

Diversity of Yeasts Associated with Natural Environments in Korea

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Biodiversity of yeasts in various natural environments including soils, swamps and plants was investigated. By molecular identification methods based on the partial sequences of 26S rDNA, 69 isolates were assigned to 44 taxa including 27 known species. The remaining 17 taxa could potentially form new species. All of them were classified into Ascomycota, Hymenomycetes, Urediniomycetes and Ustilaginomycetes. Ascomycetous and ustilaginomycetous yeasts were generally isolated from flower samples, and hymenomycetous and urediniomycetous yeasts were generally isolated from soil samples. Distribution of yeast groups exhibited geographical variation. Yeast biodiversity of root soil also varied according to the associated plant species.

Key words: biodiversity, Korea, yeasts

Yeasts are very important microorganisms in food and beverage fermentation. They are also important infectious agents (Lachance and Starmer, 1998). Various yeasts have been isolated from natural and artificial environments (Lachance *et al.*, 2001; Lee *et al.*, 2001). However, the most extensive work has been focused on the population change and roles of yeasts during the fermentation process of food and beverages (Nunez *et al.*, Strauss *et al.*, 2001). Studies of yeast biodiversity in the natural environment have been neglected because yeasts have been regarded as playing a rather minor role in the biosphere, compared with other microorganisms that may act as primary producers, predators, pathogens, or important agents of nutrient cycling in natural environments (Lachance and Starmer, 1998).

As a result of extensive phylogenetic studies on ascomycetous (Kurtzman and Robnett, 1998) and basidiomycetous yeasts (Fell *et al.*, 2000), identification of yeasts based on the 26S rDNA sequence data became possible. From the nucleotide comparison, it was predicted that strains showing greater than 1% substitutions in the ca. 600-nucleotide D1/D2 domain are likely to be different species and that strains with 0-3 nucleotide differences are either conspecific or sister species (Kurtzman and Robnett, 1998). Identification of yeasts based on the 1% substitution criterion of D1/D2 sequence is very quick and reliable in contrast to the identification based on morphological or physiological data.

In this study, we investigated the biodiversity of yeasts from various natural sources in Korea, using molecular identification methods based on the phylogenetic analysis of D1/D2 domain of 26S rDNA

Materials and methods

Isolation of yeasts

Yeasts were isolated from various natural habitats including algae, flowers, fruits, plants, sediment, soil and water from Changnyong, Kunwi, Daejeon, Wando and Yeongju regions in Korea (Fig. 1). Flowers, plants, sediment and soil samples were suspended in distilled water, mixed by vortex at high speed and allowed to settle for 1 min. The supernatants were spread on YM agar (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose, 2% agar, pH3.7 adjusted with HCl after autoclaving). Water samples were directly spread on YM agar. The plates were incubated at 20°C or 24°C for 3 days. Single colonies were transferred to new YM agar plates and pure colonies were stored at -70°C in 10% glycerol.

DNA extraction and sequence determination

Yeast cells were broken with glass beads and a TOMY micro tube mixer (TOMY, Seiko, Japan). Total DNAs were extracted using a Genomic DNA Isolation Kit (Nucleogen, Ansan, Korea) according to the supplier's guide. The D1/D2 domain (ca. 600-nucleotide) of 26S rDNA was amplified using the primer pair, No.4 (ACCCG CTGAA YTTAA GCAT AT) and No.11 (CTCCT TGGTC

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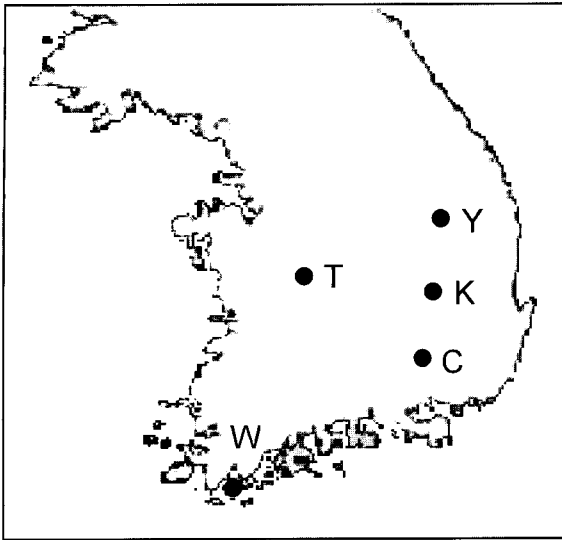


Fig. 1. Distribution of 5 sampling sites in Korea. Abbreviation: C, Changnyong; K, Kunwi; T, Daejeon; W, Wando and Y, Yeongju.

CGTGT TTCAA GACGG) (Van der Auwera *et al.*, 1994) and purified using a Wizard PCR prep kit (Promega, Madison, WI, U.S.A.). The nucleotide sequences were determined with BigDye terminator cycle sequencing kits (Applied Biosystems, Foster City, CA, U.S.A.) following the manufacturer's instructions using the same primers. The gel electrophoresis and data collection were performed on an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.). The sequences were proofread, edited, and merged into composite sequences using the PHYDIT program version 3.1 (Chun, 1995) (available at <http://plaza.snu.ac.kr/~jchun/phydit>).

Phylogenetic analysis and identification of yeast isolates

The 26S rDNA sequences of isolates were aligned with those of neighboring taxa based on secondary structure information using the PHYDIT program. Phylogenetic trees were reconstructed with Kimura's 2-parameter distance model (Kimura, 1980) and the neighbor-joining method (Saitou and Nei, 1987) using the PHYLIP 3.57c package (Felsenstein, 1995). Confidence levels for the individual branches of the resulting tree were assessed by bootstrap analysis (Felsenstein, 1985) in which 1000 bootstrapped trees were generated from the resampled data. The resultant phylogenetic trees were visualized using the TreeView program (Page, 1996). Yeast isolates were identified by the 99% similarity criteria of 26S rDNA D1/D2 domain (Kurtzman and Robnett, 1998) with reference to the phylogenetic relationships.

Results and Discussion

Details of 69 yeast isolates among 125 identified strains

are presented in Table 1. Yeast isolates with the same 26S rDNA sequences from identical samples were omitted in the table. The list contains ascomycetous, hymenomycetous, urediniomycetous, and ustilaginomycetous yeasts from various natural samples. Ascomycetous yeasts were isolated from flowers (5 isolates), fruits (2 isolates), soils (2 isolates), and water samples (1 isolate). Hymenomycetous yeasts were isolated from soil samples (26 isolates), water samples (7 isolates), and flower samples (6 isolates). Urediniomycetous yeasts were isolated from soil samples (13 isolates), water samples (3 isolates), and flower samples (2 isolates). Two ustilaginomycetous yeasts were isolated from flowers. It was found that ascomycetous and ustilaginomycetous yeasts were usually isolated from flower samples. In contrast, hymenomycetous and urediniomycetous yeasts were usually isolated from soil samples.

It is not practical to discuss the geographical distribution of yeasts from the above observation since the sample sources for isolation varied from region to region. However, it is notable that only one ascomycetous yeast was found out of 34 isolates in the Yeongju region. It could be related with the fact that ascomycetous yeasts were usually found in flowers, and that yeasts were isolated mostly from soil samples in Yeongju. However, considering that the soil sample from Wando contained two ascomycetous yeasts out of six isolates, it suggests geographical inequality in yeast distribution.

In order to study the interaction of yeasts with plants, yeast distribution of the rhizosphere soil of apple trees (*Malus pumila* var. *dulcissima*), ginseng (*Panax ginseng*), and Chinese balloon flowers (*Platycodon grandiflorum*) from the same locality were investigated. The rhizosphere soil of apple trees contained yeasts grouped into Cystofilobasidiales (1 isolate), Filobasidiales (4 isolates), Trichosporonales (1 isolate), and *Sporidiobolus* clade (2 isolates). The rhizosphere soil of ginseng contained yeasts grouped into Cystofilobasidiales (2 isolates), Tremellales (7 isolates), *Microbotryum* clade (2 isolates), and *Sporidiobolus* clade (2 isolates). The rhizosphere soil of Chinese balloon flowers contained yeasts grouped into Cystofilobasidiales (1 isolate), Filobasidiales (1 isolate), Trichosporonales (1 isolate), Tremellales (2 isolates), *Microbotryum* clade (1 isolate), and *Sporidiobolus* clade (1 isolate). It is interesting that the rhizosphere soil of Chinese balloon flowers showed an even distribution of the six major lineages of basidiomycetous yeasts in contrast to the concentration of the Filobasidiales in the rhizosphere soil of apple trees and of the Tremellales in the rhizosphere soil of ginseng. It is not certain, however, whether the difference in yeast distribution originated from specific interaction with plants or physico-chemical characteristics of the soil. In order to elucidate the interaction of yeasts and plants, it is necessary to compare the biodiversity of yeasts in various rhizosphere soil samples of the same kind of plant collected from different regions.

Table 1. List of yeast species isolated from various natural environments

Species Name	Strain No.	26S rDNA Acc. No.	Location	Habitat	Sampling Date
Ascomycetous yeasts					
<i>Aureobasidium</i> sp.	KCTC 26209	AF459654	Daejeon	Flower of <i>Taraxacum platycarpum</i> H. Dahlist.	2000. 3. 13
	KCTC 26211	AF459656	Yeongju	Flower of <i>Duchesnea chrysantha</i> (Zoll. et Morr.) Miq.	2000. 3. 16
<i>Candida railenensis</i> Ramirez & Gonzalez	KCTC 7835	AF257274	Kunwi	Fruit of <i>Prunus persica</i> (L.) Batsch	1999. 8. 4
<i>Candida tropicalis</i> (Castellani) Berkhout	KCTC 7830	AF257268	Wando	Soil	1999. 6.15
<i>Candida</i> sp.	KCTC 17041	AF399527	Kunwi	Flower of <i>Ipomoea batatas</i> Lam.	1999. 8. 4
<i>Hanseniaspora uvarum</i> (Niehaus) Shehata et al. ex Smith	KCTC 7834	AF257273	Kunwi	Fruit of <i>Prunus persica</i> (L.) Batsch	1999. 8. 4
<i>Metschnikowia korensis</i> Hong et al.	KCTC 7828	AF257272	Kunwi	Flower of <i>Ipomoea batatas</i> Lam.	1999. 8. 1
<i>Pichia guilliermondii</i> Wickerham	KCTC 7998T	AF296438	Kunwi	Flower of <i>Lilium</i> sp.	1999. 8. 4
<i>Williopsis saturnus</i> (Kloecker) Zender	KCTC 7832	AF257270	Wando	Soil	1999. 6. 15
	KCTC 17042	AF459657	Changnyong	Swamp sediment	1999. 1. 19
Hymenomycetous yeasts					
<i>Bullera sinensis</i> Li	KCTC 17043	AF459658	Yeongju	Rhizosphere soil of <i>Panax ginseng</i> C.A. meyer	2000. 3. 16
<i>Bulleromyces albus</i> Boekhout & Fonseca	KCTC 17044	AF459659	Yeongju	Rhizosphere soil of <i>Panax ginseng</i> C.A. meyer	2000. 3. 16
<i>Cryptococcus laurentii</i> (Kufferath) Skinner	KCTC 7831	AF257269	Wando	Soil	1999. 6. 15
	KCTC 17045	AF459660	Changnyong	Mixture of aquatic plant and green algae	2000. 1. 19
	KCTC 17046	AF459661	Changnyong	Floating matter in a swamp	2000. 1. 19
	KCTC 17047	AF459662	Changnyong	Aquatic plant	2000. 1. 19
<i>Cryptococcus macerans</i> (Frederiksen) Phaff & Fell	KCTC 17048	AF459663	Yeongju	Rhizosphere soil of <i>Panax ginseng</i> C.A. meyer	2000. 3. 16
	KCTC 17049	AF459664	Changnyong	Aquatic plant	2000. 1. 19
	KCTC 17050	AF459665	Changnyong	Aquatic plant	2000. 1. 19
	KCTC 17051	AF459666	Yeongju	Rhizosphere soil of <i>Panax ginseng</i> C.A. meyer	2000. 3. 16
	KCTC 17052	AF459667	Yeongju	Rhizosphere soil of <i>Platycodon grandiflorum</i> (Jacq.) A. DC.	2000. 3. 16
<i>Cryptococcus nodaensis</i> Sato et al.	KCTC 17053	AF459668	Yeongju	Rhizosphere soil of <i>Panax ginseng</i> C.A. meyer	2000. 3. 16
<i>Cryptococcus phenolicus</i> Fonseca et al.	KCTC 17054	AF459669	Yeongju	Rhizosphere soil of <i>Malus pumila</i> var. <i>dulcissima</i> Koidz	2000. 3. 16
<i>Cryptococcus podzolicus</i> (Babeva & Reshetova) Golubev	KCTC 17055	AF459670	Yeongju	Rhizosphere soil of <i>Platycodon grandiflorum</i> (Jacq.) A. DC.	2000. 3. 16
<i>Cryptococcus terreus</i> di Menna	KCTC 17056	AF459671	Yongju	Rhizosphere soil of <i>Malus pumila</i> var. <i>dulcissima</i> Koidz	2000. 3. 16
<i>Cryptococcus terricolus</i> Pedersen	KCTC 7837	AF257276	Kunwi	Soil	2000. 3. 16
	KCTC 17057	AF459672	Yeongju	Rhizosphere soil of <i>Platycodon grandiflorum</i> (Jacq.) A. DC.	1999. 8. 4
<i>Cryptococcus victoriae</i> Montes et al.	KCTC 17058	AF459673	Yeongju	Flower of <i>Duchesnea chrysantha</i> (Zoll. et Morr.) Miq.	2000. 3. 16
	KCTC 17059	AF459674	Yeongju	Rhizosphere soil of <i>Platycodon grandiflorum</i> (Jacq.) A. DC.	2000. 3. 16
<i>Cryptococcus</i> sp. [A]	KCTC 17100	AF459675	Yeongju	Rhizosphere soil of <i>Panax ginseng</i> C.A. meyer	2000. 3. 16
<i>Cryptococcus</i> sp. [B]	KCTC 17061	AF459677	Kunwi	Soil	1999. 8. 4
	KCTC 17062	AF459678	Yeongju	Rhizosphere soil of <i>Platycodon grandiflorum</i> (Jacq.) A. DC.	2000. 3. 16
<i>Cryptococcus</i> sp. [C]	KCTC 17063	AF459679	Yeongju	Rhizosphere soil of <i>Malus pumila</i> var. <i>dulcissima</i> Koidz	2000. 3. 16
	KCTC 17064	AF459680	Changnyong	Swamp water	2000. 1. 19
	KCTC 17065	AF459681	Yeongju	Flower of <i>Duchesnea chrysantha</i> (Zoll. et Morr.) Miq.	2000. 3. 16
<i>Cryptococcus</i> sp. [D]	KCTC 17066	AF459682	Daejeon	Flower of <i>Taraxacum platycarpum</i> H. Dahlist.	2000. 3. 13
	KCTC 17068	AF459684	Yeongju	Flower of <i>Duchesnea chrysantha</i> (Zoll. et Morr.) Miq.	2000. 3. 16
<i>Cryptococcus</i> sp. [E]	KCTC 17069	AF459685	Yeongju	Rhizosphere soil of <i>Panax ginseng</i> C.A. meyer	2000. 3. 16

Table 1. Continued

Species Name	Strain No.	26S rDNA Acc. No.	Location	Habitat	Sampling Date
<i>Cryptococcus</i> sp. [E]	KCTC 17070	AF459686	Yeongju	Rhizosphere soil of <i>Panax ginseng</i> C.A. meyer	2000. 3. 16
<i>Cryptococcus</i> sp. [F]	KCTC 17071	AF459687	Changnyong	Mixture of aquatic plants and green algae	2000. 1. 19
<i>Cryptococcus</i> sp. [G]	KCTC 17074	AF459690	Yeongju	Flower of <i>Duchesnea chrysantha</i> (Zoll. et Morr.) Miq.	2000. 3. 16
<i>Cryptococcus</i> sp. [H]	KCTC 17077	AF459693	Daejeon	Flower of <i>Taraxacum platycarpum</i> H. Dahlst.	2000. 3. 13
<i>Cryptococcus</i> sp. [I]	KCTC 17078	AF459694	Wando	Soil	1999. 6. 15
<i>Cryptococcus</i> sp. [J]	KCTC 17079	AF459695	Yeongju	Rhizosphere soil of <i>Panax schinseng</i> Nees	2000. 3. 16
<i>Cryptococcus</i> sp. [K]	KCTC 17080	AF459696	Yeongju	Rhizosphere soil of <i>Malus pumila</i> var. <i>dulcissima</i> Koidz	2000. 3. 16
<i>Filobasidium floriforme</i> Olive	KCTC 17081	AF459697	Yeongju	Rhizosphere soil of <i>Malus pumila</i> var. <i>dulcissima</i> Koidz	2000. 3. 16
<i>Hyalodendron lignicola</i> Diddens	KCTC 17082	AF459698	Yeongju	Rhizosphere soil of <i>Pinus</i> sp.	2000. 3. 16
<i>Trichosporon pullulans</i> (Lindner) Diddens & Lodder	KCTC 7836	AF257275	Kunwi	Soil	1999. 8. 4
	KCTC 17101	AF459699	Yeongju	Rhizosphere soil of <i>Malus pumila</i> var. <i>dulcissima</i> Koidz	2000. 3. 16
Urediniomycetous yeasts					
<i>Rhodosporiidium azoricum</i> Gadanho <i>et al.</i>	KCTC 17083	AF459700	Kunwi	Flower of <i>Lilium</i> sp.	1999. 8. 4
<i>Rhodosporiidium toruloides</i> Banno	KCTC 7833	AF257271	Wando	Soil	1999. 6. 15
<i>Rhodotorula creatinivora</i> Golubev	KCTC 17084	AF459701	Yeongju	Rhizosphere soil of <i>Panax schinseng</i> Nees	2000. 3. 16
<i>Rhodotorula glutinis</i> var. <i>daiarensis</i> Hasegawa & Banno	KCTC 17085	AF459702	Changnyong	Soil	2000. 1. 19
<i>Rhodotorula graminis</i> di Menna	KCTC 17086	AF459703	Yeongju	Rhizosphere soil of <i>Panax schinseng</i> Nees	2000. 3. 16
	KCTC 17087	AF459704	Yeongju	Rhizosphere soil of <i>Platycodon grandiflorum</i> (Jacq.) A. DC.	2000. 3. 16
	KCTC 17088	AF459705	Yeongju	Rhizosphere soil of <i>Malus pumila</i> var. <i>dulcissima</i> Koidz	2000. 3. 16
<i>Rhodotorula ingenirosa</i> (di Menna) van Arx & Weijman	KCTC 17089	AF459706	Changnyong	Soil	2000. 1. 19
	KCTC 17090	AF459707	Yeongju	Rhizosphere soil of <i>Panax schinseng</i> Nees	2000. 3. 16
<i>Rhodotorula mucilaginosa</i> (Joergensen) Harrison	KCTC 7829	AF257267	Wando	Soil	1999. 6. 15
<i>Rhodotorula</i> sp.	KCTC 17091	AF459708	Yeongju	Rhizosphere soil of <i>Platycodon grandiflorum</i> (Jacq.) A. DC.	2000. 3. 16
<i>Sporidiobolus pararoseus</i> Fell & Tallman	KCTC 17092	AF459709	Changnyong	Mixture of aquatic plants and green algae	2000. 1. 19
<i>Sporobolomyces</i> sp.	KCTC 17093	AF459710	Kunwi	Flower of <i>Lilium</i> sp.	1999. 8. 4
	KCTC 17094	AF459711	Changnyong	Mixture of aquatic plants and green algae	2000. 1. 19
	KCTC 17095	AF459712	Changnyong	Floating matter in a swamp	1999. 1. 19
	KCTC 17096	AF459713	Changnyong	Soil	2000. 1. 19
	KCTC 17097	AF459714	Yeongju	Rhizosphere soil of <i>Panax schinseng</i> Nees	2000. 3. 16
	KCTC 17098	AF459715	Yeongju	Rhizosphere soil of <i>Malus pumila</i> var. <i>dulcissima</i> Koidz	2000. 3. 16
Ustilaginomycetous yeasts					
<i>Microstroma</i> sp.	KCTC 17099	AF459716	Daejeon	Flower of <i>Taraxacum platycarpum</i> H. Dahlst.	2000. 3. 13
<i>Tilletiopsis</i> sp.	KCTC 26212	AF459717	Kunwi	Flower of <i>Erigeron annuus</i> (L.) Pers	1999. 8. 1

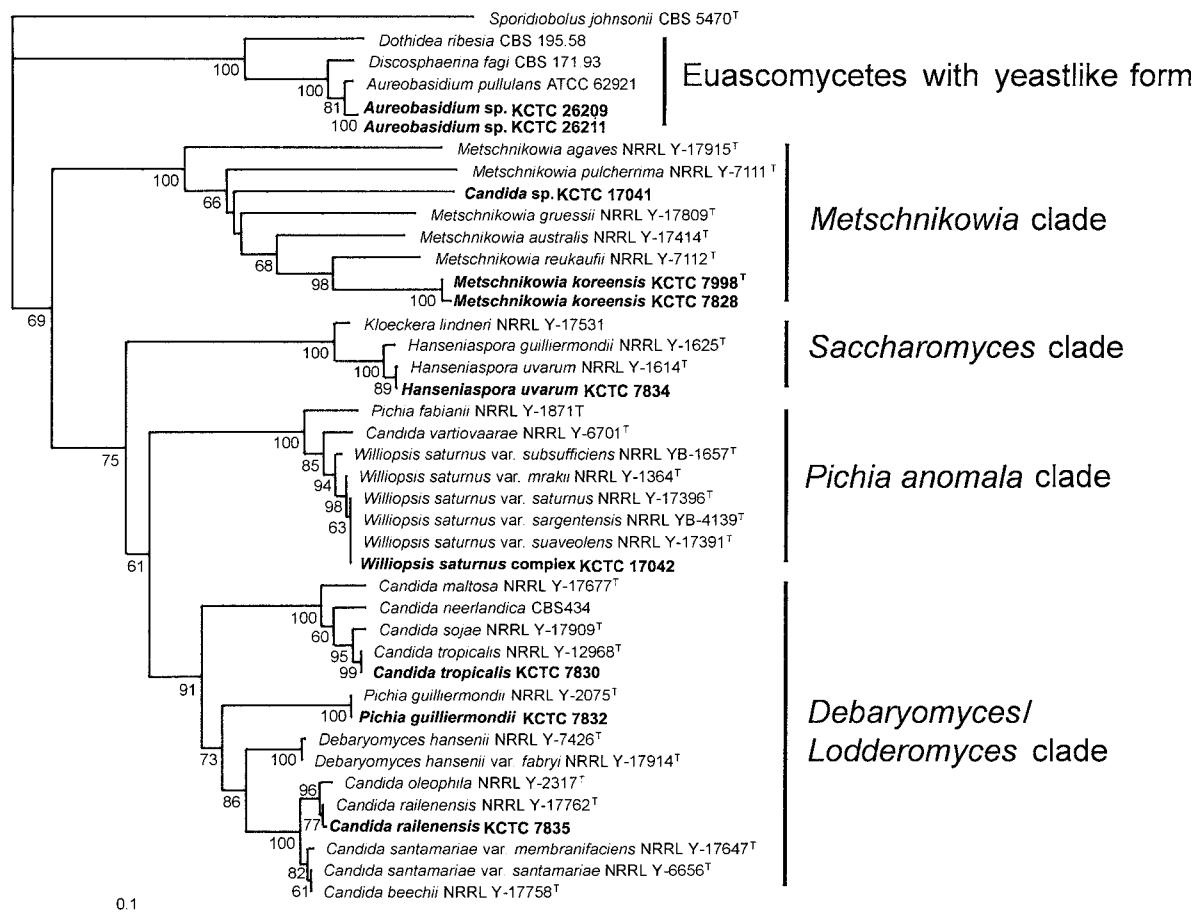


Fig. 2. Ascomycetous yeasts. A phylogenetic tree was reconstructed by a neighbor-joining algorithm based on the distances calculated by Kimura's two-parameter model from the sequences of D1/D2 domain of 26S rDNA. Bootstrap values greater than 50% are shown alongside the branch considered.

The phylogenetic relationship of yeast isolates and neighboring species is presented in Figs 2-4. Ten ascomycetous yeast isolates were distributed in a Euascomycetes clade with yeast-like form and four yeast clades including *Metschnikowia*, *Saccharomyces*, *Pichia anomala*, and *Debaryomyces/Lodderomyces* clades out of ten major clades defined by Kurtzman and Robnett (1998). Among them, *Metschnikowia koreensis* was first described with the two isolates (KCTC 7828 and KCTC 7998) included in this study (Hong *et al.*, 2001). *Candida* sp. KCTC 17041 (86.42% similarity with *Metschnikowia australis*) is also a species first found in Korea. Many yeast species with affinities to the genus *Metschnikowia* have been found on flowers and associated vector insects (Miller and Phaff, 1998). Two strains of *M. koreensis* and *Candida* sp. KCTC 17041 were also isolated from flowers of *Ipomoea batatas* and/or *Lilium* sp.

Hymenomycetous yeast isolates were distributed in four orders, Cystofilobasidiales, Filobasidiales, Trichosporonales, and Tremellales. The biodiversity of 39 hymenomycetous yeasts identified in this study covers nearly all the major phylogenetic lineages of hymenomycetous yeasts.

Among them, sixteen strains were not matched to any established species by the 99% similarity criterion of D1/D2 domain of 26S rDNA. These strains could be grouped into eleven species. *Cryptococcus* sp. [A] KCTC 17100 isolated from soil samples was closely related to *Cystofilobasidium infirmominatum* (98.69%) and *Cystofilobasidium bisporidii* (98.83%). Three strains labeled as *Cryptococcus* sp. [B] were closely related to *Cryptococcus nyarrowii* (96.39%) and *Holtermannia corniformis* (97.21%). They were related to the soil environment. Six strains labeled as *Cryptococcus* sp. [C], *Cryptococcus* sp. [D] or *Cryptococcus* sp. [E] were closely related to *Cryptococcus victoriae* (99.02%, 98.20%, and 96.73% respectively). *Cryptococcus* sp. [C] was isolated from swamp water and flowers. *Cryptococcus* sp. [D] was isolated from two flower samples. Two strains of *Cryptococcus* sp. [E] were isolated from the same root sample but showed one base difference in the D2 domain of the 26S rDNA. *Cryptococcus* sp. [F] KCTC 17071 and *Cryptococcus* sp. [G] KCTC 17074 were closely related to *Cryptococcus luteolus* (95.91% and 97.87% respectively) and *Bullera sinensis* (96.22% and 96.72% respectively). The

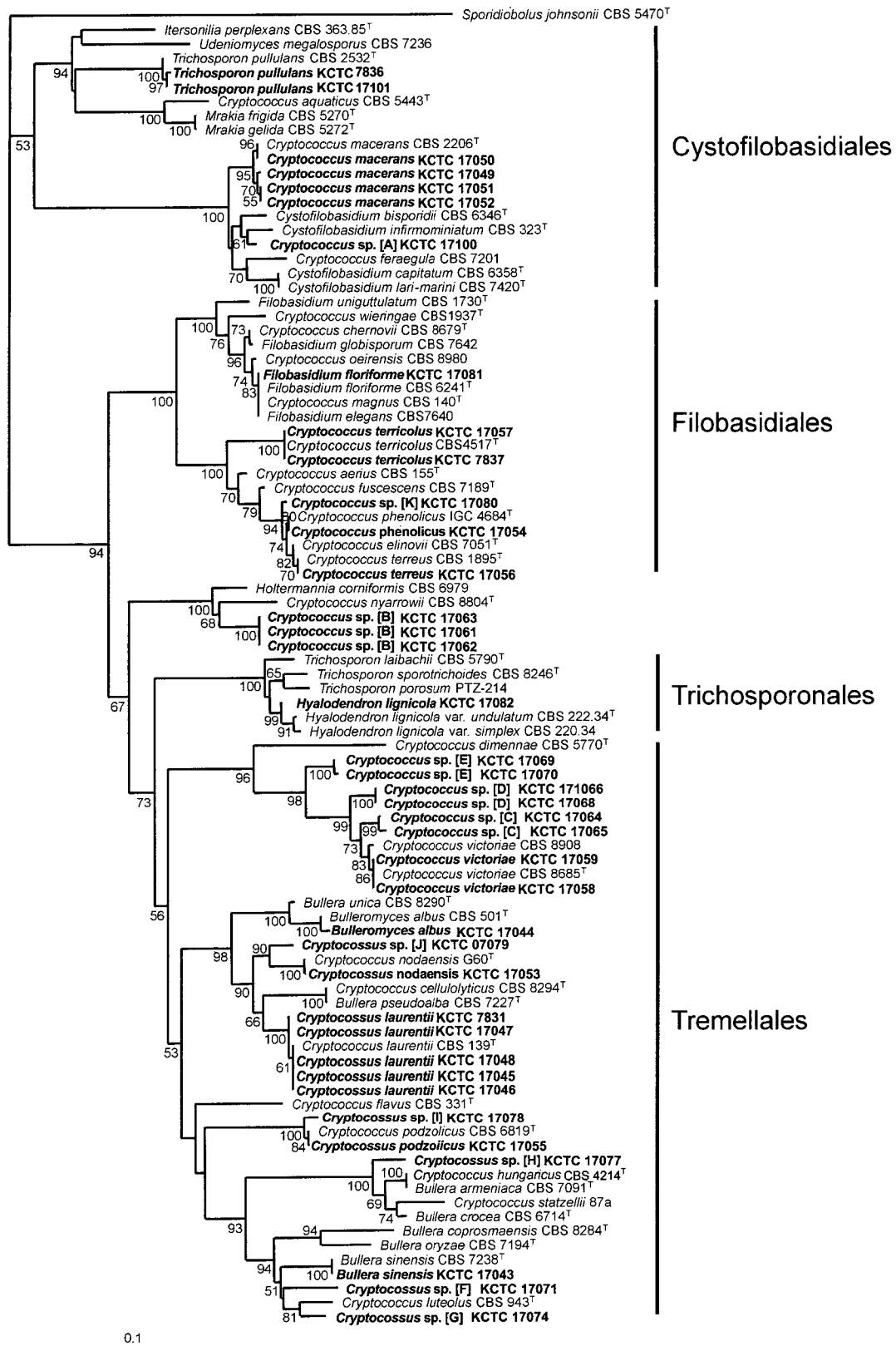


Fig. 3. Hymenomycetous yeasts. A phylogenetic tree was reconstructed by a neighbor-joining algorithm based on the distances calculated by Kimura's two-parameter model from the sequences of D1/D2 domain of 26S rDNA. Bootstrap values greater than 50% are shown alongside the branch considered.

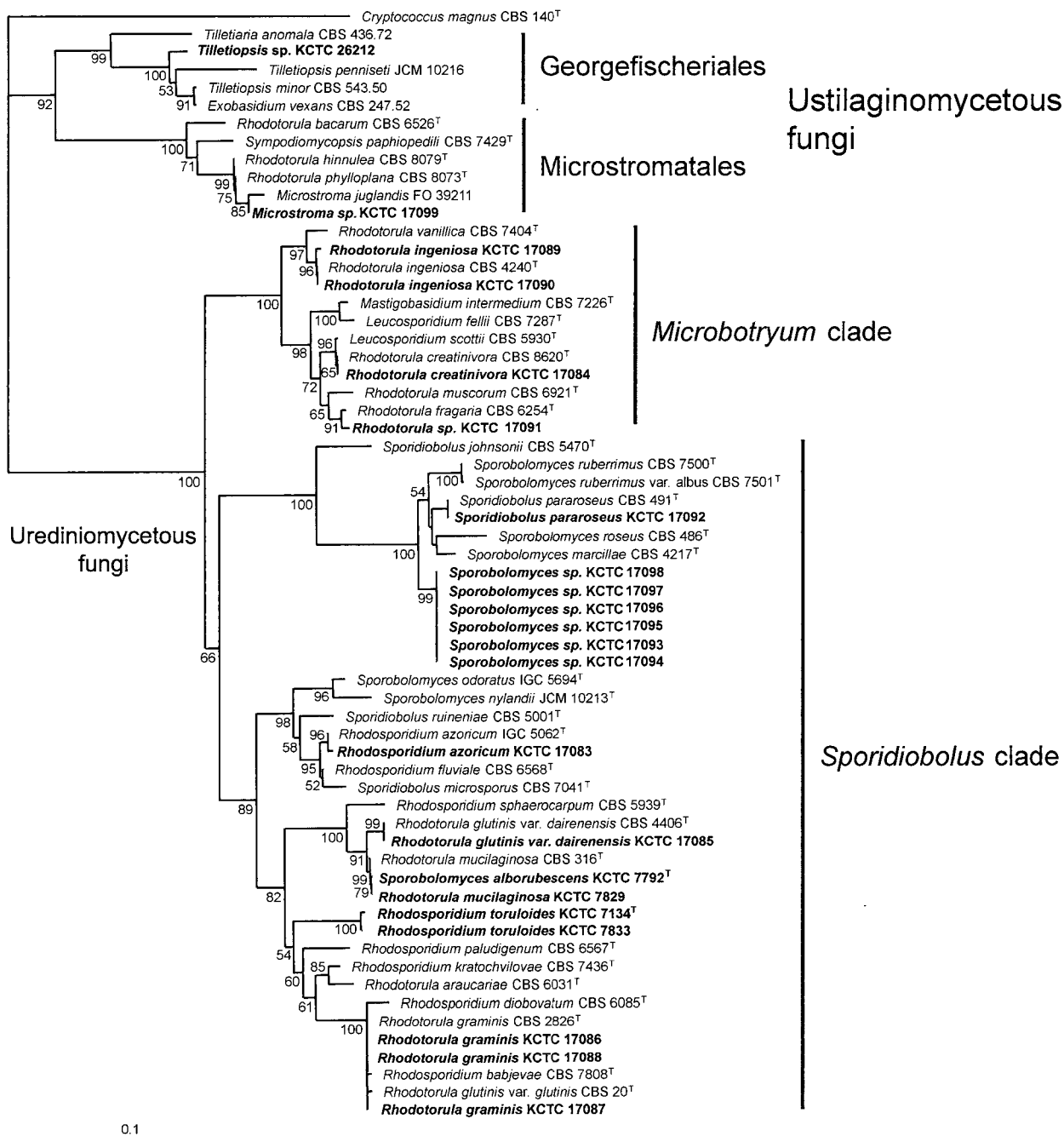


Fig. 4. Urediniomycetous yeasts, ustilaginomycetous yeasts and related fungi. A phylogenetic tree was reconstructed by a neighbor-joining algorithm based on the distances calculated by Kimura's two-parameter model from the sequences of D1/D2 domain of 26S rDNA. Bootstrap values greater than 50% are shown alongside the branch considered.

strain KCTC 17071 was isolated from an aquatic sample and strain KCTC 17074 was isolated from a flower sample. Flower-associated yeast *Cryptococcus* sp. [H] KCTC 17077 was closely related to *Bullera armeniaca* (97.71%), *Bullera crocea* (96.73%), *Cryptococcus hungaricus* (97.72%), and *Cryptococcus statzellii* (96.57%). *Cryptococcus* sp. [I] KCTC 17077 isolated from soil samples was closely related to *Cryptococcus podzolicus*

(99.32%). Soil-associated yeast *Cryptococcus* sp. [J] KCTC 17079 was closely related to *Cryptococcus nodaensis* (97.82%). *Cryptococcus* sp. [K] KCTC 17080 was closely related to *Cryptococcus elinovii* (99.50%), *Cryptococcus phenolicus* (99.50%), and *Cryptococcus treus* (99.33%).

Cryptococcus laurentii, *Cryptococcus macerans*, and *Cryptococcus* sp. [B] were each isolated from more than

two samples. In particular, *C. laurentii* was found to be a widespread species.

Urediniomycetous yeasts were recovered in the two major clades, *Microbotryum* and *Sporidiobolus* clades, among four major clades (Fell *et al.*, 2000). The major taxa was *Sporobolomyces* sp. isolated from various sources including soil, flowers, and water samples. Two ustilaginomycetous fungi were related to *Tilletiopsis minor* and *Microstroma juglandis*. They were regarded as potentially new species based on the 26S rDNA data.

There have been many studies for bacterial diversity to elucidate distribution and role in natural environments. However, diversity and ecology of yeasts in natural environments have been neglected because they have been regarded as playing a minor role in the biosphere. From this study, we can suggest that there might be geographical inequality in yeast distribution and host-specific interaction between yeasts and plants. In addition, the high ratio of new species (39% of 44 taxa) suggests that there is an urgent need for extensive studies on the biodiversity and the ecological roles of yeasts in natural environments

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