree of confidence to particular taxa and clarifying their taxonomic position (Figs. 1, 2). A phylogenetic analysis of the *doenjang* library is shown in Fig. 1. All of the sequences were phylogenetically placed within the *Saccharomycotina* class. This class may contribute to *doenjang* aroma and function. The DY I group (sixteen clones) was associated to the isolated strain *D. hansenii*. The DY II group (DY03 and DY17) was related to the typical fermented soybean yeast, *Z. pseudorouxii*. The DY III (DY07) and DY IV (DY11) groups were related to the *moromi* (Japanese fermented soybean food) isolates *C. versatilis* and *C. etchellsii*, respectively (Suezawa et al. 2006). The DY V group (one clone) clustered with the cultured isolate of *D. castellii*. The results of phylogenetic analysis of the *kanjang* library are shown in Fig. 2. In this library, the majority of the sequences were placed within the *Saccharomycotina* class. This class may also contribute to the *kanjang* aroma and function. The KY I group (eight clones) was associated with the isolated strain *D. hansenii*. The KY II group (KY02, KY10, KY14, and KY16) clustered with the halophilic yeast, *S. halophilus*. The KY III group (five clones) was associated with the lipophilic yeast, *M. rextricta*. The KY IV group (KY05) was related to the typical fermented soybean yeast, *Z. pseudorouxii*. The KY V (KY12) and KY VI (KY13 and KY19) were related to the *C. oeirensis* and *P. triangularis*, respectively.

Yeast distribution from the two libraries

The distribution of yeast species from the *doenjang* and *kanjang* are shown in Table 3. *Debaryomyces hansenii* was the predominant species in the two fermented soybean foods: 76 % of the species in the *doenjang* library and 38 % of the species in the *kanjang* library. *C. etchellsii* (4.8 %), *C. versatilis* (4.8 %), *D. castellii* (4.8 %), and *Z. pseudorouxii* (9.6 %) were also detected in the *doenjang* library, and *C. magnus* (4.8 %), *M. restricta* (23.8 %), *P. triangularis* (9.6 %), *S. halophilus* (19.0 %), and *Z. pseudorouxii* (4.8 %) were detected in the *kanjang* library. *D. hansenii* and *Z. pseudorouxii* were found in both libraries. The *doenjang* yeast (DY) clones only appeared in the *Saccharomycotina* class. In contrast, the twenty-one *kanjang* yeast (KY) clones were observed in the *Saccharomycotina* (52.4 %), *Urediniomycetes* (19.0 %), *Ustilaginomycetes* (23.8 %), and *Hymenomycetes* (4.8 %) classes (Table 4).

Discussion

Compared with other ecosystems, there have been only a few studies examining yeast in traditional Korean foods, such as *doenjang* and *kanjang*. However, the various kinds of yeast participate in the fermentation of *doenjang* and their unique flavors and tastes are attributed to the decomposed products of soybean proteins by microbial action during fermentation. There have been a few reports

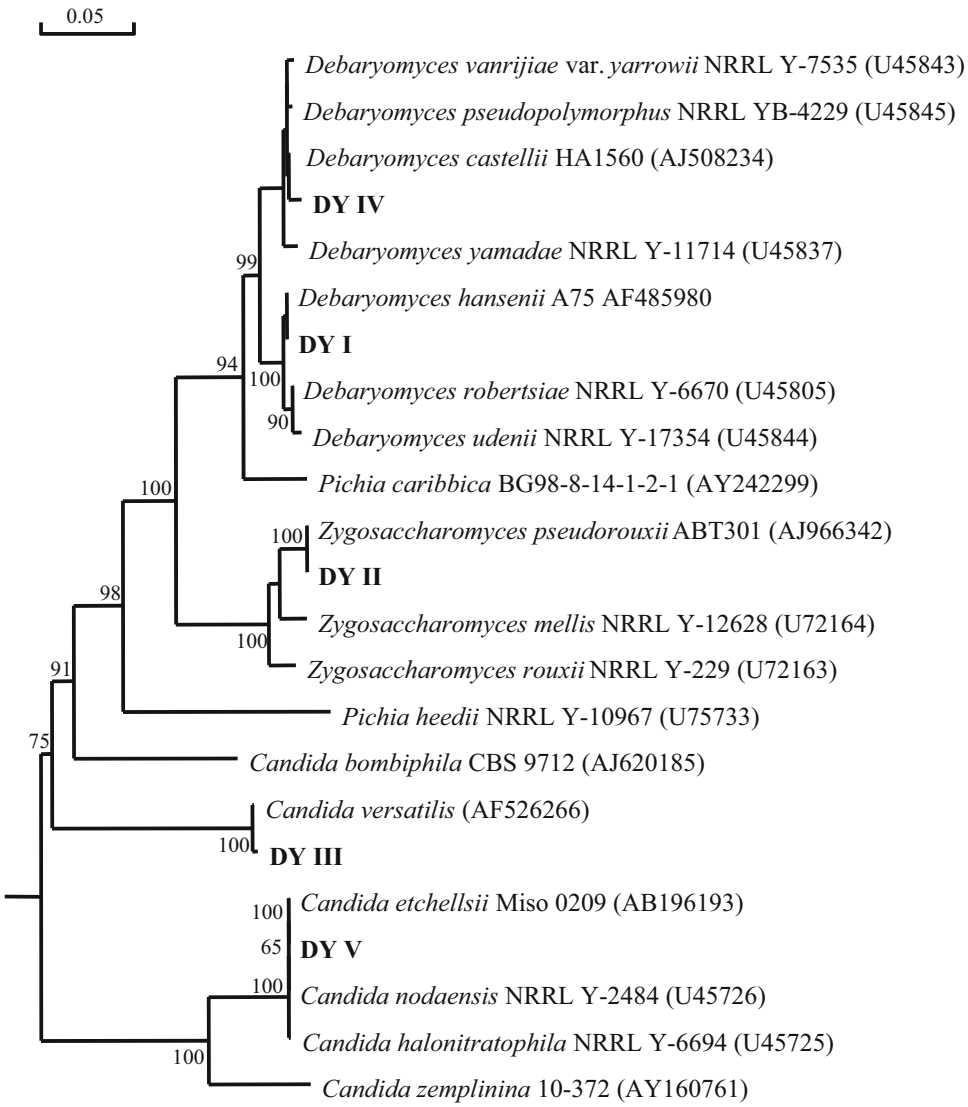

Fig. 1 Phylogenetic placement of 26S rRNA sequences from *doenjang*. Numbers above each node are confidence levels (%) generated from 1,000 bootstrap trees. The scale bar is in fixed nucleotide substitutions per sequence position. Only values of 60 % or above are shown. *DY doenjang* yeast

of microbial diversity in Korean traditional fermented soybean food (*doenjang* and *kanjang* etc.) using culture-independent methods. Previously, we only reported the bacterial diversity in two different Korean traditional fermented soybean foods (Cho and Seo 2007). Recently, Kim et al. (2009) reported the bacterial and yeast diversities in *doenjang*, but the yeast diversity in *kanjang* has not yet been reported. Additionally, both *doenjang* (12.4–13.8 %) and *kanjang* (18.6–22.3 %) represent extremely halophilic environments among Korean traditional fermented foods. Extreme environments are interesting for the study of microbial diversity, the identification of novel microorganisms, and the understanding of the functioning of their ecosystems.

In the yeast analysis, yeasts most closely related to *D. hansenii* and *Z. pseudorouxii* were found in the both libraries (Table 3). *Debaryomyces hansenii* was found to be the predominant yeast in the *doenjang* and *kanjang* samples. *Debaryomyces hansenii* had previously been isolated and identified in the fermented foods kanjang-koji and mash (Lee et al. 1970; Lee and Lee 1970) and nuruk, a traditional Korean starter for rice wine (Jo and Lee 1997). Recently, Kim et al. (2009) reported that culture-independent approaches found *D. hansenii* to be the predominant yeast in several *doenjang* samples. This yeast is also attractive for study due to its ability to grow under extreme conditions, such as very low temperatures, widely varying pH, and high salt concentrations (Cabrera-Orefice et al. 2010). Under saline conditions, *D. hansenii* accumulates large amounts of Na^+ without being intoxicated. K^+ is also present at a low concentration in the environment (Chao et al. 2009). In addition, the various peptidase and protease of *D. hansenii* have been described, suggesting that it likely participates in the ripening of dry-cured meat products (Bolumar et al. 2003, 2008). The genus *Zygosaccharomyces* was found on fermented soybean foods. Some

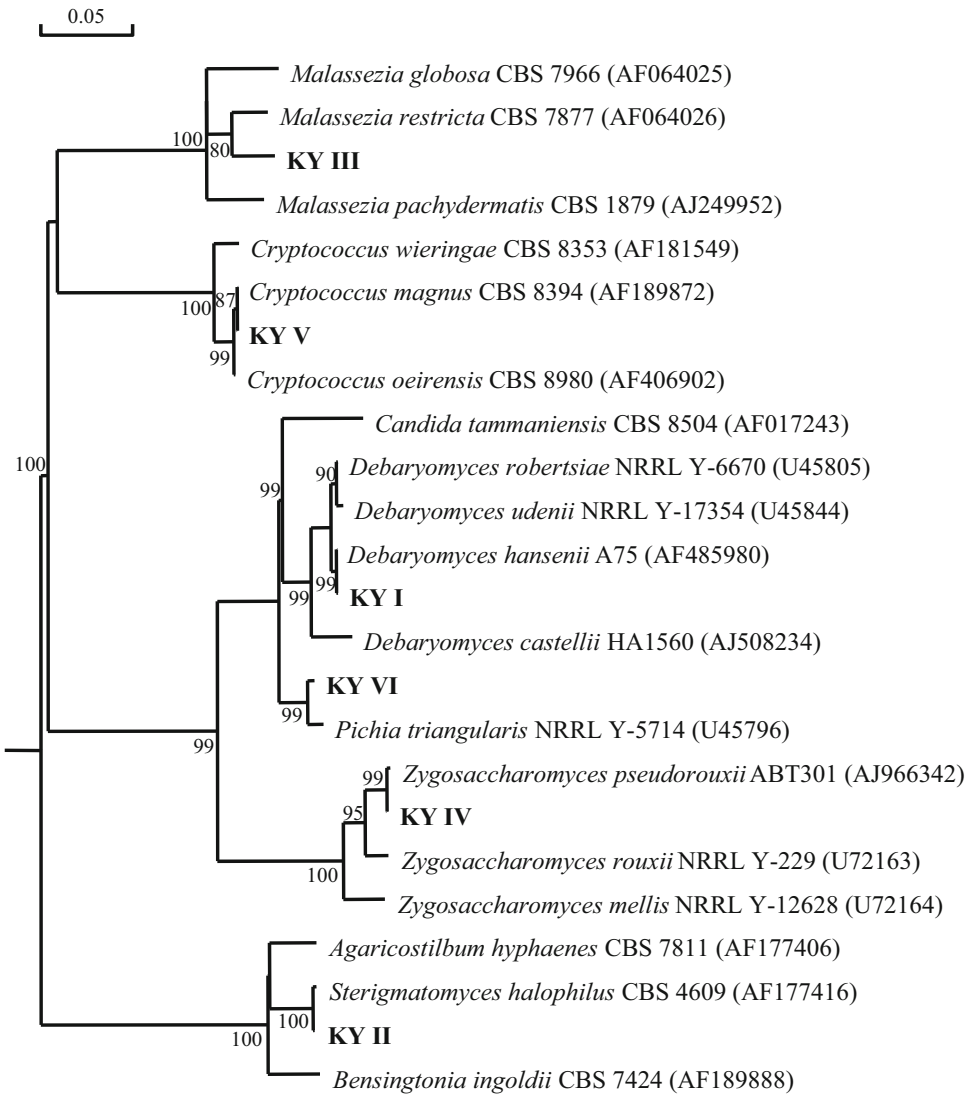

Fig. 2 Phylogenetic placement of 26S rRNA sequences from *kanjang*. Numbers above each node are confidence levels (%) generated from 1,000 bootstrap trees. The scale bar is in fixed nucleotide substitutions per sequence position. Only values of 60 % or above are shown. *KY kanjang* yeast

Table 3 Yeast distribution of *doenjang* and *kanjang*

Species	DY n/%	KY n/%	Number of total clones n/%[a]
Candida etchellsii	1/4.8		1/2.4
Candida versatilis	1/4.8		1/2.4
Cryptococcus magnus		1/4.8	1/2.4
Debaryomyces castellii	1/4.8		1/2.4
Debaryomyces hansenii	16/76.0	8/38.0	24/57.1
Malassezia restricta		5/23.8	5/11.9
Pichia triangularis		2/9.6	2/4.8
Sterigmatomyces halophilus		4/19.0	4/9.5
Zygosaccharomyces pseudorouxii	2/9.6	1/4.8	3/7.1
Number of total clones n/%	21/100	21/100	42/100

[a] *DY doenjang* yeast, *KY kanjang* yeast

strains of *Zygosaccharomyces* (*Z. rouxii*) are known to produce about 3 % (w/v) alcohol, and several compounds add the characteristic aroma to *shoyu* (soybean sauce) and *miso* (soybean paste) (Aidoo et al. 2006). A provisional new species, Z. *pseudorouxii*, has recently been named but not formally described and may be a distinct species that

Table 4 Overview of relationship between phylum and class analysis results each of library

Phylum class	DY n/%	KY n/%	Number of total clones n/%[a]
Ascomycota			
Saccharomycotina	21/100	11/52.4	32/76.2
Basidiomycota			
Urediniomycetes		4/19.0	4/9.5
Ustilaginomycetes		5/23.8	5/11.9
Hymenomycetes		1/4.8	1/2.4
Number of total clones n/%	21/100	21/100	42/100

[a] *DY doenjang* yeast, *KY kanjang* yeast

exists on its own and as a hybrid with Z. *rouxii* (Gordon and Wolfe 2008; Harrison et al. 2011). To the best of our knowledge, this is the first report to reveal *Z. pseudorouxii* in traditional Korean fermented soybean foods.

The *doenjang* library was unique in containing members of the species *C. etchellsii*, *C. versatilis*, and *D. castellii*, while the *kanjang* library was the only one that yielded members from the species *C. magnus*, *M. restricta*, *P. triangularis*, and *S. halophiles* (Table 3). The salt-tolerant yeasts *C. etchellsii* and *C. versatilis* produce 4-ethylguaiacol (4EG) and 4-ethylphenol (4EP), which are characteristic flavors of the Japanese seasonings *miso* and *shoyu* (Suezawa et al. 2006; Watanabe et al. 2008). Additionally, *D. castellii* produces the phytase that hydrolyses the six phytate-bound phosphates (Ragon et al. 2008). Thus, *D. castellii* will probably degrade phytic acid in soybeans during *doenjang* fermentation.

On the other hand, *Malassezia* species are lipophilic yeasts that are part of the normal flora of human skin and are found in 75–98 % of healthy adults. These yeasts are the cause of *pityriasis versicolor* and *Malassezia folliculitis* and appear to be involved in the pathogenesis of common skin disorders such as *Seborrheic dermatitis* (SD), psoriasis, and atopic dermatitis (Lee et al. 2011). In particular, *M. restricta* are considered to be the most important pathogenic organisms in the development of SD (Ko et al. 2011). What accounts for the presence of *M. restricta* in the *kanjang*? Homemade *kanjang* is usually made from *meju*, a rectangular cake of boiled and mashed soybeans. Because the *meju* is fermented in a natural environment, several microorganisms may be involved in its colonization. Homemade *kanjang* fermentation begins as an open ecosystem and becomes closed during the fermentation process, with each batch of fermented *kanjang* having various microbial populations, depending on the length of the fermentation period. Furthermore, the various pathogens can contaminate the *meju* or the *kanjang* was fermented while being made. In the future, the microbial diversity will necessarily be investigated if the *meju* is manufactured or ripened or if the *kanjang* is fermented while being made. Previously, *Cryptococcus, Pichia, and Sterigmatomyces* species were found in fermented foods such as wine, cheese, sausage, and *doenjang* (Callon et al. 2007; Cocolin et al. 2006; Kim et al. 2009).

The question remains as to why there are different yeast species in *doenjang* and *kanjang*. Generally, the qualities of *doenjang* and *kanjang* products are determined by the different raw materials (e.g., soybean, wheat, and salt), fermentation conditions (solid or liquid type), fermentation periods and different types of microorganisms that exist during processing. We previously reported that the predominant bacterial species were *Staphylococcus equorum* (60.6 %) in *doenjang* and *Haloanaerobium* sp. (37.5 %) and *Haloanaerobium fermentans* (37.5 %) in *kanjang* (Cho and Seo 2007). Tiquia (2005) suggested that physiology and biochemical characteristics cause the microorganisms to adapt to extreme conditions. These finding suggest that different microbial communities may play specific roles in different soybean fermentation conditions.

This study revealed the yeast diversity in *doenjang* and *kanjang* by analyzing yeast 26S rRNA sequences in a culture-independent manner. Most of the sequence derived from the *doenjang* and *kanjang* libraries were related to those of the *Saccharomycotina* class. It is interesting that 21 clones of the *doenjang* library were classified into only the *Saccharomycotina* class and that the *Saccharomycotina* were predominant in *kanjang* (Table 4). It is also assumed that the five-mixer sample used in the present study is not enough to cover the diversity of the yeast in fermented soybean foods. To reveal the exact population of yeasts in fermented soybean foods, it is necessary to compare the biodiversity of yeasts in various fermented soybean food samples collected from different homemade or commercial products. The scope of functional roles and the extent of yeast diversity have yet to be understood considering that most yeasts in fermented soybean foods remain undescribed. The PCR primers described in this report provide unique tools to further characterize this important group of organisms (Borneman and Hartin 2000). Considering the variation in each position amounts to 5 %, when eukaryotic nuclear large subunit (LSU) rRNA was amplified with *S. cerevisiae* as reference organism and using various

universal primers (Shin et al. 2004), the PCR primer to amplify 26S rRNA used in this study was not enough to cover most phyla of yeast in fermented soybean foods. A more specific set of phylogenetic primers for the microorganism will be used in future studies to more fully resolve the character of microbial diversity.

In conclusion, we have shown that culture-independent methods can be successfully applied to explore the yeast diversity in *doenjang* and *kanjang*. The results reveal that the diversity of yeast communities in *doenjang* and *kanjang* are different and provide novel insights into the yeast communities in fermented soybean foods. The data presented in this study may provide a useful frame of reference for further analysis of microbial population dynamics in soybean fermentation, flavor development, and control of the fermentation process.

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