

**S1-2**

## **Diversity of Yeasts Associated with Rhizosphere of *Panax ginseng***

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Biodiversity of yeasts were investigated in the ginseng cultivation field. Hymenomycetous yeasts were the most frequently isolated yeast group. Among 34 isolates considered in this study, 26 isolates were included in the hymenomycetous yeast group. They were classified into 12 species including 4 species which don't have clear affiliation to the established species. Seven isolates were classified into three ascomycetous yeasts and one isolate was identified as a urediniomycetous yeast. Most of the species reported in this study have been isolated from soil and/or plant materials, but two new species candidates of the genus *Cryptococcus* and *Rhodotorula slooffiae* were newly reported from soil samples in this study.

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. Based on the medicinal effects by empirical and experimental results, ginseng became one of the most popular natural herbs. 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. Therefore, there is an increasing demand for alternative methods to control disease.

Yeasts have been developed to control mould diseases on post-harvest fruit, vegetables and grains. As antagonistic mechanisms, cell wall hydrolysis by  $\beta$ -1,3-glucanases and inhibiting either pathogen growth or metabolic activity by sequestering iron were proposed. 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 in soil environment of ginseng cultivation field and to develop biocontrol agents against diseases of ginseng.

Details of 34 yeast isolates among 51 strains isolated from the ginseng cultivation field are presented. 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. *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. 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. Sequence differences in the D1/D2 domain of 26S rDNA are 0-1 base pairs 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 and 3 year old ginseng fields showed 0-1 nucleotide differences from *W. saturnus* var. *saturnus*.

*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 soil of 5 year old ginseng.

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

*Trichosporon pullulans* was included in the Cystofilobasidiales and has been isolated from air, beverages, plant materials, soil, and clinical materials. 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. In this study, *C. laurentii* was isolated from rhizosphere and non-rhizosphere soil samples of 1, 3, and 5 year old ginseng, including ginseng with red skin disease.

*Cryptococcus flavescens*, which has been recognized as *Torula flavescens*, *Cryptococcus laurentii* var. *flavescens*, or *Cryptococcus nodaensis* has been isolated from air, plant materials, or clinical materials. In this study, *C. flavescens* was isolated from rhizosphere soil of 5 year old ginseng with red skin disease. *Cryptococcus podzolicus* is a species isolated from forest soil samples in Siberia. In this study, it was isolated from rhizosphere soil of 3 year old ginseng.

*Cryptococcus waticus* is a species described with two strains isolated from soil and shell samples in Antarctica. In this study, *C. waticus* was isolated from rhizosphere soil of 3 year old ginseng. *Cryptococcus* sp. [I] 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. In this study, *C. gastricus* was isolated from non-rhizosphere soil of 3 year old ginseng. *Cryptococcus terricolus* has long been regarded as 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 separate species. 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. They have no or 1 nucleotide differences in D1/D2 domain of 26S rDNA from *C. terreus*. *Cryptococcus phenolicus* has 3 nucleotides difference in D1/D2 domain of 26S rDNA from *C. terreus* and was raised as a new species based on the carbon assimilation difference. *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 satoi* and showed 7 nucleotides difference 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 an urediniomycetous yeast and related to the order Erythrobasidium. *Rhodotorula slooffiae* has been considered as synonym of *Rhodotorula minuta*, but later it was reinstated as separate species based on the sequence data. 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 nucleotides difference 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. 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.

## References

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