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NOTE

Diversity of Yeasts Associated with *Panax ginseng*

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Biodiversity of yeasts was investigated in the ginseng cultivation field. Among 34 isolates tested in this study, 26 isolates belonged to the hymenomycetous yeast group. These 26 strains were classified into 12 species including four new-species candidates that did not have clear affiliation to any established species. Seven isolates among the remaining strains were classified into three ascomycetous yeast species, and one isolate was identified as a urediniomycetous yeast species.

Keywords: yeast, ginseng, soil, rhizosphere

Ginseng has been used as one of the most valuable natural medicines in Asian countries for thousands of years. In the modern medical and biological studies, several pharmacological effects of ginseng have been reported, including effects on cancer, diabetes, and sexual dysfunction (Murphy and Lee, 2002; Yeh *et al.*, 2003; Yun, 2003). Based on the medicinal effects by empirical and experimental results, ginseng became one of the most popular natural herbs. Markets for ginseng and related products are estimated to be 3.5 billion dollars worldwide. However, cultivation of ginseng is very difficult because long duration of cultivation is needed to achieve good quality of products, which poses serious problems of plant diseases, including red skin and root rot diseases. Heavy application of agricultural chemicals to prevent those diseases has caused problems concerning remaining chemicals on the products because of the consumer resistance to chemical residues and concern for environmental safety. Therefore, there is an increasing demand for alternative methods to control such diseases.

Yeasts have been developed to control mould diseases on postharvest fruit, vegetables and grains (Kurtzman and Droby, 2001; Druvefors *et al.*, 2002; Fredlund *et al.*, 2002; Nunes *et al.*, 2002). As antagonistic mechanisms, cell wall hydrolysis by β-1,3-glucanases and inhibiting either pathogen growth or metabolic activity by sequestering iron were proposed (Riquelme, 1996; Masih and Paul, 2002).

In contrast to the study on surfaces of fruits, vegetables, and grains, there have been few researches on yeasts in soil environments. In this study, we investigated biodiversity of yeasts related to the soil environment of ginseng cultivation field, which is prerequisite to understand role of yeasts and to develop biocontrol agents against diseases of ginseng.

Soil samples were taken from rhizosphere (within 1 cm distance from roots of ginseng) and non-rhizosphere soils (in the same ridge but more than 20 cm apart from roots of ginseng) of 1, 3, and 5 year-old ginsengs in the ginseng cultivation fields at Gumsan Agricultural Development and Technology Center located at Naeburi, Kunbukmyun, Geumsan, Chungnam on 21 January 2002. Soil samples were suspended in sterile distilled water, mixed by vortexing at high speed for 1 min and allowed to settle down for 1 min. The supernatant was serially diluted and spread on acidified YM agar (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose, 2% agar, pH 3.7 adjusted with HCl after autoclave). The plates were incubated at 20°C for 3 days. Single colonies were transferred to new YM agar plates and pure colonies were stored at −70°C in 10% glycerol.

Yeast cells were broken by shaking with glass beads

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Table 1. Yeast species isolated from ginseng fields

Species name	Strain no. (GenBank No.)	nt differences (GenBank No.)*	Habitat
Ascomycetous yeasts			
Candida vartiovaarae (Capriotti) van Uden and Buckley *ex* Meyer and Ahearn	SG02-033 (EF068202)	0 (U69875)	3 years, non-rhizosphere
Schizoblastosporion starkeyi-henricii Ciferri	SG02-007 (EF068185)	0 (U40089)	1 year, rhizosphere
	SG02-046 (EF068211)	0 (U40089)	5 years, non-rhizosphere
Williopsis saturnus (Kloecker) Zender	SG02-008 (EF068186)	0 (U75958)	1 year, rhizosphere
	SG02-010 (EF068188)	0 (U75958)	1 year, non-rhizosphere
	SG02-026 (EF068197)	1 (U75958)	3 years, rhizosphere
	SG02-034 (EF068203)	0 (U75958)	3 years, non-rhizosphere
Hymenomycetous yeasts			
Cryptococcus flavescens (Saito) Skinner	SG02-048 (EF068213)	4 (AB035042)	5 years, rhizosphere, red skin disease
Cryptococcus gastricus Reidesöl and di Menna	SG02-040 (EF068206)	0 (AF137600)	3 years, non-rhizosphere
Cryptococcus laurentii (Kufferath) Skinner	SG02-009 (EF068187)	2 (AF075469)	1 year, rhizosphere
	SG02-017 (EF068191)	2 (AF075469)	1 year, non-rhizosphere
	SG02-021 (EF068194)	1 (AF075469)	3 years, rhizosphere
	SG02-038 (EF068205)	1 (AF075469)	3 years, non-rhizosphere
	SG02-041 (EF068207)	1 (AF075469)	5 years, non-rhizosphere
	SG02-049 (EF068214)	1 (AF075469)	5 years, rhizosphere, red skin disease
Cryptococcus podzolicus (Bab'eva and Reshetova) Golubev	SG02-025 (EF068196)	3 (AF075481)	3 years, rhizosphere
Cryptococcus terreus di Menna	SG02-043 (EF068209)	0 (AF075479)	5 years, non-rhizosphere
Cryptococcus terricolus Pedersen	SG02-013 (EF068189)	0 (AF181520)	1 year, non-rhizosphere
	SG02-028 (EF068198)	0 (AF181520)	3 years, rhizosphere
	SG02-035 (EF068204)	0 (AF181520)	3 years, non-rhizosphere
Cryptococcus watticus Guffogg *et al.*	SG02-030 (EF068199)	2 (AY138478)	3 years, rhizosphere
Cryptococcus sp. [I]	SG02-032 (EF068201)	5 (*C. podzolicus*: AF075481)	3 years, non-rhizosphere
	SG02-044 (EF068210)	5 (*C. podzolicus*: AF075481)	5 years, non-rhizosphere
	SG02-006 (EF068184)	6 (*C. podzolicus*: AF075481)	1 year, rhizosphere
	SG02-047 (EF068212)	6 (*C. podzolicus*: AF075481)	5 years, non-rhizosphere
Cryptococcus sp. [K]	SG02-020 (EF068193)	3 (*C. phenolicus*: AF181523)	1 year, non-rhizosphere
	SG02-051 (EF068216)	3 (*C. phenolicus*: AF181523)	5 years, rhizosphere, red skin disease
Cryptococcus sp. [L]	SG02-001 (EF068183)	7 (*C. saitoi*: AF181540)	1 year, rhizosphere
	SG02-015 (EF068190)	7 (*C. saitoi*: AF181540)	1 year, non-rhizosphere
	SG02-024 (EF068195)	7 (*C. saitoi*: AF181540)	3 years, rhizosphere
	SG02-031 (EF068200)	7 (*C. saitoi*: AF181540)	3 years, non-rhizosphere
Cryptococcus sp. [M]	SG02-042 (EF068208)	3 (*C. terreus*: AF075479)	5 years, non-rhizosphere
Trichosporon pullulans (Lindner) Diddens and Lodder	SG02-019 (EF068192)	1 (AF105394)	1 year, non-rhizosphere
Urediniomycetous yeasts			
Rhodotorula slooffiae (Saito) Harrison	SG02-50 (EF068215)	2 (AF189965)	5 years, rhizosphere, red skin disease

*Nucleotide difference to the type strain of the species when it was identified or to the closest relative presented in the parenthesis when it was not identified. Accession numbers of reference sequences were presented in the parentheses.

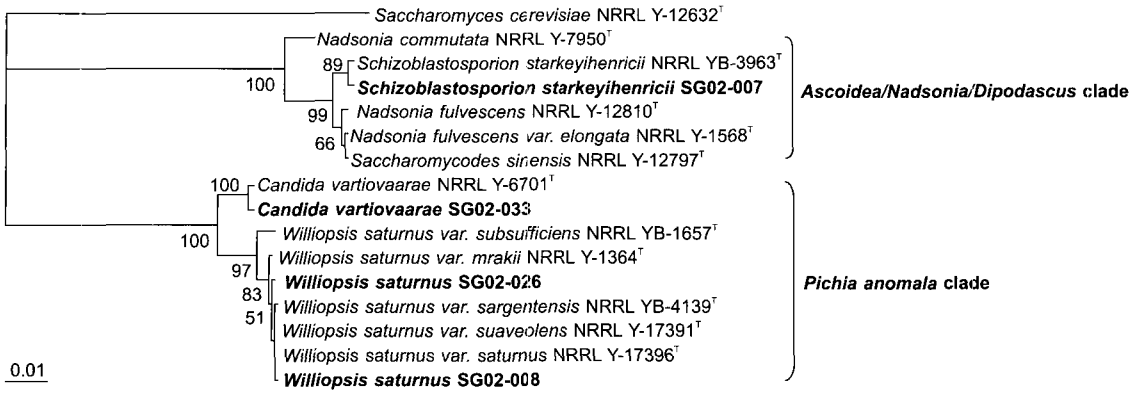

Fig. 1. Ascomycetous yeasts. A phylogenetic tree was reconstructed by neighbor-joining algorithm based on the distances calculated by Kimura's two-parameter model from the sequences of D1/D2 domain of 26S rDNA. Bootstrap values greater than 50% are presented alongside the branch.

on the TOMY micro tube mixer (TOMY, Japan). Genomic DNA was extracted using a Genomic DNA Isolation Kit (Nucleogen, Korea) according to the supplier's guide. The D1/D2 domain (ca. 600-nucleotides) of 26S rDNA was amplified using forward primer (5'-ACCCG CTGAA YTTAA GCAT AT-3') and reverse primer (5'-CTCCT TGGTC CGTGT TTCAA GACGG-3') (Van der Auwera *et al.*, 1994), and purified using a Wizard PCR prep kit (Promega, USA). The nucleotide sequences were determined with BigDye terminator cycle sequencing kits (Applied Biosystems, USA) 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, USA). 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).

The 26S rDNA sequences of isolates were aligned with those of closely related taxa based on the secondary structural 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 trees were generated from the 1,000 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. Labeling of unidentified new species candidates followed a previous study (Hong *et al.*, 2002).

Details of 34 yeast isolates from the ginseng cultivation field are presented in Table 1. Information for yeast isolates with the same 26S rDNA sequences from identical samples was not presented in the table. The list includes 3 ascomycetous, 12 hymenomycetous, and 1 urediniomycetous yeast species.

Candida vartiovaarae and *Williopsis saturnus* are related to the *Pichia anomala* clade circumscribed by Kurtzman and Robnett (1998), and *Schizoblastosporion starkeyihenricii* is related to the *Ascoidea/Nadsonia/Dipodascus* clade (Fig. 1). *Candida vartiovaarae* is an anamorphic species classified in the heterogeneous and polymorphic ascomycetous genus *Candida* and has been isolated from soil, water, or cider in Finland, USA and UK (Meyer *et al.*, 1998). The strain isolated from non-rhizosphere soil of 3 year-old ginseng field had identical sequence with the type strain NRRL Y-6701. *Williopsis saturnus* is a teleomorphic ascomycetous yeast species and has five varieties. Discrimination among varieties is based on the fermentation or assimilation of carbon or nitrogen sources and requirement for vitamins (Kurtzman, 1998). Sequence differences in the D1/D2 domain of 26S rDNA are 0-1 base pair among varieties *mrakii, saturnus, sargentensis*, and *suaveolens,* and 4-5 base pairs between variety *subsufficiens* and the other four varieties. In this study, four strains isolated from rhizosphere and non-rhizosphere soils of 1 year and 3 year-old ginseng fields showed 0-1 nucleotide difference from *W. saturnus* var. *saturnus*. Without physiological data, variety was not determined. *Williopsis saturnus* has been isolated from soil, water, or plant materials in Africa, Asia, Australia, Europe, and North American continents (Kurtzman, 1998; Hong *et al.*, 2002). *Schizoblastosporion starkeyihenricii* is the only species of the anamorphic genus *Schizoblastosporion* and has been isolated from various soil samples of New Zealand, Norway, and Germany (Smith, 1998). In this study, *S. starkeyihenricii* was isolated from rhizosphere soil of 1 year old ginseng and non-rhizosphere

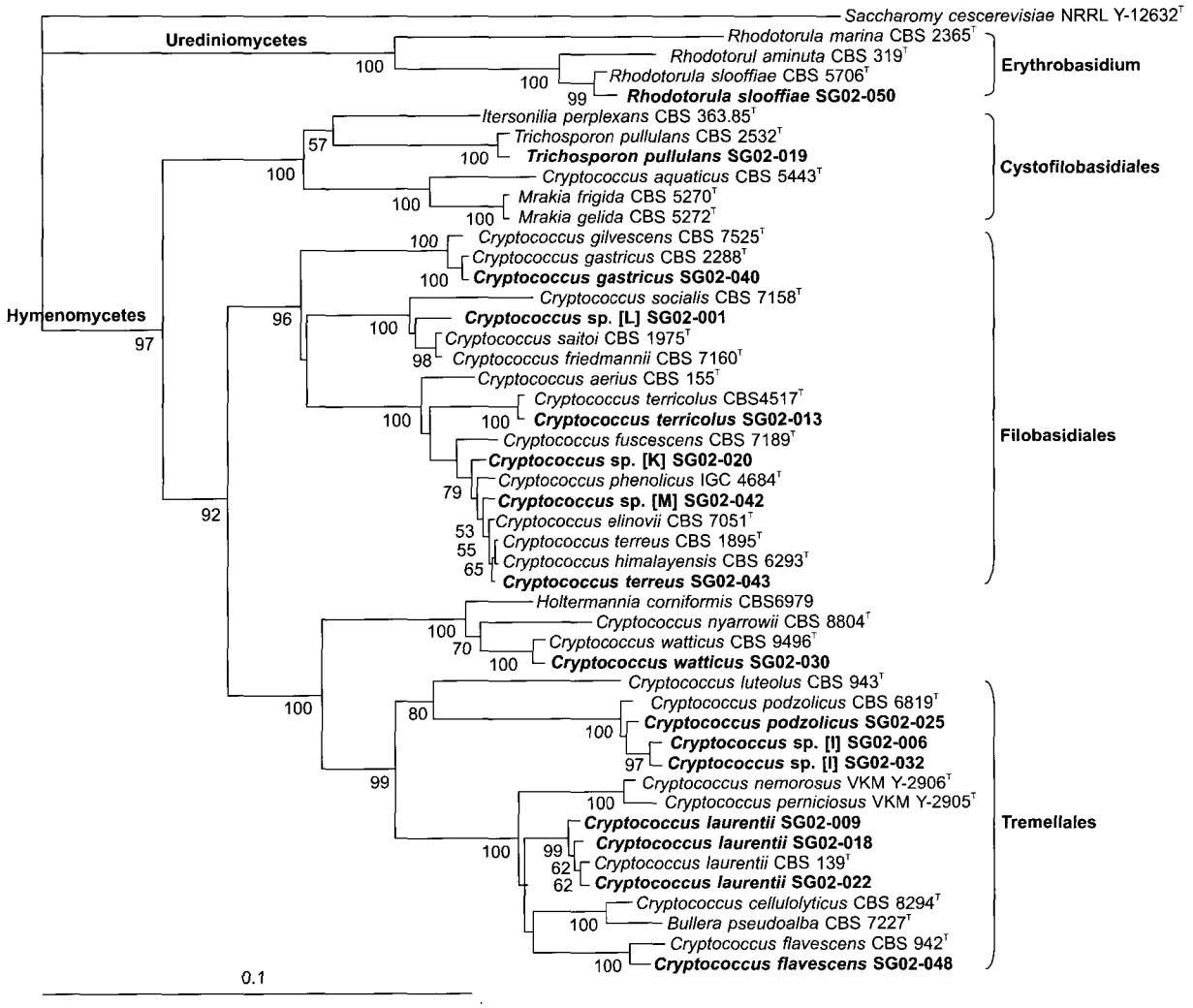

Fig. 2. Basidiomycetous yeasts. A phylogenetic tree was reconstructed by neighbor-joining algorithm based on the distances calculated by Kimura's two-parameter model from the sequences of D1/D2 domain of 26S rDNA. Bootstrap values greater than 50% are presented alongside the branch.

soil of 5 year-old ginseng.

Eleven hymenomycetous yeast species including four new species candidates were related to Cystofilobasidiales, Filobasidiales, or Tremellales. One hymenomycetous yeast species, *Cryptococcus watticus*, was related to the group of uncertain affiliation including *Holtermannia corniformis* and *Cryptococcus nyarrowii* (Fig. 2).

Trichosporon pullulans was included in the Cystofilobasidiales, and has been isolated from air, beverages, plant materials, soil, and clinical materials (Guého *et al.*, 1998; Hong *et al.*, 2002). In this study, *T. pullulans* was isolated from non-rhizosphere of 1 year old ginseng.

Cryptococcus laurentii, Cryptococcus flavescens, Cryptococcus podzolicus, and *Cryptococcus* sp. [I] were included in the Tremellales. *Cryptococcus laurentii* has been isolated from air, sea water, soil, plant material, clinical materials, and insects (Fell and Statzel-Tallman, 1998a; Hong *et al.*, 2002). In this study, *C. laurentii* was isolated from rhizosphere and non-rhizosphere soil samples of 1, 3, and 5 year-old gingeng, including ginseng with red skin disease. *Cryptococcus flavescens*, which has been recognized as *Torula flavescens* (Saito, 1922), *Cryptococcus laurentii* var. *flavescens* (Lodder and Kreger-van Rij, 1952), or *Cryptococcus nodaensis* (Sato *et al.*, 1999) has been isolated from air, plant materials, or clinical materials (Takashima *et al.*, 2003). In this study, *C. flavescens* was isolated from rhizosphere soil of 5 year-old ginseng with red skin disease. Interestingly, two closely related species *C. laurentii* and *C. flavescens* were isolated from ginseng cultivation field in Yeongju, too (Hong *et al.*, 2002). *Cryptococcus podzolicus* is a species isolated from forest soil samples in Siberia

(Bab'eva and Reshetova, 1975). In Korea, it was found from rhizosphere soil of Chinese balloon flower (*Platycodon grandiflorum*) (Hong *et al.*, 2002). In this study, it was isolated from rhizosphere soil of 3 year-old ginseng.

Cryptococcus watticus is a species described with two strains isolated from soil and shell samples in Antarctica (Guffogg *et al.*, 2004). This species was reported as *Cryptococcus* sp. [B] from rhizosphere soil of Chinese balloon flowers and apple tree (*Malus pumila* var. *dulcissima*) in the previous study (Hong *et al.*, 2002). In this study, *C. watticus* was isolated from rhizosphere soil of 3 year-old ginseng. *Cryptococcus* sp. [I], which has been reported from soil sample in Wando (Hong *et al.*, 2002), was isolated from rhizosphere soil of 1 year old and non-rhizosphere soil of 3 and 5 year-old ginseng. Sequence of 26S rDNA D1/D2 domain of this species showed 5 or 6 nucleotide differences from that of the most closely related species, *Cryptococcus podzolicus*.

Cryptococcus gastricus, Cryptococcus terreus, Cryptococcus terricolus, Cryptococcus sp. [K], *Cryptococcus* sp. [L], and *Cryptococcus* sp. [M] were included in the Filobasidiales. The type strain of *C. gastricus* was isolated from clinical material and several strains have been isolated from soil in New Zealand (Fell and Statzell-Tallman, 1998a). In this study, *C. gastricus* was isolated from non-rhizosphere soil of 3 year-old ginseng. *Cryptococcus terricolus* has long been regarded as a synonym of *Cryptococcus albidus*. However, based on the sequence analysis and inability to assimilate ribitol as carbon source and L-lysine as nitrogen source, it was reinstated as a separate species (Fonseca *et al.*, 2000). In this study, *C. terricolus* was isolated from non-rhizosphere soil of 1 year old ginseng and rhizosphere and non-rhizosphere soil of 3 year-old ginseng. *Cryptococcus himalayensis* and *C. elinovii* have been considered as synonyms of *C. terreus* based on the similarity of sequence information and physiological characteristics (Fell and Statzell-Tallman, 1998a; Fonseca *et al.*, 2000). They have no or 1 nucleotide difference in D1/D2 domain of 26S rDNA from *C. terreus*. *Cryptococcus phenolicus* has 3 nucleotide differences in D1/D2 domain of 26S rDNA from *C. terreus* and was raised as a new species based on the carbon assimilation difference (Fonseca *et al.*, 2000). *Cryptococcus sp.* [K] has 3 and 4 nucleotide differences from *C. phenolicus* and *C. terreus*, respectively. *Cryptococcus sp.* [M] has 3, 3, and 4 nucleotide differences from *Cryptococcus sp.* [K], *C. terreus*, and *C. phenolicus*, respectively. Without physiological data, they were left unidentified and could be separate species considering the relationship between *C. phenolicus* and *C. terreus*. The lineage comprising *Cryptococcus aerius*, *C. terricolus*, *C. fuscescens*, *C. phenolicus*, and *C. terreus* is composed of species usually isolated from soil samples. The most closely related species of *Cryptococcus* sp. [L] was *Cryptococcus saitoi* and showed 7 nucleotide differences in the D1/D2 domain of 26S rDNA. This species was isolated from rhizosphere and non-rhizosphere soil of 1 and 3 year-old ginseng. This is the first report for this species.

Rhodotorula slooffiae is a urediniomycetous yeast and related to the order Erythrobasidium. *Rhodotorula slooffiae* has been considered as a synonym of *Rhodotorula minuta* (Fell and Statzell-Tallman, 1998b), but later it was reinstated as a separate species based on the sequence data (Fell *et al.*, 2000). When substrate information is available, all of the strains deposited in CBS culture collection as *R. slooffiae* were isolated from clinical materials. In this study, this species was isolated from rhizosphere soil of 5 year-old ginseng with red skin disease. The strain identified as *R. slooffiae* in this study showed 2 nucleotide differences from the type strain in the D1/D2 domain of 26S rDNA. At this point, it is not clear if this difference implies adaptation of the stain to specific environment with distinct physiological and ecological state.

Among 34 strains considered in this study, 7 strains were ascomycetous yeasts, 26 strains were hymenomycetous yeasts, and 1 strain was urediniomycetous yeast. This result is consistent with the previous study, in which it was reported that soil environments were dominated by hymenomycetous yeasts (Hong *et al.*, 2002). Most of the species reported in this study have been isolated from soil and/or plant materials with the exception of *Cryptococcus* sp. [K], *Cryptococcus* sp. [M], and *R. slooffiae*. Two *Cryptococcus* species are newly reported in this study and future studies are needed to reveal if they are general inhabitants of broad substrate or specifically adapted to ginseng cultivation field.

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References

Bab'eva, I.P. and I.S. Reshetova. 1975. A new yeast species *Candida podzolica* sp. n. from the soil. *Microbiology* (trasl. of *Mikrobiologiya*) 44, 294-333.

Chun, J. 1995. Computer-assisted classification and identification of actinomycetes. Ph. D. Thesis. University of Newcastle.

Druvefors, U., N. Jonsson, M.E. Boysen, and J. Schnurer. 2002. Efficacy of the biocontrol yeast *Pichia anomala*

during long-term storage of moist feed grain under different oxygen and carbon dioxide regimens. *FEMS Yeast Res.* 3, 389-394.

Fell, J.W., T. Boekhout, A. Fonseca, G. Scorzetti, and A. Statzell-Tallman. 2000. Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. *Int. J. Syst. Evol. Microbiol.* 50, 1351-1371.

Fell, J.W. and A. Statzell-Tallman. 1998a. *Cryptococcus* Vuillemin. p. 742-767. *In* Kurtzman, C.P. and J.W. Fell (eds.) The yeasts, a taxonomic study. 4th ed. Elsevier, Amsterdam.

Fell, J.W. and A. Statzell-Tallman. 1998b. *Rhodotorula* F.C. Harrison. p. 800-827. *In* Kurtzman, C.P. and J.W. Fell (eds.) The yeasts, a taxonomic study. 4th ed. Elsevier, Amsterdam.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783-791.

Felsenstein, J. 1995. PHYLIP (Phylogeny Inference Package) Version 3.57c, University of Washington.

Fonseca, A., G. Scorzetti, and J.W. Fell. 2000. Diversity in the yeast *Cryptococcus albidus* and related species as revealed by ribosomal DNA sequence analysis. *Can. J. Microbiol.* 46, 7-27.

Fredlund, E., U. Druvefors, M.E. Boysen, K.J. Lingsten, and J. Schnurer. 2002. Physiological characteristics of the biocontrol yeast *Pichia anomala* J121. *FEMS Yeast Res* 2, 395-402.

Guého, E., M.T. Smith, and G.S. de Hoog. 1998. *Trichosporon* Behrend. p.854-872. *In* Kurtzman, C.P. and J.W. Fell (eds.) The yeasts, a taxonomic study. 4th ed. Elsevier, Amsterdam.

Guffogg, S.P., S. Thomas-Hall, P. Holloway, and K. Watson. 2004. A novel psychrotolerant member of the hymenomycetous yeasts from Antarctica: *Cryptococcus watticus* sp. nov. *Int. J. Syst. Evol. Microbiol.* 54, 275-277.

Hong, S.G., K.H. Lee, and K.S. Bae. 2002. Diversity of yeasts associated with natural environments in Korea. *J. Microbiol.* 40, 55-62.

Kimura, M. 1980. A simple mehtod for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16, 111-120.

Kurtzman, C.P. 1998. *Williopsis* Zender. p. 413-419. *In* Kurtzman, C.P. and J.W. Fell (eds.) The yeasts, a taxonomic study. 4th ed. Elsevier, Amsterdam.

Kurtzman, C.P. and C.J. Robnett. 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie Van Leeuwenhoek* 73, 331-371.

Kurtzman, C.P. and S. Droby. 2001. *Metschnimowia fructicola*, a new ascosporic yeast with potential for biocontrol of postharvest fruit rots. *Syst. Appl. Microbiol.* 24, 395-399.

Lodder, J. and N.J.W. Kreger-van Rij. 1952. The yeasts, a taxonomic study. Elsevier, Amsterdam.

Masih, E.I. and B. Paul. 2002. Secretion of beta-1,3-glucanases by the yeast *Pichia membranifaciens* and its possible role in the biocontrol of *Botrytis cinerea* causing grey mold disease of the grapevine. *Curr. Microbiol.* 44, 391-395.

Meyer, S.A., R.W. Payne, and D. Yarrow. 1998. *Candida* Berkhout. p. 454-573. *In* Kurtzman, C.P. and J.W. Fell (eds.) The yeasts, a taxonomic study. 4th ed. Elsevier, Amsterdam.

Murphy, L.L. and T.J. Lee. 2002. Ginseng, sex behavior, and nitric oxide. *Ann. NY Acad. Sci.* 962, 372-377.

Nunes, C. J. Usall, N. Teixido, and I. Vinas. 2002. Improvement of *Candida sake* biocontrol activity against post-harvest decay by the addition of ammonium molybdate. *J. Appl. Microbiol.* 92, 927-935.

Page, R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. *Comp. Appl. Biosci.* 12, 357-358.

Riquelme, M. 1996. Fungal siderophores in plant-microbe interactions. *Microbiologia* 12, 537-546.

Saito, K. 1922. Untersuchungen über die atmosphärischen Pilzkeime. *Mitt. Jpn. J. Bot.* 1, 1-54.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstruction phylogenetic trees. *Mol. Biol. Evol.* 4, 406-425.

Sato, I., H. Kobayashi, Y. Hanya, S. Murakami, G. Scorzetti and J.W. Fell. 1999. *Cryptococcus nodaensis* sp. nov., a yeast isolated from soil in Japan that produces a salt-tolerant and thermostable glutaminase. *J. Ind. Microbiol. Biotechnol.* 22, 127-132.

Smith, M.T. 1998. *Schizoblastosporion* Ciferri. p. 602. *In* Kurtzman, C.P. and J.W. Fell (eds.) The yeasts, a taxonomic study. 4th ed. Elsevier, Amsterdam.

Takashima, M., T. Sugita, T. Shinoda, and T. Nakase. 2003. Three new combinations from the *Cryptococcus laurentii* complex: *Cryptococcus aureus*, *Cryptococcus carnescens* and *Cryptococcus peneaus*. *Int. J. Syst. Evol. Microbiol.* 53, 1187-1194.

Van der Auwera, G., S. Chapelle, and R. De Wachter. 1994. Structure of the large ribosomal subunit RNA of *Phytophthora megasperma*, and phylogeny of the Oomycetes. *FEBS Lett.* 338, 133-136.

Yeh, G.Y., D.M. Eisenberg, T.J. Kaptchuk, and R.S. Phillips. 2003. Systematic review of herbs and dietary supplements for glycemic control in diabetes. *Diabetes Care* 26, 1277-1294.

Yun, T.K. 2003. Experimental and epidemiological evidence on non-organ specific cancer preventive effect of Korean ginseng and identification of active compounds. *Mutat. Res.* 523, 63-74.