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Minireview

# Genomic and functional features of yeast species in Korean traditional fermented alcoholic beverage and soybean products

Da Min Jeong, Hyeon Jin Kim, Min-Seung Jeon, Su Jin Yoo, Hye Yun Moon, Eun-joo Jeon, Che Ok Jeon, Seong-il Eyun, Hyun Ah Kang*

Department of Life Science, College of Natural Science, Chung-Ang University, Seoul 06974, South Korea
*Corresponding author. Department of Life Science, College of Natural Science, Chung-Ang University, Seoul 06974, South Korea. Tel.: +82-2-820-5863; Fax: +82-2-825-5206; E-mail: hyunkang@cau.ac.kr
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## Abstract

In this review, we describe the genomic and physiological features of the yeast species predominantly isolated from Nuruk, a starter for traditional Korean rice wines, and Jang, a traditional Korean fermented soy product. Nuruk and Jang have several prevalent yeast species, including *Saccharomycopsis fibuligera*, *Hyphopichia burtonii*, and *Debaryomyces hansenii* complex, which belong to the CUG clade showing high osmotic tolerance. Comparative genomics revealed that the interspecies hybridization within yeast species for generating heterozygous diploid genomes occurs frequently as an evolutional strategy in the fermentation environment of Nuruk and Jang. Through gene inventory analysis based on the high-quality reference genome of *S. fibuligera*, new genes involved in cellulose degradation and volatile aroma biosynthesis and applicable to the production of novel valuable enzymes and chemicals can be discovered. The integrated genomic and transcriptomic analysis of *Hyphopichia* yeasts, which exhibit strong halotolerance, provides insights into the novel mechanisms of salt and osmo-stress tolerance for survival in fermentation environments with a low-water activity and high-concentration salts. In addition, Jang yeast isolates, such as *D. hansenii*, show probiotic potential for the industrial application of yeast species beyond fermentation starters to diverse human health sectors.

**Keywords:** Korean fermented foods, yeast species, genome structure, saccharification, volatile flavor, osmotolerance, probiotic potential

## Introduction

In Asia, numerous types of fermented foods are made from cereals, leguminous seeds, vegetables, meat, and fish (Nout and Aidoo 2002). The representative fermented food products in Korea include kimchi (fermented cabbage), doenjang (fermented soybean paste), ganjang (fermented soy sauce), gochujang (fermented chili paste), jeotgal (fermented fish sauce), and makgeolli (fermented alcoholic beverage), whose fermentation can take several months to several years (Table 1). Many different fermented products either occur naturally or through the addition of a starter culture. Nuruk and Meju, which are traditional Korean fermentation starters of rice wine and Jang products, respectively, are rich sources of microbiota; therefore, different bacterial and yeast species are present in each starter, contributing to unique flavors and textures in fermented foods. Several nonconventional yeast species isolated in Nuruk and Meju contribute to the hydrolytic breakdown of cereal starches and other polysaccharides (Carroll et al. 2017, Chun et al. 2021). In fermented foods, volatile desired flavor compounds, such as branched alcohols, esters, and aldehydes, are synthesized through amino acid degradation or transformation by yeast strains (Song et al. 2015).

Yeast species involved in traditional food fermentation have been subjected to genomic studies because of their great biotechnological potential and implication on human health. In recent years, we have performed *de novo* whole genome (WG) sequencing analysis of yeast isolates from traditional Korean fermented foods, particularly Nuruk and Jang, to establish reference genomes with high-quality annotation. In this review, we describe the genomic features and physiological characteristics of the predominant yeast species from Nuruk and Jang, including *Saccharomycopsis fibuligera*, *Hyphopichia* yeast species, *Wickerhamomyces anomalus*, and *Debaryomyces hansenii* complex, which were subjected for the complete WG sequencing and chromosome-level assembly to find common and unique genomic characteristic of yeast species in major Korean fermented foods (Choo et al. 2016, Chun et al. 2021, Lee et al. 2021, Jeong et al. 2022). The obtained genome information from the yeast species serves as a good basis for comparative genomics to elucidate the evolutionary consequences of yeast species in fermentation environments. Furthermore, combined with other omics analysis, genomic data can be used to establish a useful platform for the identification and functional analyses of useful genetic resources. We also discuss the probiotic potential of yeast species isolated from Korean fermentation foods as an extended future application.

## Diversity of yeast species in Nuruk and Jang

The traditional Korean wine makgeolli, frequently called Takju, is made by fermenting the starch materials of rice, which has been the staple food of Korea (Jang 1989). To produce makgeolli, the fermentation starter Nuruk should be prepared from several kinds of

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**Table 1.** List of Korean traditional fermented food and alcoholic beverages.

| Name | Description |
|---|---|
| **Fermented food** | |
| **Kimchi (fermented cabbage)** | |
| Napa cabbage kimchi | Traditional Korean side dish of salted and fermented vegetables, such as napa cabbage, Korean radish, and cucumber |
| Radish kimchi | |
| Cucumber kimchi | |
| **Jang (soy-based products)** | |
| Cheonggukjang (fermented soybean) | Fermented boiled soybean product with short term fermentation (2∼3 days) |
| Gochujang (fermented chili paste) | A savory, sweet, and spicy condiment, fermented with red chili powder, glutinous rice and Meju powder |
| Doenjang (fermented soybean paste) | Fermented soybean products from Meju following a complex fermentation process that separates the products into solid (doenjang) and liquid (ganjang) states. Used as a basic seasoning |
| Ganjang (fermented soy sauce) | |
| **Jeotgal (fermented fish sauce)** | |
| Saeu-jeot (shrimp jeotgal) | Salted preserved dishes made with salt, red chili pepper, and seafood such as shrimps, roe, oysters, and so on. Used as a side dish or as an ingredient of kimchi |
| Myeongnan-jeot (salted pollock roe) | |
| Guljeot (salted oyster) | |
| Jogae-jeot (salted clam) | |
| Gejang (salted crab) | |
| Ojingeo-jeot (salted squid) | |
| Myeolchi-jeot (salted anchovy) | |
| **Fermented alcoholic drinks** | |
| **Makgeolli (rice wine)** | Fermented with boiled rice (or wheat) and Nuruk, soaked in water nondistilled cloudy alcohol (4%∼6% of alcohol content) |
| **Yakju (cheongju)** | Clear liquid refined from makgeolli |
| **Soju (distilled liquor)** | |
| Distilled soju | Liquor (16%∼41% of alcohol content), distilled after makgeolli fermentation. |
| Insamju (ginseng medicinal wine) | Matured wine with ginseng and soju |
| **Gwasilju (fruit wine)** | |
| Bokbunja (raspberry wine) | Fruit wine (5%∼15% of alcohol content), fermented with fruits such as black raspberry, green plum, and Korean red grape. |
| Maesilju (green plum liquor) | |
| Meoruju (Korean red grape wine) | |

grains, especially rice grains, by using airborne microorganisms (Fig. 1A). These microorganisms provide a source of several hydrolytic enzymes required for starch degradation during saccharification to produce glucose and other organic acids (Yang et al. 2011, Song et al. 2013, Bal et al. 2014, 2015, Park et al. 2016). Then, glucose is fermented by *Saccharomyces cerevisiae* to produce alcohol. Thus, the quality of alcohol in makgeolli critically depends on the characteristics of Nuruk used for fermentation.

Korean fermented soybean products, namely, gochujang, ganjang, and doenjang, have been used as essential flavors and nutritional bases of authentic Korean cuisine. Soy sauce, a traditional fermented liquid condiment that originated in East Asia, is popularly used in cooking worldwide (Devanthi and Gkatzionis 2019). It varies in flavor and taste among different countries because of differences in raw materials and manufacturing processes (O'toole 2019). In Korea, soy sauce is made using soybeans alone; in Japan and China, it is made using a combination of soybeans and wheat flour (Lioe et al. 2010). In traditional fermentation, soy sauce is produced through a two-step fermentation process, i.e. solid-state fermentation (Koji in Japan, Meju in Korea, and Qu in China) and brine fermentation (Fig. 1B). In soybean product preparation, Meju (fermented soybean block) is mixed with high-salt brine (∼18%) and ripened in a porcelain pot. The liquid portion is separated and boiled after ∼2 months, resulting in ganjang. Doenjang is the remaining solid portion, which is subsequently mashed and fermented for 1 month to 180 days in the porcelain pot (Shin and Jeong 2015). The metabolism of Meju microbiome is diversified during soybean fermentation with bacteria and fungi, including yeast.

A variety of yeast species have been isolated from the fermentation starter Nuruk for makgeolli and the fermented Jang products, and several yeast species were commonly identified in both Nuruk and Jang (Table 2). The yeast species reported to be isolated from Nuruk include *S. fibuligera*, *Candida glabrata*, *Pichia kudriavzevii* (*syn. C. krusei*; *Issatchenkia orientalis*), *Candida tropicalis*, *Clavispora lusitaniae*, *Kluyveromyces lactis*, *Pichia fabianii*, *P. guilliermondii*, *Millerozyma farinosa* (*syn. Pichia sorbitophila*; *Pichia farinosa*), *W. anomalus*, *Hyphopichia burtonii*, *H. pseudoburtonii*, *P. membranifaciens*, and *S. cerevisiae* (Song et al. 2013, Bal et al. 2015, Carroll et al. 2017, Choi et al. 2017, Lee et al. 2021, Yoon et al. 2022). The yeast species isolated from Jang products include *D. hansenii* complex, *Candida versatilis*, *W. anomalus*, *Zygosaccharomyces rouxii*, *Millerozyma farinosa*, *C. lusitaniae*, *Galactomyces geotrichum*, *Candida mogii*, *C. etchellsii*, *C. intermedia*, *P. kudriavzevii*, *C. glucosophila*, *H. burtonii*, and *H. pseudoburtonii* (Kim et al. 2009, Song et al. 2015, Chun et al. 2021). The yeast species from Nuruk and Jang are classified mostly under Ascomycota, and a few species are assigned to Basidiomycota at the phylum level (Fig. 2). The major families of yeast member s found in Nuruk and Jang are Debaryomycetaceae and Saccharomycetaceae.

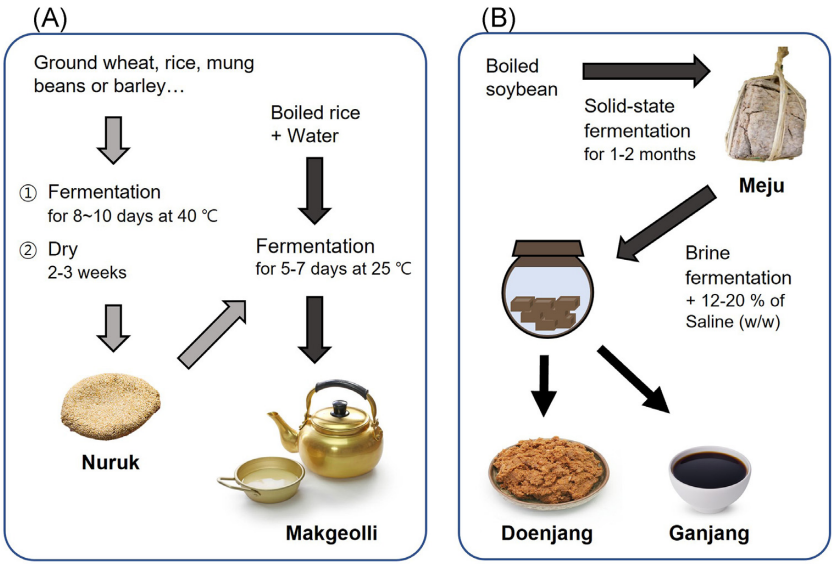


**Figure 1.** Fermentation of the traditional Korean rice wine makgeolli and the fermented soy product Jang. **(A)** Makgeolli fermentation. A traditional fermentation starter called Nuruk, which is a dough made of coarsely ground grain and water naturally inoculated with bacteria, fungi, and yeast, is dried to become hard as a rock. The Nuruk cake is added to boiled rice and fermented in a liquid state. **(B)** Jang fermentation. A traditional Korean fermented soybean brick called Meju is made with boiled soybeans and dried in a warm, dry place for days. For the first fermentation, Meju is hung by a string in a sunny and dry place with a high amount of airflow upward for a month. The aged meju is mixed with salt-water brine, and the mixture is fermented additionally. Next, the solid part, doenjang, and the liquid part, ganjang, are divided.

## Genomic features of yeast isolates from traditional Korean fermented foods

### *Saccharomycopsis fibuligera* isolates from Nuruk

The amylolytic yeast *Saccharomycopsis fibuligera* is the most abundant species among several yeast species isolated from wheat-based Nuruk samples collected from various provinces in Korea (Bal et al. 2014, Carroll et al. 2017). *S. fibuligera*, a member of the subphylum Saccharomycotina of the phylum Ascomycota of the kingdom Fungi (Fig. 2), is also commonly found as a dominant yeast species in traditional Asian alcoholic starters for producing rice wine, such as "Daqu" in China (Zheng et al. 2012), "Ragi" in Indonesia (Hesseltine et al. 1988), "Loogpang" in Thailand (Sukhumavasi et al. 1975), and "Banh Men" in Vietnam (Thanh et al. 2008). Moreover, this yeast has been isolated as a spoilage fungus causing chalk mold defects, which are commonly found on dark bread popular in continental Europe and the UK (Deschuyffeleer et al. 2011).

We performed the *de novo* WG sequencing of two *S. fibuligera* isolates from wheat-based Nuruk samples from Jeju (KJJ81) and Pohang (KPH12) in Korea, which were chosen for based on their high saccharification activity, and performed a complete genome assembly with high-quality (Choo et al. 2016). As a dimorphic yeast, *S. fibuligera* KJJ81 and KPH12 grow exclusively as filamentous forms with a minor fraction of budding yeast cells. The KPH12 genome is a haploid composed of seven chromosomes with a total length of 19.56 Mb (Table 3). Intriguingly, the KJJ81 genome with a total size of 38.51 Mb was revealed as a hybrid between the KPH12 genome (subgenome A) and another unidentified genome (subgenome B) sharing 88.1% nucleotide identity with the KPH12 genome (Fig. 3A). The sequence divergence observed between subgenomes A and B of the KJJ81 genome is 10.84% at the nucleotide level between syntenic regions, which is equivalent to the divergence described between the genomes of *S. cerevisiae* and *S. paradoxus*, which are two distinct *Saccharomyces* species. Thus, the formation of this heterozygous diploid genome of *S. fibuligera* KJJ81 is speculated to involve hybridization between KPH12 and a yet-unidentified parent strain with subgenome B via inter(sub)species mating. The highly conserved synteny over the WG in the *S. fibuligera* KJJ81 hybrid genome strongly indicates that hybridization occurred recently.

Additional WG sequencing of *S. fibuligera* ATCC36309, an isolate from chalky rye bread in Germany, revealed that the ATCC36309 genome is highly identical to the KPH12 genome with 97.97% sequence identity (Choo et al. 2016). Interestingly, despite such high identity, reciprocal translocation between chromosomes 3 and 5 occurred with the loss of ~250 paralogous genes between the ATCC36309 and KPH12 genomes. This finding indicated that sequence divergence occurred during growth adaptation to different environments such as Nuruk in Korea and chalky rye bread in Germany. Our previous phylogeny analysis, inferred on the basis of genome sequences analyzed by ClustalW and Neighbor-Joining method, *S. fibuligera* was positioned as an early divergent of the subphylum Saccharomycotina, which was separated from a common ancestor much earlier before the divergence of Saccharomycetaceae and the CUG clade (Choo et al. 2016). However, in this study when we constructed the phylogenetic tree of 33 yeast species from Nuruk and Jang using iterative refinement (MAFFT and MUSCLE, respectively) and Maximum Likelihood (ML)-based tree construction, *S. fibuligera* was positioned to be diverged after the separation of CUG-Ser1 group (Fig. 2). Notably, *Saccharomycopsis* yeasts were recently reassigned as a subclade in the CUG clade, namely, CUG-Ser2 group, of yeasts, which was positioned to be separated from Saccharomycetaceae in the phylogenomic tree of 52 yeast species (Krassowski et al. 2018). The phylogenetic tree in the present study supports the reassignment of Saccharomycopsidaceae including *S. fibuligera* as the CTG-Ser2 group, i.e. a sister clade to Saccharomycetaceae, not a subclade of the CUG-Ser1 clade.

**Table 2.** Yeast species isolated in traditional Korean fermentation starter (Nuruk) and fermented soy product (Jang).

| Yeast taxa | Nuruk | Jang |
|---|---|---|
| **Ascomycota** | **Debaryomycetaceae**<br>*C. tropicalis*<br>*H. burtonii*<br>*H. pseudoburtonii*<br>*Meyerozyma guilliermondii*<br>*M. farinosa*<br>**Metschnikowiaceae**<br>*C. lusitaniae*<br>**Pichiaceae**<br>*P. membranifaciens*<br>*P. kudriavzevii*<br>**Phaffomycetaceae**<br>*Cyberlindnera jadinii*<br>*P. fabianii*<br>*W. anomalus*<br>**Saccharomycetaceae**<br>*C. glabrata*<br>*K. lactis*<br>*S. cerevisiae*<br>*Torulaspora delbrueckii*<br>**Saccharomycopsidaceae**<br>*S. fibuligera* | **Debaryomycetaceae**<br>*Candida albicans*<br>*Candida parapsilosis*<br>*Candida temnochilae*<br>*Candida zeylanoides*<br>*D. hansenii*<br>*H. burtonii*<br>*H. pseudoburtonii*<br>*Meyerozyma guilliermondii*<br>*M. farinosa*<br>**Dipodascaceae**<br>*Geotrichum candidum*<br>**Metschnikowiaceae**<br>*C. intermedia*<br>*C. mogii*<br>*C. lusitaniae*<br>**Pichiaceae**<br>*P. kudriavzevii*<br>**Phaffomycetaceae**<br>*Cyberlindnera jadinii*<br>*W. anomalus*<br>**Saccharomycetaceae**<br>*Kazachstania exigua*<br>*Lachancea kluyveri*<br>*S. cerevisiae*<br>*Tetrapisispora blattae*<br>*Torulaspora delbrueckii*<br>*Z. rouxii*<br>**Trichomonascaceae**<br>*Wickerhamiella (Candida) versatilis*<br>**Not classified**<br>*C. glucosophila*<br>*Diutina rugosa*<br>*Kuraishia capsulata*<br>*Nakazawaea holstii*<br>*Starmerella etchellsii* |
| **Basidiomycota** | | *Microbotryum violaceum*<br>*Naganishia albida*<br>*Rhodotorula mucilaginosa*<br>*Rhodotorula glutinis*<br>*Sterigmatomyces halophilus* |
| **References** | Song et al. (2013), Carroll et al. (2017), Choi et al. (2017), Kang et al. (2014), Lee et al. (2021), Yoon et al. (2022) | Oh and Lee (1996), Kim et al. (2009), Shin and Jeong (2015), Song et al. (2015a), Chun et al. (2021) |

*Underlined yeast species were commonly identified in Nuruk and Jang.

## *Hyphopichia burtonii* and *H. pseudoburtonii* from Nuruk

*Hyphopichia burtonii* is common in nature and strongly competitive in several harsh conditions (Kurtzman 2005). It belongs to the CUG-Ser1 clade grouped with several yeast species found in Nuruk and Jang in phylogenetic trees generated using genome sequence information (Fig. 2 and Table 3). *H. burtonii* is a spoilage yeast, along with other amylolytic yeasts such as *S. fibuligera* and *W. anomalus*, and it causes chalk mold defects in partially baked breads and traditional cookies (Deschuyffeleer et al. 2011, Berni and Scaramuzza 2013). It has been isolated from various foods, such as fermented baking products with a high sugar content and a low water activity (Bonjean and Guillaume 2003). It is one of the dominant yeast species in amylolytic fermentation starters used to produce traditional alcoholic drinks, such as "Murcha" in Nepal (Takeuchi et al. 2006) and "Nuruk" in Korea (Carroll et al. 2017). In addition, *H. burtonii* is a major isolate in dried substrates with high starch contents, such as Japanese dry noodle (Ohisa et al. 2012) and white rice stored at low temperatures (Lee et al. 2018a); it is also found in salty foods, such as Italian ham (Simoncini et al. 2007) and Korean traditional fermented soybean food "Jang" (Kim et al. 2011).

We conducted genomic and transcriptomic analyses to characterize two *Hyphopichia* yeast isolates, namely, *H. burtonii* KJJ43

**Table 3.** General information on the whole-sequenced genomes of yeast species isolated in Korean fermented foods.

| Yeast species | Genome size (Mb) | GenBank # | G + C content (%) | Ploidy (Chromosome #) | Protein account | Genetic code change | Source (localization) | References |
|---|---|---|---|---|---|---|---|---|
| *S. fibuligera* KJJ81 | 38.51 | CP012809-CP012822 | 38.5 | Heterozygous diploid (14) | 12,185 | CUG-Ser2 clade | Nuruk (Jeju) | Choo et al. (2016) |
| *S. fibuligera* KPH12 | 19.56 | CP012823-CP012829 | 38.2 | Haploid (7) | 6,155 | CUG-Ser2 clade | Nuruk (Pohang) | Choo et al. (2016) |
| *H. burtonii* KJJ43 | 12.49 | CP024759-CP024766 | 35 | Haploid (8) | 6,418 | CUG-Ser1 clade | Nuruk (Jeju) | Lee et al. (2021) |
| *H. pseudoburtonii* KJS14 | 15.54 | CP024751-CP024758 | 36 | Haploid (8) | 6,202 | CUG-Ser1 clade | Nuruk (Kangwon-do) | Lee et al. (2021) |
| *Debaryomyces* sp. KD2 | 12.91 | CP046876-CP046882 | 36.52 | Haploid (7) | 6,000 | CUG-Ser1 clade | Ganjang (Jeongeup) | Jeong et al. (2022) |
| *Debaryomyces* sp. C11 | 24.84 | Pri: JADOBC000000000 Alt: JADOBD000000000 | 36.41 | Heterozygous diploid (12) | 11,901 | CUG-Ser1 clade | Ganjang (Gyeongju) | Jeong et al. (2022) |
| *Wickerhamomyces* sp. KG16 | 23.96 | Pri: JAHTLX000000000 Alt: JAHTLY000000000 | 34.53 | Heterozygous (partial) diploid (14) | 11,166 | CUG-Leu1 clade | Ganjang (Jeongeup) | Chun et al. (2021) |
| *M. farinosa* KCTC27753 | 21.25 | ASM219676v1 | 41.1 | Heterozygous diploid | 10,910 | CUG-Ser1 clade | Nuruk (Kangwon-do) | Yoon et al. (2022) |

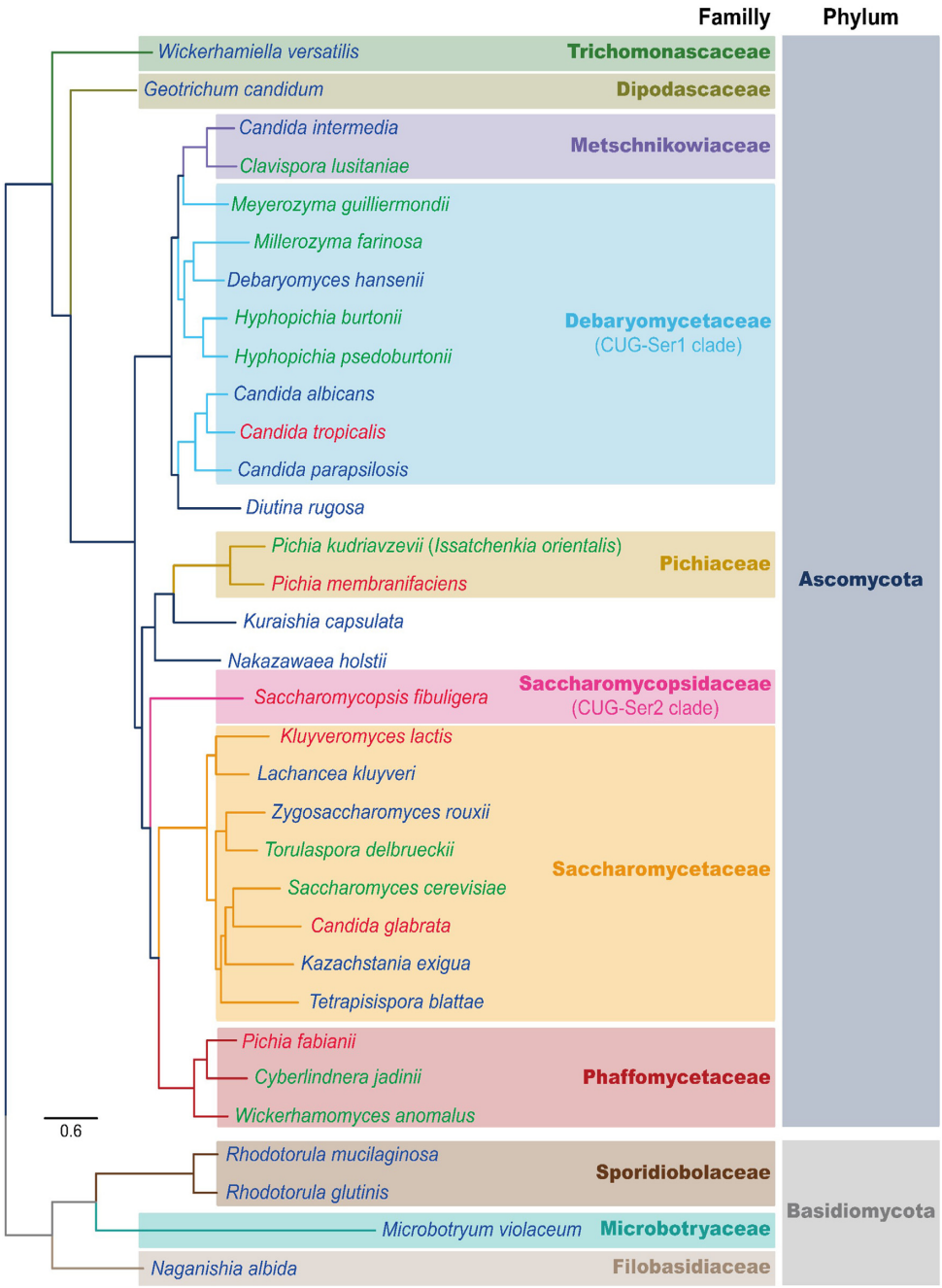


**Figure 2.** Phylogenetic tree analysis of yeast species isolated from Nuruk and Jang. The phylogenetic relationship was reconstructed by the maximum-likelihood method using RAxML (ver. 8.2.12) with LG + F + I + G4 matrix. Total 13 genes were selected to recapitulate the genome-based phylogeny of *S. cerevisiae* strains (Ramazzotti et al. 2012) and concatenated for further phylogenetic analyses. Among 39 yeast species (Table 2), six yeast species (*C. glucosophila*, *C. mogii*, *C. temnochilae*, *C. zeylanoides*, *S. etchellsii*, and *S. halophilus*), whose genome information is unavailable, were not included. Nuruk, Jang, and both derived strains were presented in red, blue, and green, respectively.

and *H. pseudoburtonii* KJS14, which were isolated as major fungal species with a high saccharification activity from wheat-based Nuruk samples in Korea (Lee et al. 2021). *H. pseudoburtonii* KJS14 morphologically resembles *H. burtonii*, and both *Hyphopichia* yeasts grow exclusively in the filamentous form with few budding cells upon cultivation under normal growth conditions. The WGs of *H. burtonii* KJJ43 and *H. pseudoburtonii* KJS14 have lengths of 12.5 Mb and 15.5 Mb, respectively, and both genomes are haploids consisting of eight chromosomes (Table 3). *H. burtonii* and *H. pseudoburtonii* show a sequence identity of 85.2% with ~10.3 Mb of synteny blocks between them. However, numerous chromosomal rearrangements between the two genomes suggest their relatively early divergence from a common ancestor.

## *Debaryomyces hansenii* complex isolates from Jang

*Debaryomyces hansenii*, belonging to Hemiascomycetes, is grouped as a member of the CUG-Ser1 clade (Riley et al. 2016; Fig. 2 and Table 3). It has been isolated from saline water, foods, fruits, and even the human gut (Hallen-Adams and Suhr 2017). Particularly, *D. hansenii* is among the yeast species with the highest preva-

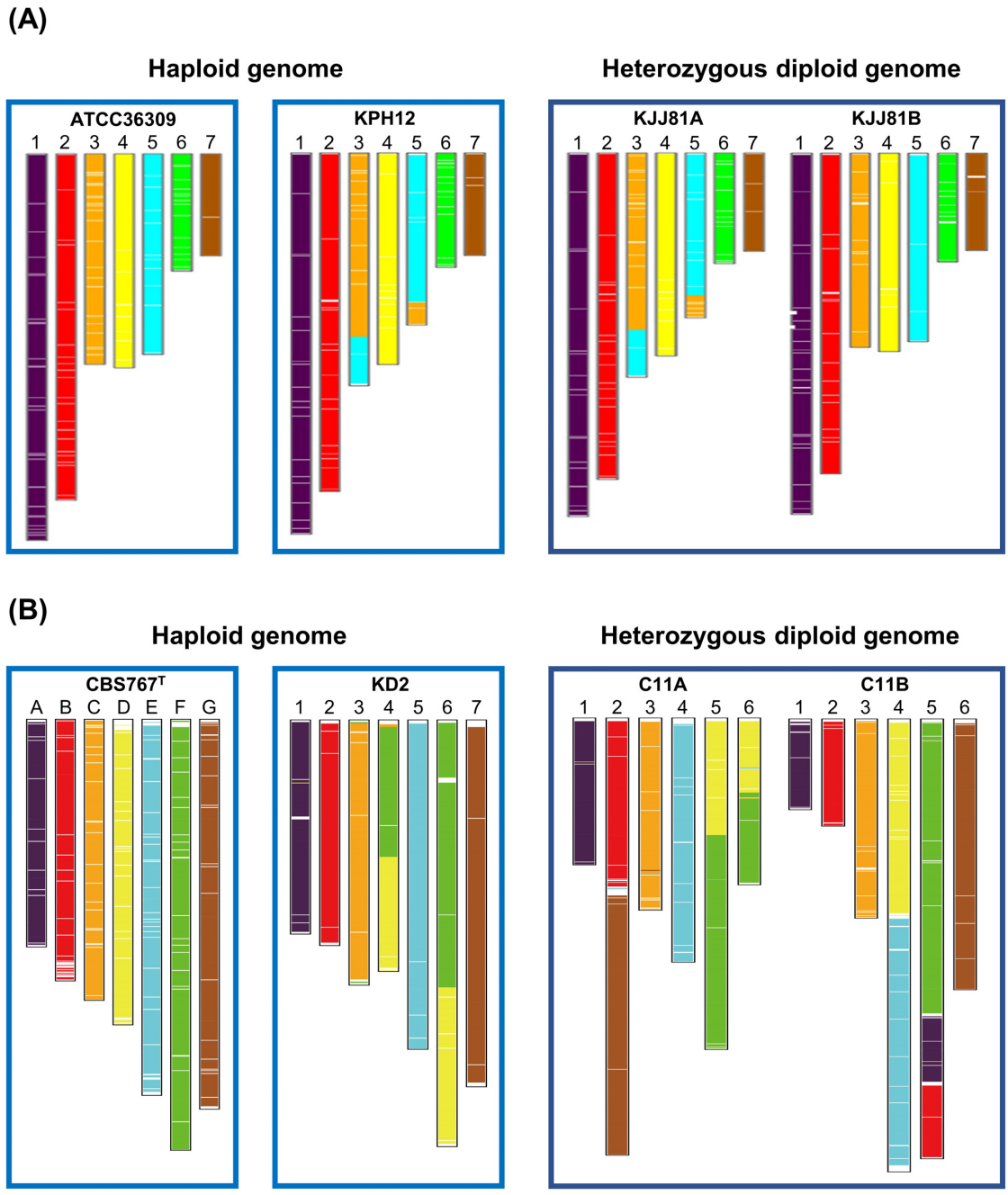


**Figure 3.** Representative haploid and heterozygous diploid genomes of *S. fibuligera* and *D. hansenii* isolates from Nuruk and Jang. Synteny blocks of *S. fibuligera* genomes **(A)** and *D. hansenii* genomes **(B)** Synteny blocks were visualized through chromosome painting in SynChro (Drillon et al. 2014), which are modified from the previous synteny analysis data of *S. fibuligera* (Choo et al. 2016) and *D. hansenii* (Jeong et al. 2022).

lence on surface-ripened cheeses (Fröhlich-Wyder et al. 2019) and dry-aged beef (Ryu et al. 2018). *Debaryomyces* spp. are also identified as the most highly abundant fungal groups between 40 and 60 days during the Korean traditional ganjang fermentation period (Chun et al. 2021). We performed a genomic analysis of two *D. hansenii* ganjang isolates KD2 and C11, which show halotolerance to concentrations of up to 15% NaCl and improved growth in the presence of salt (Jeong et al. 2022). Notably, ploidy and WG sequencing analyses indicated that the KD2 genome is haploid, whereas the C11 genome is a heterozygous diploid with two distinctive subgenomes. The *D. hansenii* KD2 genome is composed of seven chromosomes with a total length of 12.91 Mb. Conversely, the *D. hansenii* species complex C11 genome consists of two subgenomes A and B, each containing six contigs. Chromosome rearrangement was observed in all chromosomes of C11 subgenomes except for chromosome 3, which corresponds to chromosome C in *D. hansenii* CBS767$^T$. The synteny block construction shows various changes of chromosomal structures in the C11 diploid genome (Fig. 3B). Based on such genomic features, the *D. hansenii* species complex C11 is considered a heterozygous diploid, which resulted from hybridization between two strains that diverged from the common ancestor of *Debaryomyces* spp., followed by a loss of heterozygosity and massive chromosomal rearrangements.

## *Wickerhamomyces anomalus* from Jang and *Millerozyma farinosa* from Nuruk

*Wickerhamomyces anomalus*, previously known as *Pichia anomala* or *Hansenula anomala*, is a yeast frequently associated with the spoilage or processing of food and grain products (Sabel et al. 2013). *W. anomalus* is a member of the family Phaffomycetaceae in the order Saccharomycetales of the phylum Ascomycota (Fig. 2). Along with *H. burtonii* and *S. fibuligera*, *W. anomalous* is known as a spoilage yeast that causes chalk mold defects (Berni and Scaramuzza 2013). It is one of the predominant yeast isolates in Korean fermented foods, namely, Nuruk and Jang (Carroll et al. 2017, Chun et al. 2021), and it is frequently isolated as a flavor yeast in Chinese liquor fermentation starter and wine microbial consortia (Renouf et al. 2007, Shi et al. 2022). The genome sequencing of *W. anomalus* haploid strains reported the WG size as about 15 Mb (Riley et al. 2016, Shi et al. 2022), but the complete assembly of genomes at a chromosomal level has yet to be described. The draft genome sequence of the diploid neotype strain *W. anomalus* DSM 6766 has a total genome size as 25.47 Mb (Schneider et al. 2012). Our WG sequencing analysis of several *W. anomalus* isolated from Jang revealed that it has a genome size of 23.96 Mb composed of two separable subgenomes (Table 3), indicating the frequent presence of heterozygous diploid genomes in *W. anomalus*.

*Millerozyma farinosa*, formerly known as *P. farinosa* (synonym: *Pichia sorbitophila*), is a member of the CUG-Ser1 group of Saccharomycotina and closely related to *D. hansenii* in phylogenetic tree analysis (Fig. 2). It is known as a multistress tolerant yeast that particularly exhibits a high-salt tolerant and osmotolerant phenotype (Fig. 6). *M. farinosa* (syn. *P. sorbitophila*) is found mainly in food, including fermented alcoholic beverages, and on various substrates, such as laboratory media and highly concentrated sorbitol solutions. The complete WG sequencing with detailed analyses of *M. farinosa* (*P. sorbitophila*) CBS7064, isolated from a concentrated sorbitol solution in the industry, reported its genome as a hybrid genome composed of two subgenomes (seven chromosomes per subgenome), which is inherited separately from two distinct progenitors with high-level nucleotide polymorphism (10.84% of divergence) but well-conserved synteny (Louis et al. 2012). The diploid genome with a size of 21.5 Mb appears to be generated by a recent hybridization during evolution, followed by subsequent genomic changes, including loss of heterozygosity, unilateral loss of rDNA, and reciprocal exchange. The draft genome sequence of *M. farinosa* KCTC27753, a strain isolated from Nuruk, revealed that the genome length is 21.3 Mb with a total of 10 910 plausible gene-coding regions, indicating that the strain is also a heterozygous diploid. Therefore, *M. farinosa* exists as a population of haploids and hybrids in Nuruk and Jang, as observed in other prevalent yeast species, including *S. fibuligera*, *D. hansenii*, and *W. anomalus*.

## Genetic resources of the Nuruk yeast *S. fibuligera* for industrial applications of cellulase degradation genes

The enzymatic degradation of cellulose, which is the most abundant polymer on Earth, includes the joint action of exoglucanases or cellobiohydrolases, endoglucanases, and $\beta$-glucosidases (BGLs). As an amylolytic yeast, *S. fibuligera* has served as a good source of enzymes and genes involved in saccharification (Chi et al. 2009). For example, the *S. fibuligera BGL1* gene, encoding $\beta$-glucosidase 1, has been heterologously expressed in *S. cerevisiae* to construct cellobiose-growing and fermenting strains (Moon et al. 2022). Gene inventory analysis based on the high-quality reference genome of *S. fibuligera* KJJ81 and KPH12 has revealed new genes involved in cellulose degradation (Choo et al. 2016). The *S. fibuligera* genome retains several genes encoding hydrolytic enzymes, which are found in many cellulolytic fungi. A total of four or five copies of the genes encode BGL, which acts mainly on cellobiose, in the *S. fibuligera* KPH12 and KJJ81 genomes. The *S. fibuligera* KJJ81 genome (Fig. 4A) contains two previously reported genes (*SfBGL1* and *SfBGL2*), three novel genes encoding a protein with considerable homology to SfBGL1 (55% identity; designated *SfBGL3*), and two additional genes encoding homologs of *Schizosaccharomyces pombe* putative $\beta$-glucosidase (designated *SfBGL4* and *SfBGL5*). The *S. fibuligera* BGLs belong to glycosyl hydrolase family 3 or 17 (PF00933 or PF00332). The fibronectin type III-like domain (PF14310) associated with the glycoside hydrolase 3 domain is present in SfBGL1, 2, and 3, but its function is yet unknown. All the SfBGLs except SfBGL4 have a native signal peptide and multiple *N*-/*O*-glycosylation sites, indicating that they are secretory glycoproteins. Notably, three *S. fibuligera* homologs to thermophilic *Trichoderma reesei* and *Sporotrichum thermophile* Cel61A proteins, which are copper-dependent polysaccharide monooxygenases (PMOs), are identified in the diploid genome of *S. fibuligera* KJJ81 (Fig. 4B). The *S. fibuligera* Cel61A proteins have a signal peptide except for SfCel61A(A)1 and several *N*-/*O*-glycosylation sites. PMOs belong to the auxiliary activity family 9 (PF03443), formerly glycosyl hydrolase (GH61), and they constitute a novel class of enzymes that catalyze the $O_2$-dependent oxidative cleavage of recalcitrant polysaccharides (Dimarogona et al. 2013). PMOs have been explored for biotechnological applications because they enhance the efficiency of common cellulases, resulting in increased hydrolysis yields while reducing the protein loading needed for plant polysaccharide degradation. Therefore, their substrate specificity and the biological functions of putative *S. fibuligera* Cel61A homologs should be defined.

A homolog of *Aspergillus nidulans* abfC, encoding a probable $\alpha$-L-arabinofuranosidase C involved in the degradation of hemicellulose polymers with arabinosyl residues, is present in *S. fibuligera* KJJ81 and KPH12 genomes. The abfC proteins in *S. fibuligera* KJJ81 have the $\alpha$-L-arabinofuranosidase domain (PF06964) and an active site in Glu 305 (Fig. 4C). The acidic residue of the active site is required for substrate recognition (Souza et al. 2011). Our transcriptome analysis revealed that the expression of this *S. fibuligera* abfC homolog is highly induced under glucose-limited and sulfur-limited conditions (Choo et al. 2016). We tested the enzymatic activity of the *S. fibuligera* abfC homolog, probably encoding $\alpha$-L-arabinofuranosidase (E.C. 3.2.1.55) isozymes (ABFs) that belong to the glycoside hydrolase (GH) family 51. The diploid strain *S. fibuligera* KJJ81 has two abfC homologs with 92.3% amino acid sequence identity, which is named *SfABF51A* and *SfABF51B*. The open reading frame of both *SfABF51* isogenes encodes a protein of 517 amino acids with a molecular mass of ~59 kDa, sharing 92.3% amino acid sequence identity with each other. These isozymes share ~49% amino acid sequence identity with eukaryotic ABFs from filamentous fungi. The recombinant $SfABF51_A$ and $SfABF51_B$ proteins, were expressed and purified from *Escherichia coli*; they were shown to hydrolyze arabinoxylo-oligosaccharides (AXOS) and arabino-oligosaccharides (AOS) to produce only L-arabinose. They can catalyze the versatile hydrolysis of $\alpha$-(1,2)- and $\alpha$-(1,3)-L-arabinofuranosidic linkages of AXOS and the hydrolysis of $\alpha$-(1,2)-, $\alpha$-(1,3)-, and $\alpha$-(1,5)-linkages of linear and branched AOS (Park et al. 2021). Thus, *S. fibuligera* abfC-encoded ABFs likely play important roles in the degradation and utilization of hemicellulosic biomass by *S. fibuligera*. The retention of a subset of cellulose degradation genes in *S. fibuligera* is quite noteworthy because

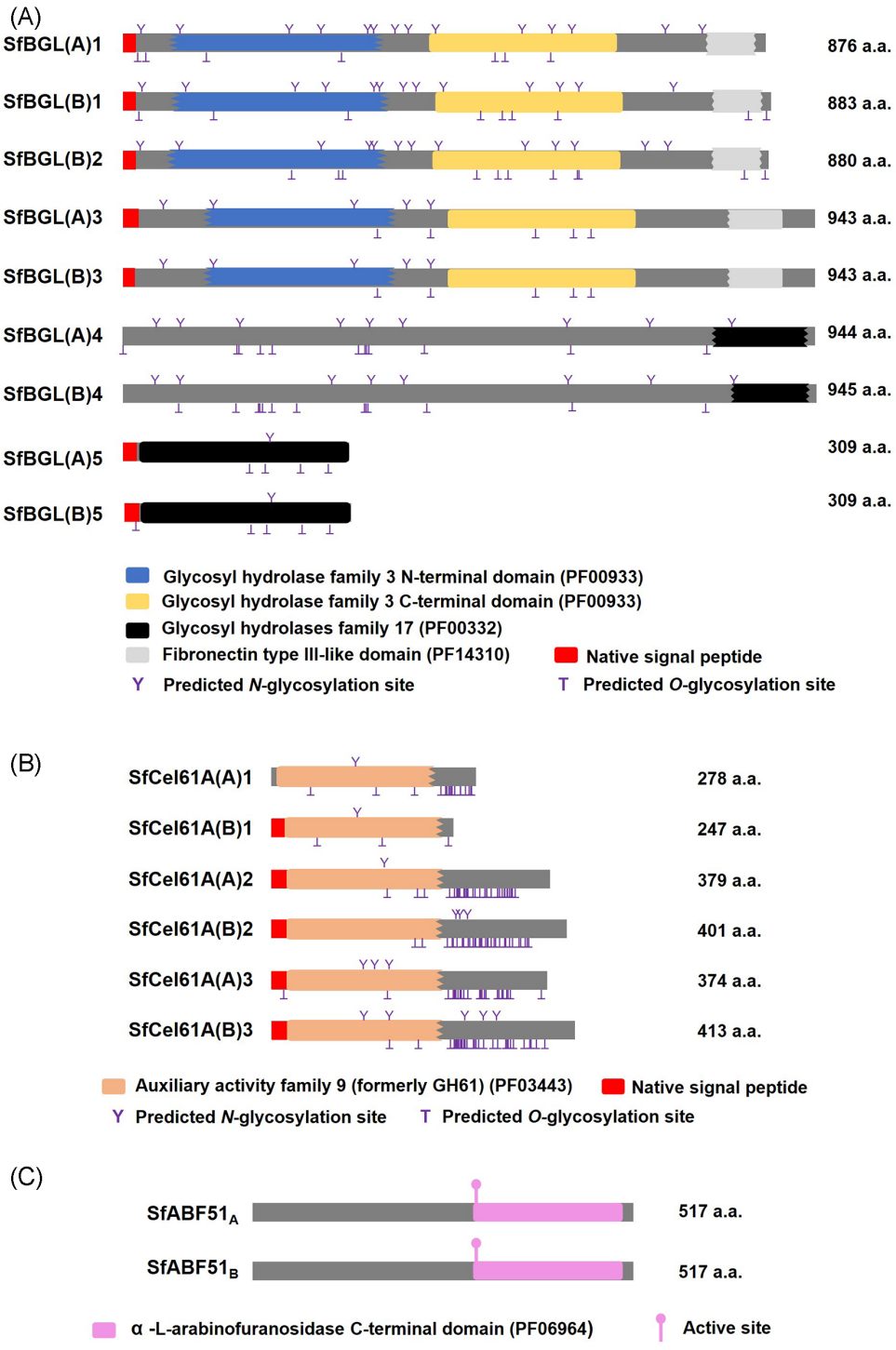


**Figure 4.** Domain analysis of cellulase degradation enzymes identified by *in silico* analysis of *S. fibuligera* KJJ81 genome by using Pfam domain matching (http://hmmer.org). **(A)** The β-glucosidases (BGL) in the *S. fibuligera* KJJ81 genome, predicted as glycosyl hydrolase family (PF00933 or PF00332). The N- and O-glycosylation sites were predicted with NetNglyc (ver. 1.0) and YinOYang (ver. 1.2), respectively. The native signal peptide was analyzed using SignalP (ver. 5.0). **(B)** *S. fibuligera* Cel61A homologs, belonging to an auxiliary activity family 9 (PF03443) of a family of lytic PMOs. **(C)** *S. fibuligera* abfC homologs carrying the α-L-arabinofuranosidase domain (PF06964) and an active site.

most yeast species belonging to the Saccharomycotina subphylum have lost the gene sets involved in cellulose degradation.

## Genes involved in volatile aroma ester formation

Aroma ester components, which are responsible for the fruity character of fermented foods, are produced by fermenting yeast cells via alcohol acyl/acetyltransferase (AATase)-catalyzed intracellular reactions. Two major aroma-esters are present in fermented foods: acetate esters and medium-chain fatty acid (MCFA) ethyl esters (Saerens et al. 2010, Hu et al. 2018). Acetate esters are produced from acetic acid (acetate) with ethanol or a higher alcohol (fusel alcohol) through the action of AATases (EC 2.3.1.84) (Yoshioka and Hashimoto 1981). Fusel alcohols and acetyl coen-

zyme A (acetyl-CoA) can be condensed via the action of alcohol-O-acetyltransferases (Atfs). In *S. cerevisiae*, two Atf enzymes (Atf1p and Atf2p) catalyze these reactions. MCFA-ethyl ester, a second group of aroma-esters, is produced in *S. cerevisiae* through the condensation of the acyl chain from fatty acid-coenzyme A and ethanol by Eeb1p and Eht1p, encoding acyl-CoA:ethanol *O*-acyltransferase (Saerens et al. 2006).

In the culture supernatant of *S. fibuligera* KJJ81, an isolate from wheat-based Nuruk, some volatile metabolites derived from phenylalanine, such as phenethyl alcohol, phenethyl acetate, and ethylphenyl acetate, are predominantly found (Lee et al. 2018b). We identified 12 *ATF* orthologs (*SfATF*s) encoding putative AATases with the AATase Pfam domain (PF07247) in the diploid genome of *S. fibuligera* KJJ81 (Fig. 5A). The identified *SfATF* proteins (SfAtfp) display low-sequence identities with *S. cerevisiae* Atf1p (between 13.3% and 27.0%). All the identified SfAtfp, except SfAtf(A)4p and SfAtf(B)4p, contain the activation domain (HXXXD) conserved in other Atf proteins. The recombinant *S. cerevisiae* strains expressing SfAtf(A)2p, SfAtf(B)2p, and SfAtf(B)6p produce high levels of isoamyl and phenethyl acetates (Moon et al. 2021). The volatile aroma profiles generated by SfAtf proteins were distinct from that of *S. cerevisiae* Atf1p, implying differences in substrate preference. A previous study on the genome sequencing of *W. anomalus* DSM 6766 reported the presence of six AATases with the Pfam domain PF07247 (Schneider et al. 2012). The presence of multiple *ATF* genes might partly contribute to the increased levels of acetate ester in *S. fibuligera* as the case of *W. anomalus*. In addition to *ATF* family genes, *S. fibuligera* KJJ81 genome contains the homologs of *EEB1* and *IMO32* encoding another group of AATases, which possess an $\alpha/\beta$ hydrolase fold structure (Pfam domain: PF00561 or PF12697) and the Ser-Asp/Glu-His catalytic triad (Fig. 5B). In *S. cerevisiae*, Imo32p is an alternative ethyl acetate biosynthetic enzyme of the major enzyme Eat1p, whereas Eeb1p is involved in MCFA-ethyl ester formation (Kruis et al. 2017). The presence of multiple gene families involved in ester formation indicates the high potential of *S. fibuligera* as a producer of high-level volatile aroma and source of novel genes for diverse flavor production.

## Unique osmotolerance mechanisms employed by Nuruk yeast *H. burtonii*

The tolerance of most yeast isolates from Nuruk and Jang to salt and sugar is higher than that of *S. cerevisiae*. Particularly, *H. burtonii* and *M. farinosa* retain their vigorous growth at high concentrations of NaCl, KCl, and sorbitol. Interestingly, *H. burtonii* on YPD plates supplemented with salts and sorbitol formed white expanding colonies with farinose and wrinkled surface because of induced hyperfilamentous growth in response to osmostress conditions (Fig. 6). Unique mechanisms employed by *Hyphopichia* yeasts for high osmotolerance and halotolerance have been proposed through the integrated genomic and transcriptomic analysis (Lee et al. 2021). *H. burtonii* KJJ43 and *H. pseudoburtonii* KJS14 genomes show the enrichment of novel genes carrying ATP-binding cassette (ABC) and gene families of amino acid permeases, including oligopeptide transporter proteins. ABC transporters, enriched with the MRP subfamily, display dynamic expression patterns during osmotic stress. Moreover, the high-osmolarity glycerol (HOG) signaling pathway and filamentous growth (FG)-mediated signaling pathways are activated even under nonosmotic stress conditions. Particularly, the enhanced glycerol accumulation during osmotic stress is mediated by its efficient production via cytosolic Gpd1p, its active import by multiple Stl1p, and its decreased leakage as *Hyphopichia* yeasts do not possess the glycerol channel protein Fps1p. Associated with hyperfilamentation growth under high osmotic conditions, a set of genes in the *FLO* family with induced expression in response to NaCl, KCl, and sorbitol supplementation were identified. Furthermore, the increased *BAT2* expression suggests that changes in amino acid metabolism, including the pooling of hydroxy acid metabolites of branched-chain amino acids, are involved in salt-specific stress response in *Hyphopichia* yeasts.

Glycerol plays a role as the major osmoprotective molecule in most of the yeast species (Stratford et al. 2019), but the synthesis and intracellular accumulation of glycerol occur in distinctive ways in *Hyphopichia* yeasts with highly strong osmotolerance, leading to extremely high glycerol productivity. This capacity of *H. burtonii* KJJ43 to accumulate high glycerol levels inside cells under osmotic stress could be exploited to develop this yeast as a promising host for commercial glycerol production. Based on their ability to accumulate large amounts of glycerol as the main intracellular osmoprotectant, several osmotolerant yeast species belonging to *Candida*, *Debaryomyces*, *Pichia*, *Torulaspora*, or *Zygosaccharomyces* have been considered for glycerol production (Semkiv et al. 2020).

## Probiotic potential of Jang yeast isolates *D. hansenii*

In addition to its role in producing fermented foods, several yeast species have been used in several biotechnological processes (Breuer and Harms 2006, Chi et al. 2009, Riley et al. 2016). In recent years, the biological application of yeast has been extended as probiotic candidates. Since yeast species in fermented foods can affect the gut microbiota as an autochthonous species, probiotic and potentially probiotic yeasts have been screened from foods (Kumura et al. 2004). During the last decade, some species isolated from food and environmental habitats have been assessed to possess probiotic characteristics and abilities, which include yeast species from *Debaryomyces*, *Hanseniaspora*, *Pichia*, *Meyerozyma*, and *Torulaspora* (Staniszewski and Kordowska-Wiater 2021, Tamang and Lama 2022). These potentially probiotic yeasts can be used to produce new fermented food with enhanced nutritional and sensory properties, presenting the potential for new probiotic products with novel properties.

Live *D. hansenii* strains have been recognized as potential human probiotics because they secrete higher levels of IL-10/IL-12 in human dendritic cells (hDCs) than *Saccharomyces boulardii* does (Ochangco et al. 2016). They have also been considered as fish probiotics based on the probiotic effects of marine *D. hansenii* CBS8339 on innate immune and antioxidant parameters in newborn goats (Angulo et al. 2019). *D. hansenii* is the most abundant species in the neonatal gut during breastfeeding (Schei et al. 2017), and it is included in the Top 10 most prevalent fungi found in metagenomic WG sequences in fecal metagenomic sequencing data of Human Microbiome Project (Nash et al. 2017), supporting the possibility of *D. hansenii* as human probiotic candidates. We evaluated the potential of the ganjang isolates *D. hansenii* KD2 and *D. hansenii* species complex C11 as probiotic candidates by examining the tolerance of the strains to low pH and bile salt, adherence ability to Caco-2 cells, and immunomodulatory activity (Jeong et al. 2022). KD2 and C11 were viable in the presence of bile salts and at low pH, strong adhesion to Caco-2 cells, and beneficial immunomodulatory activity to induce high levels of the anti-inflammatory cytokine IL-10. Furthermore, the safety of the Jang yeast isolates was confirmed by analyzing virulence and acute oral toxicity. This

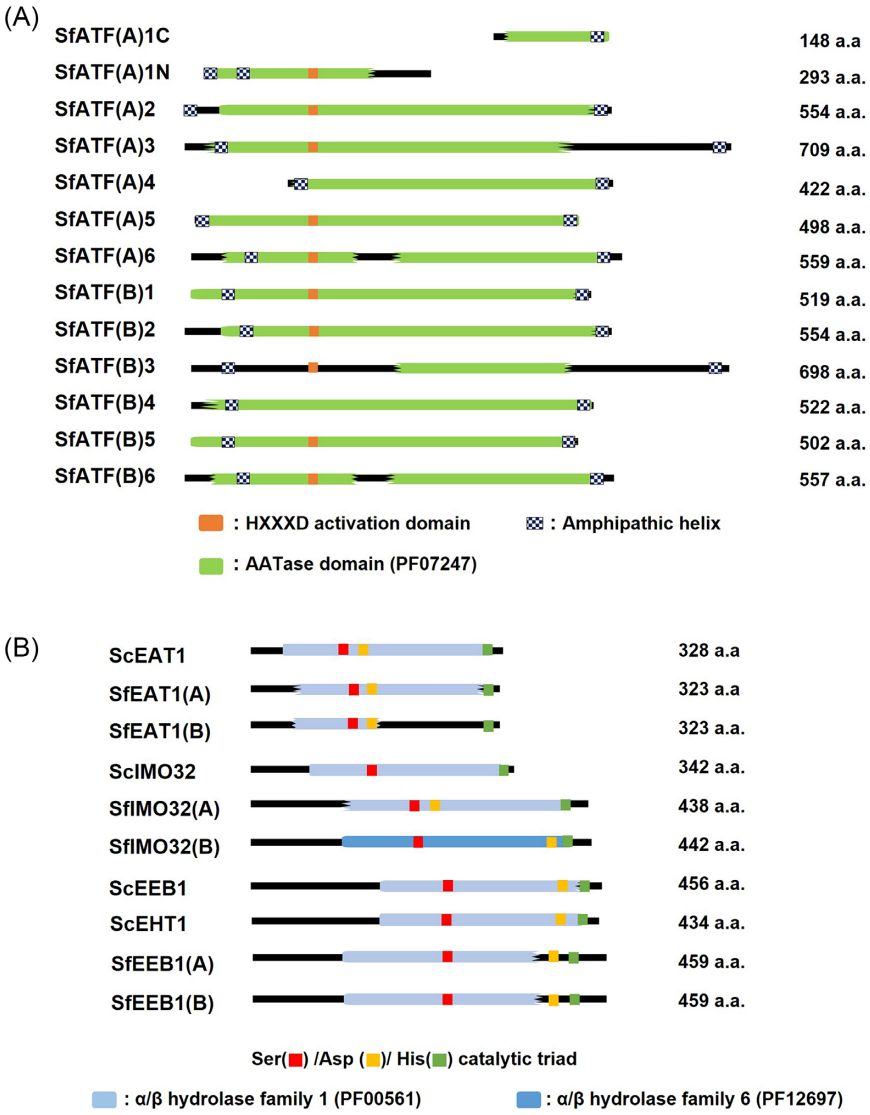


**Figure 5.** Domain structure analysis of acyl/acetyl transferases (AATases) identified by *in silico* analysis of *S. fibuligera* KJJ81 genome via Pfam domain matching (http://hmmer.org/). N- and O-glycosylation sites were predicted with NetNglyc 1.0 and YinOYang 1.2 program, respectively. The native signal peptide was analyzed using SignalP 5.0. **(A)** Atf protein family predicted on the basis of the *S. fibuligera* KJJ81 genome. The domain features of SfAtf proteins were partly obtained from a previous study (Moon et al. 2021). **(B)** Eat1, Imo32, and Eeb1 proteins predicted on the basis of the *S. fibuligera* KJJ81 genome.

result supported their potential as probiotics for enhancing the functional properties of fermented foods.

## Conclusion and perspectives

In the Asian region, indigenous fermented foods are important in daily life, and numerous diverse yeast species have been isolated from various fermented foods. *De novo* WG sequencing and comparative genomics of yeast species prevalently isolated from Nuruk and Jang provide a useful basis for elucidating and documenting evolutionary consequences, revealing intriguing aspects of genetic diversity and population structure. Notably, the coexistence of haploid and heterozygous diploid genomes is not rare among Nuruk and Jang yeast species (Table 3), strongly indicating that interspecies hybrids are frequently generated in yeast populations in Nuruk and Jang environments. Since the hybrid nature of a *Saccharomyces* yeast used in the production of lager beer was revealed in the late 1980s (Vaughan Martini and Kurtzman 1985), the number of identified interspecies hybrids of *Saccharomyces* hybrids in fermented beverages and foods has been increased, as genome sequencing technologies have rapidly developed (Hittinger et al. 2018). Beyond the genus *Saccharomyces sensu stricto*, many hybrids have been identified also in other yeast genera of food industry, including *Zygosaccharomyces* (Solieri et al. 2007), *Dekkera* (Curtin et al. 2012), *Pichia* (Smukowski Heil et al. 2018), *Millerozyma* (Louis et al. 2012), *Saccharomycopsis* (Choo et al. 2016), and *Debaryomyces* (Jeong et al. 2022), suggesting that interspecies hybrids may be common in human-associated strains across the broader yeast phylogeny (Gabaldón T 2020). Furthermore, many Nuruk and Jang yeast species belong to the CUG-Ser clade (Fig. 2). Several CUG clade species are ubiquitous, showing strong osmotolerance and halotolerance. It is noticeable that several CUG yeast species have high biotechnological potentials, such as promising producers of industrial enzymes, single-cell protein, bioethanol, vitamins, sweeteners, lipids, and other metabolites of medicinal and nutritional values (Papon et al. 2014). These unique traits might enable

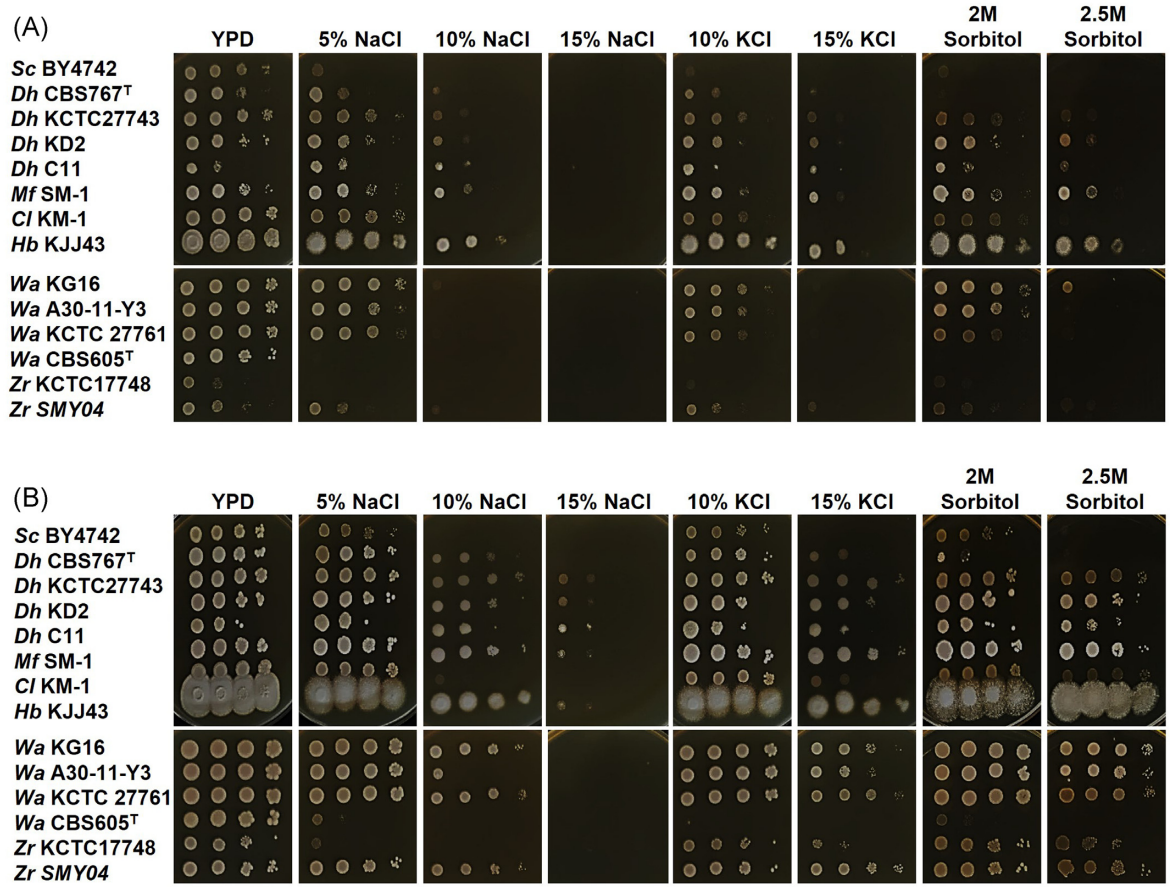


**Figure 6.** Halotolerance and osmotolerance of yeast species isolated from Jang. Yeast cells, including *S. cerevisiae* BY4742 (*Sc*, a laboratory strain), *D. hansenii* CBS767$^T$ (*Dh*, a type strain), KCTC27743 (from Nuruk), KD2 (from Jang), and complex C11 (from Jang), *M. farinosa* SM-1 (*Mf*, from Jang), *C. lusitaniae* KM-1 (*Ch*, from Jang), *H. burtonii* KJJ43 (*Hb*, from Nuruk), *W. anomalus* KG16 (*Wa*, from Jang), A30-11-Y3 (from Jang), KCTC27761 (from Nuruk), CBS605$^T$ (a type strain), *Z. rouxii* KCTC17748 (*Zr*, from red miso), and SMY-04 (from Jang), cultivated in YPD, were subjected to 10-fold serial dilutions from $OD_{600} = 1$, spotted on plates with YPD alone or YPD supplemented with various osmolytes, and incubated at 26°C for 2 days **(A)** and 4 days **(B)**.

them to thrive in fermentation environments. As more information on complete WG sequences with high-quality annotation and assembly is accumulated, more yeast species in fermented foods would be discovered to have hybrid genomes and to employ non-canonical genetic codes.

An important foundation for the functional inference of each yeast species is established on the basis of the completely sequenced genome with high-quality annotation and RNA-Seq analysis. New genes with biotechnological potential, such as multiple genes involved in cellulose degradation and volatile aroma formation, are discovered via gene inventory analysis on the reference genomes of Nuruk and Jang yeast species, indicating their key roles in fermentation mash degradation and flavor production. Integrated genomic and transcriptomic analysis also reveals multiple novel mechanisms involved in the halotolerant and osmotolerant behavior of *Hyphopichia* yeasts, which are abundantly present in Nuruk and Jang. Such advanced information and new genetic resources will be applicable to the development of engineered yeast strains for simultaneous saccharification/fermentation processes, along with improved starter strains tailored to produce fermented products with desired flavor profiles or an enhanced nutraceutical composition. Moreover, yeast isolates from Jang possess several physiological properties beneficial to be developed as probiotic candidates, such as tolerance to gastric conditions, strong adhesion to intestinal epithelial cells, and beneficial immunomodulatory activity. These results indicate that Korean fermented foods are good sources of yeast species that can exert various beneficial effects on human health. Therefore, yeast species isolated in fermented foods will be further applied not only to food starters but also to new probiotics and commercialized globally.

In Korean fermented foods, diverse yeast species other than *S. cerevisiae*, which are so-called nonconventional yeasts, participate in fermentation with defined and as yet-unidentified functions. Although limited to a few yeast species from Nuruk and Jang, the high-quality WG information with systematic characterization of their physiological features has led to the unraveling of several interesting traits with industrial potentials. Implementation of functional genomics platforms, including genomics, transcriptomics, and metabolomics, in Nuruk and Jang yeast species is expected to facilitate the exploitation of huge biotechnological potential of these yeast species in development of functional foods and bioprocesses for bioactive compound production. Particularly, systems and synthetic biology approach, which has been mainly carried out for *S. cerevisiae*, would be valuable in the development of novel cell factories for food ingredients using Nuruk and Jang yeast species.

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## References


Angulo M, Reyes-Becerril M, Cepeda-Palacios R *et al.* Probiotic effects of marine *Debaryomyces hansenii* CBS 8339 on innate immune and antioxidant parameters in newborn goats. *Appl Microbiol Biotechnol* 2019;**103**:2339–52.

Bal J, Yun SH, Song HY *et al.* Mycoflora dynamics analysis of Korean traditional wheat-based nuruk. *J Microbiol* 2014;**52**:1025–29.

Bal J, Yun SH, Choi MS *et al.* Pyrosequencing reveals bacterial diversity in Korean traditional wheat-based nuruk. *J Microbiol* 2015;**53**:812–19.

Berni E, Scaramuzza N. Effect of ethanol on growth of *Chrysonilia sitophila* ('the red bread mould') and *Hyphopichia burtonii* ('the chalky mould') in sliced bread. *Lett Appl Microbiol* 2013;**57**:344–49.

Bonjean B, Guillaume LD. Yeast in bread and baking products. In: Boekhout T, Robert V (eds.), *Yeasts in Food, Beneficial and Detrimental Aspects*, Amsterdam: Elsevier. 2003, 289–307.

Breuer U, Harms H. *Debaryomyces hansenii*–an extremophilic yeast with biotechnological potential. *Yeast* 2006;**23**:415–37.

Carroll E, Trinh TN, Son H *et al.* Comprehensive analysis of fungal diversity and enzyme activity in nuruk, a Korean fermenting starter, for acquiring useful fungi. *J Microbiol* 2017;**55**:357–65.

Chi Z, Chi Z, Liu G *et al. Saccharomycopsis fibuligera* and its applications in biotechnology. *Biotechnol Adv* 2009;**27**:423–31.

Choi DH, Park EH, Kim MD. Isolation of thermotolerant yeast *Pichia kudriavzevii* from nuruk. *Food Sci Biotechnol* 2017;**26**:1357–62.

Choo JH, Hong CP, Lim JY *et al.* Whole-genome de novo sequencing, combined with RNA-Seq analysis, reveals unique genome and physiological features of the amylolytic yeast *Saccharomycopsis fibuligera* and its interspecies hybrid. *Biotechnol Biofuels* 2016;**9**:246.

Chun BH, Han DM, Kim HM *et al.* Metabolic features of ganjang (a Korean traditional soy sauce) fermentation revealed by genome-centered metatranscriptomics. *mSystems* 2021;**6**:e0044121.

Curtin CD, Borneman AR, Chambers PJ *et al.* De-novo assembly and analysis of the heterozygous triploid genome of the wine spoilage yeast *Dekkera bruxellensis* AWRI1499. *PLoS ONE* 2012;**7**:e33840.

Deschuyffeleer N, Audenaert K, Samapundo S *et al.* Identification and characterization of yeasts causing chalk mould defects on par-baked bread. *Food Microbiol* 2011;**28**:1019–27.

Devanthi PVP, Gkatzionis K. Soy sauce fermentation: microorganisms, aroma formation, and process modification. *Food Res Int* 2019;**120**:364–74.

Dimarogona M, Topakas E, Christakopoulos P. Recalcitrant polysaccharide degradation by novel oxidative biocatalysts. *Appl Microbiol Biotechnol* 2013;**97**:8455–65.

Drillon G, Carbone A, Fischer G. SynChro: a fast and easy tool to reconstruct and visualize synteny blocks along eukaryotic chromosomes. *PLoS ONE* 2014;**9**:e92621.

Fröhlich-Wyder MT, Arias-Roth E, Jakob E. Cheese yeasts. *Yeast* 2019;**36**:129–41.

Gabaldón T. Hybridization and the origin of new yeast lineages. *FEMS Yeast Res* 2020;**20**:foaa040.

Hallen-Adams HE, Suhr MJ. Fungi in the healthy human gastrointestinal tract. *Virulence* 2017;**8**:352–58.

Hesseltine CW, Rogers R, Winarno FG. Microbiological studies on amylolytic oriental fermentation starters. *Mycopathologia* 1988;**101**:141–55.

Hittinger CT, Steele JL, Ryder DS. Diverse yeasts for diverse fermented beverages and foods. *Curr Opin Biotechnol*. 2018;**49**:199–206.

Hu K, Jin GJ, Mei WC *et al.* Increase of medium-chain fatty acid ethyl ester content in mixed *H. uvarum/S. cerevisiae* fermentation leads to wine fruity aroma enhancement. *Food Chem* 2018;**239**:495–501.

Jang JH. History of Korean traditional rice wine. *Korean J Diet Cult* 1989;**4**:271–74.

Jeong DM, Yoo SJ, Jeon MS *et al.* Genomic features, aroma profiles, and probiotic potential of the *Debaryomyces hansenii* species complex strains isolated from Korean soybean fermented food. *Food Microbiol* 2022;**105**:104011.

Kang SH, Kim HR, Kim JH *et al.* Identification of wild yeast strains and analysis of their $\beta$-glucan and glutathione levels for use in makgeolli brewing. *Mycobiology* 2014;**42**:361–67.

Kim TW, Lee JH, Kim SE *et al.* Analysis of microbial communities in doenjang, a Korean fermented soybean paste, using nested PCR-denaturing gradient gel electrophoresis. *Int J Food Microbiol* 2009;**131**:265–71.

Kim JY, Yeo SH, Baek SY *et al.* Molecular and morphological identification of fungal species isolated from bealmijang meju. *J Microbiol Biotechnol* 2011;**21**:1270–79.

Krassowski T, Coughlan AY, Shen XX *et al.* Evolutionary instability of CUG-leu in the genetic code of budding yeasts. *Nat Commun* 2018;**9**:1887.

Kruis AJ, Levisson M, Mars AE *et al.* Ethyl acetate production by the elusive alcohol acetyltransferase from yeast. *Metab Eng* 2017;**41**:92–101.

Kumura H, Tanoue Y, Tsukahara M *et al.* Screening of dairy yeast strains for probiotic applications. *J Dairy Sci* 2004;**87**:4050–56.

Kurtzman C. New species and a new combination in the *Hyphopichia* and *Yarrowia* yeast clades. *Antonie Van Leeuwenhoek* 2005;**88**:121–30.

Lee JI, Kim DW, Jang GJ *et al.* Effects of different storage conditions on the metabolite and microbial profiles of white rice (*Oryza sativa* L.). *Food Sci Biotechnol* 2018a;**28**:623–31.

Lee SM, Jung JH, Seo JA *et al.* Bioformation of volatile and non-volatile metabolites by *Saccharomycopsis fibuligera* KJJ81 cultivated under different conditions-carbon sources and cultivation times. *Molecules* 2018b;**23**:2762.

Lee DW, Hong CP, Thak EJ *et al.* Integrated genomic and transcriptomic analysis reveals unique mechanisms for high osmotolerance and halotolerance in *Hyphopichia* yeast. *Environ Microbiol* 2021;**23**:3499–522.

Lioe HN, Selamat J, Yasuda M. Soy sauce and its umami taste: a link from the past to current situation. *J Food Sci* 2010;**75**:R71–6.

Louis VL, Despons L, Friedrich A *et al. Pichia sorbitophila*, an interspecies yeast hybrid, reveals early steps of genome resolution after polyploidization. *G3* 2012;**2**:299–311.

Moon HY, Kim HJ, Kim KS *et al.* Molecular characterization of the *Saccharomycopsis fibuligera* ATF genes, encoding alcohol acetyltransferase for volatile acetate ester formation. *J Microbiol* 2021;**59**:598–608.

Moon HY, Sim GH, Kim HJ *et al.* Assessment of Cre-lox and CRISPR-Cas9 as tools for recycling of multiple-integrated selection markers in *Saccharomyces cerevisiae*. *J Microbiol* 2022;**60**:18–30.

Nash AK, Auchtung TA, Wong MC *et al.* The gut mycobiome of the Human Microbiome Project healthy cohort. *Microbiome* 2017;**5**:153.

Nout MJR, Aidoo KE. Asian fungal fermented food, In: Osiewacz HD (ed.), *The Mycota*. Berlin: Springer, 2002, 23–47.

O'toole DK. The role of microorganisms in soy sauce production. *Adv Appl Microbiol* 2019;**108**:45–113.

Ochangco HS, Gamero A, Smith IM *et al.* In vitro investigation of *Debaryomyces hansenii* strains for potential probiotic properties. *World J Microbiol Biotechnol* 2016;**32**:141.

Oh NS, Lee NS. Characteristics of yeast flora and gas generation during fermentation of doenjang. *Appl Biol Chem* 1996;**39**:255–59.

Ohisa N, Suzuki N, Miura M *et al.* Halotolerant yeast participates in the cavity formation of hand-pulled dry noodles. *J Jpn Soc Food Sci* 2012;**59**:442–46.

Papon N, Courdavault V, Clastre M. Biotechnological potential of the fungal CTG clade species in the synthetic biology era. *Trends Biotechnol* 2014;**32**:167–68.

Park KH, Liu Z, Park CS *et al.* Microbiota associated with the starter cultures and brewing process of traditional Hong Qu glutinous rice wine. *Food Sci Biotechnol* 2016;**25**:649–58.

Park TH, Choi CY, Kim HJ *et al.* Arabinoxylo- and arabinooligosaccharides-specific $\alpha$-L-arabinofuranosidase GH51 isozymes from the amylolytic yeast *Saccharomycopsis fibuligera*. *J Microbiol Biotechnol* 2021;**31**:272–79.

Ramazzotti M, Berná L, Stefanini I *et al.* A computational pipeline to discover highly phylogenetically informative genes in sequenced genomes: application to *Saccharomyces cerevisiae* natural strains. *Nucleic Acids Res* 2012;**40**:3834–48.

Renouf V, Claisse O, Lonvaud-Funel A. Inventory and monitoring of wine microbial consortia. *Appl Microbiol Biotechnol* 2007;**75**:149–64.

Riley R, Haridas S, Wolfe KH *et al.* Comparative genomics of biotechnologically important yeasts. *Proc Natl Acad Sci* 2016;**113**:9882–87.

Ryu S, Park MR, Maburutse BE *et al.* Diversity and characteristics of the meat microbiological community on dry aged beef. *J Microbiol Biotechnol* 2018;**28**:105–8.

Sabel A, Martens S, Petri A *et al. Wickerhamomyces anomalus* AS1: a new strain with potential to improve wine aroma. *Ann Microbiol* 2013;**64**:483–91.

Saerens SM, Verstrepen KJ, Van Laere SD *et al.* The *Saccharomyces cerevisiae* EHT1 and EEB1 genes encode novel enzymes with medium-chain fatty acid ethyl ester synthesis and hydrolysis capacity. *J Biol Chem* 2006;**281**:4446–56.

Saerens SM, Delvaux FR, Verstrepen KJ *et al.* Production and biological function of volatile esters in *Saccharomyces cerevisiae*. *Microb Biotechnol* 2010;**3**:165–77.

Schei K, Avershina E, Øien T *et al.* Early gut mycobiota and mother-offspring transfer. *Microbiome* 2017;**5**:107.

Schneider J, Rupp O, Trost E *et al.* Genome sequence of *Wickerhamomyces anomalus* DSM 6766 reveals genetic basis of biotechnologically important antimicrobial activities. *FEMS Yeast Res* 2012;**12**:382–86.

Semkiv MV, Ruchala J, Dmytruk KV *et al.* 100 years later, what is new in glycerol bioproduction?. *Trends Biotechnol* 2020;**38**:907–16.

Shi X, Wang X, Hou X *et al.* Gene mining and flavour metabolism analyses of *Wickerhamomyces anomalus* Y-1 isolated from a Chinese liquor fermentation starter. *Front Microbiol* 2022;**13**:891387.

Shin DH, Jeong DY. Korean traditional fermented soybean products: Jang. *J Ethn Foods* 2015;**2**:2–7.

Simoncini N, Rotelli D, Virgili R *et al.* Dynamics and characterization of yeasts during ripening of typical Italian dry-cured ham. *Food Microbiol* 2007;**24**:577–84.

Smukowski Heil C, Burton JN, Liachko I *et al.* Identification of a novel interspecific hybrid yeast from a metagenomic spontaneously inoculated beer sample using Hi-C. *Yeast* 2018;**35**:71–84.

Solieri L, Cassanelli S, Giudici P. A new putative *Zygosaccharomyces* yeast species isolated from traditional balsamic vinegar. *Yeast* 2007;**24**:403–17.

Song SH, Lee C, Lee S *et al.* Analysis of microflora profile in Korean traditional nuruk. *J Microbiol Biotechnol* 2013;**23**:40–46.

Song YR, Jeong DY, Baik SH. Monitoring of yeast communities and volatile flavor changes during traditional Korean soy sauce fermentation. *J Food Sci* 2015;**80**:M2005–14.

Souza TA, Santos CR, Souza AR *et al.* Structure of a novel thermostable GH51 $\alpha$-L-arabinofuranosidase from *Thermotoga petrophila* RKU-1. *Protein Sci* 2011;**20**:1632–7.

Staniszewski A, Kordowska-Wiater M. Probiotic and potentially probiotic yeasts-characteristics and food application. *Foods* 2021;**10**:1306.

Stratford M, Steels H, Novodvorska M *et al.* Extreme osmotolerance and halotolerance in food-relevant yeasts and the role of glycerol-dependent cell individuality. *Front Microbiol* 2019;**9**:3238.

Sukhumavasi J, Kato K, Harada T. Glucoamylase of a strain of *Endomycopsis fibuligera* isolated from mould bran (Loog Pang) of Thailand. *J Ferment Technol* 1975;**53**:559–65.

Takeuchi A, Shimizu-Ibuka A, Nishiyama Y *et al.* Purification and characterization of an alpha-amylase of *Pichia burtonii* isolated from the traditional starter "murcha" in Nepal. *Biosci Biotechnol Biochem* 2006;**70**:3019–24.

Tamang JP, Lama S. Probiotic properties of yeasts in traditional fermented foods and beverages. *J Appl Microbiol* 2022;**132**:3533–42.

Thanh VN, Mai le T, Tuan DA. Microbial diversity of traditional Vietnamese alcohol fermentation starters (banh men) as determined by PCR-mediated DGGE. *Int J Food Microbiol* 2008;**128**:268–73.

Yang S, Lee J, Kwak J *et al.* Fungi associated with the traditional starter cultures used for rice wine in Korea. *J Korean Soc Appl Biol Chem* 2011;**54**:933–43.

Yoon JA, Lee YJ, Park EH *et al.* Draft genome sequence data of multi-stress tolerant yeast *Millerozyma farinosa* KCTC27753 isolated from nuruk. *Data Brief* 2022;**42**:108030.

Yoshioka K, Hashimoto N. Ester formation by alcohol acetyltransferase from brewers' yeast. *Agric Biol Chem* 1981;**45**:2183–90.

Vaughan Martini A, Kurtzman CP. Deoxyribonucleic acid relatedness among species of the genus *Saccharomyces sensu stricto*. *Int J Syst Evol Microbiol* 1985;**35**:508–11.

Zheng XW, Yan Z, Han BZ *et al.* Complex microbiota of a Chinese "Fen" liquor fermentation starter (Fen-Daqu), revealed by culture-dependent and culture-independent methods. *Food Microbiol* 2012;**31**:293–300.