26S rDNA D1/D2 領域配列に着目した酵母 Trichosporonoides megachiliensis SN G-42 株の系統解析

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報　文

Phylogenetic analysis of the yeast *Trichosporronoides megachiliensis* SN G−42 by sequencing the large subunit (26S) D1/D2 rDNA regions

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Abstract

The yeast strain SN G−42 classified in *Trichosporonoides megachiliensis* is one of the strains used industrially in the industrial production of erythritol. Analysis of the D1/D2 region of the 26S rRNA gene of the *Trichosporonoides megachiliensis* SN G−42 revealed that this strain possesses two different nucleotides in the region in comparison with the type species of the genus *Trichosporonoides* and that polyol−producing species classified into *Trichosporonoides* and *Moniliella* form a new lineage in the Ustilaginomycetidae clade of the basidiomycetous yeasts. Analysis of the D1/D2 regions of the 26S rDNA may thus provide a feasible criterion for searching for industrially useful yeasts.

Introduction

Strain SN G−42 classified in *Trichosporonoides megachiliensis* is one of the strains which have been used in the industrial production of erythritol strain SN G−42 is a mutant deribed from strain SN A−42[1)], which was originally isolated from the soil of Kyushu in Japan and formerly identified as *Aureobasidium* sp[2)]. Later, strain SN G−42 was reclassified into *T. megachiliensis*, which had been described as a new species by Inglis[3)], on the basis of the morphological (forming true hypa; multipolar budding; color turns yellowish−cream to black with lapse of time) and physiological (fermenting glucose, sucrose and maltose; assimilating glucose, sucrose, maltose, ribose, glycerol and erythritol) characteristics (Kasumi et al., unpublished data). Howeber, the exact phylogenetic lineage of SN G−42 has not been established.

Partial sequences of the D1/D2 region of the 26S rRNA gene have been used to generate phylogenetic databases for basidiomycetous yeasts[4) 5)]. In the present study, we analyzed the D1/D2 domains of the *Trichosporonoides megachiliensis* SN G−42 and compared the sequence with thosse of related polyol−producting yeasts.

Materials and Methods

Cultures were obtained from National Food Research Institute. DNA was extracted from *T. megachiliensis* SN G−42 with the standard protocol[6)]. The

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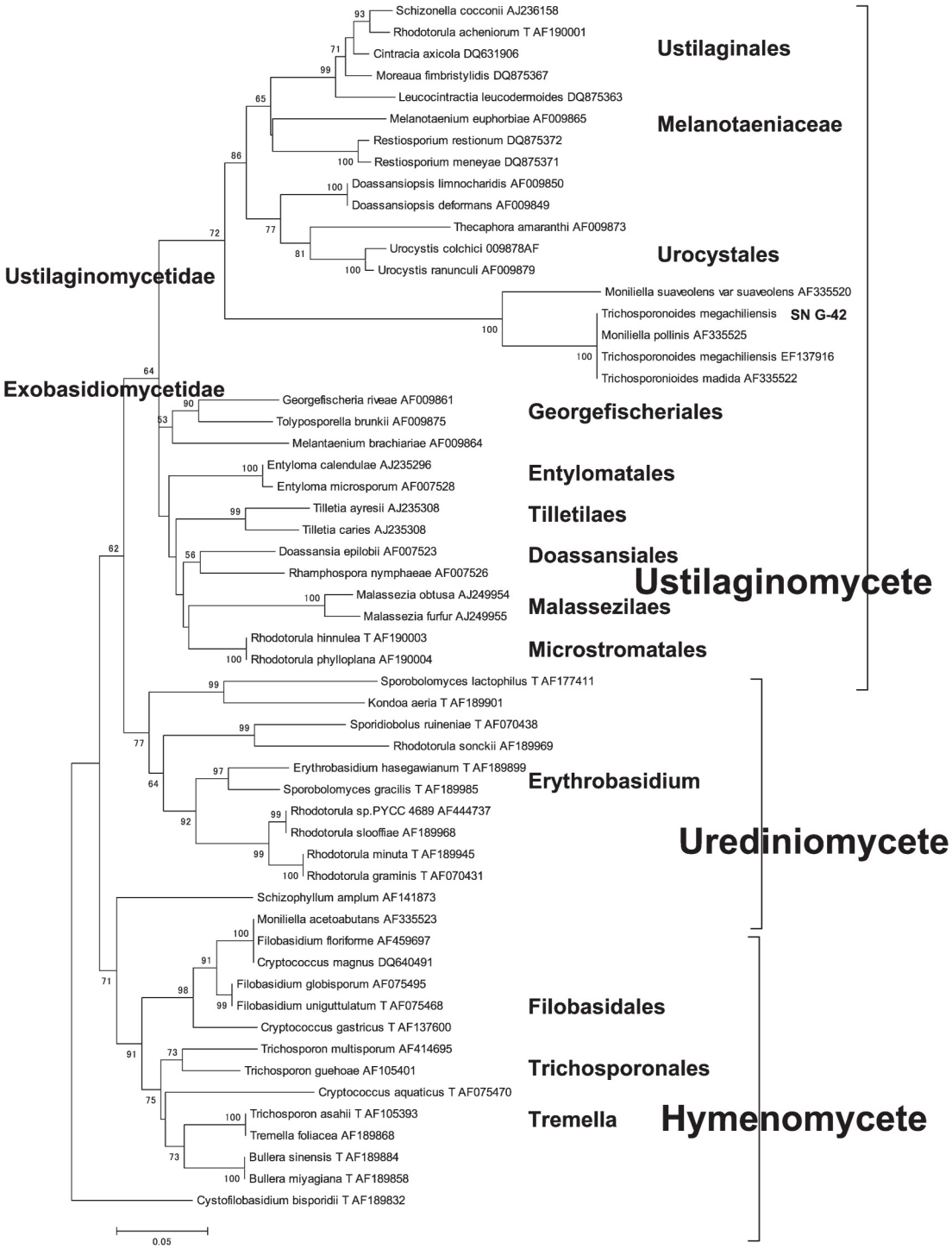

Figure 1 Neighbor-joining analysis on the basis of the D1/D2 rDNA region sequence data of the genera *Trichosporonoides* and *Moniliella*. Numbers on the branches represent bootstrap values (10,000 replicates). Values of ＜50 % are were not reported. Bars represents 0.02 substitutions per site.

D1/D2 domain of 26S rDNA from the strain SN G-42 was amplified with primers NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL-4 (5'-GGTCCGTGTTTAAGACGG-3')[7]. Amplification was performed for 35 PCR cycles, annealing at 56℃ for 1 min, extension at 72℃ for 2 min, and penetration at 94℃ for 1 min. Both strands of the rDNA regions were sequenced with the Big-Dye terminator cycle sequencing kit (Applied Biosystems Japan, Chiba). DNA sequence data were analyzed with MEGA 3.1 software[8]. Sequences were aligned with CLUSTAL W[9]. A phylogenetic tree was constructed by the neighbor-joining method in the MEGA 3.1[10]. Bootstrap values were obtained from 10,000 replicates seeding 10,000. The DNA sequences of the D1/D2 regions (550 ～ 580 bp-length) of the basidiomycetous yeasts were used for the analysis. The sequence of *Cystofilobasidium bisporidii* (GenBank number; AF189832, nucletotide number 1-579) was used as an outgroup.

Results and Discussion

The nucleotide sequence of the D1/D2 regions of *Trichosporonoides megachiliensis* SN G-42 (626 bp) differed from that of the type strain *Trichosporonoides megachiliensis* CBS 190.92 at two nucleotide positions 22 and 617. Since, Fell *et al*. reported that strains that differ by two or more nucleotides represent different taxa[4], our results suggested that the strain SN G-42 was different from *Trichosporonoieds megachiliensis* in the framework of the 26S rDNA analysis.

The sequence of strain SN G-42 was compared with known sequences using the BLAST (Basic Local Alignment Search Tool) search algorithm[11]; it showed high homology with *Trichosporonoides madida* (Expected values; 0.0), *Monilliela polinis* (0.0) and *Moniliella suaveolans var suaveolans* (1e-140), and significant homology with *Restiosporium restionum* (3e-61), *Cintracia axicola* (3e-61), *Leucocintracia leucodemmoides* (3e-61) and *Moreaua fimbrristylidis* (3e-61). The basidiomycetous yeasts are classified within the three classes: Ustilaginomycetes, Urediniomycetes and Hymenomycetes[4), 5)].

Figure 1 shows that the genera of *Trichosporonoides, Moniliella pollinis* and *Moniliella suaveolans var suaveolans* formed a new lineage in the Ustilaginomycetidae clade of the Ustilaginomycete. Interestingly, these species have been used for erythritol and polyol production at an industrial level. *M. acetoabutans*, which has not been used for commercial production of polyol, was placed in the Filobasidiales lineage of the Hymenomycete in the D1/D2 region-based tree. This result was consistent with the speculation by de Hoog, in which *Moniliella* were surmised to be closely associated with Hymenomycetous yeasts[12]. These results may, in turn, be taken as an indication that D1/D2 region analysis data can be utilized as a new criterion in searching for useful erythritol-producing yeasts.

In summary, phylogenetic analysis of *Trichosporonoides megachiliensis* SN G-42, an erythritol-producing yeast, revealed that strain SN G-42 should be classified in the Ustilaginomycetidae clade of the basidiomycetous yeasts and formed a new lineage in the Ustilaginomycetidae clade of the Ustilaginomycete with other members of the genera; *Trichosporonoides megachiliensis CBS* 190.92, *Trichosporonoides madida, Moniliella pollinis* and *Moniliella suaveolans var suaveolans*.

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要　旨

Trichosporonoides megachiliensis SN G-42 株は工業的にエリスリトールを生産している酵母菌株の一つである．この菌の 26S rDNA の D1/D2 領域を解析した結果，基準株である *Trichosporoonides megachiliensis* CBS190.92 との間で塩基配列が 2 塩基異なっていること，及びポリオールを発酵する *Trichosporonoides* 属や *Moniliella* 属の菌株は，担子菌門 Ustilaginomycetidae 目内で新たなグループを形成することがわかった．従って，26S rDNA D1/D2 領域の解析は，産業的に有用な酵母を探索するうえで，有効な手法となりえるかもしれない．