食総研報（Rep. Nat'l. Food Res. Inst）No . 72, 73−76（2008）［報　文］

報　文

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

Tetsuya Ookura[$] and Takafumi Kasumi[*]

National Food Research Institute, 2−1−12 Kan−nondai, Tsukuba, Ibaraki 305−8642
[*]Department of Bioscience and Bioengineering, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252−8510, Japan

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and maltose; assimilating glucose, sucrose, maltose, ribose, glycerol and erythritol) characteristics (Kasumi et al., unpublished data).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

2007 年 11 月 13 日受付，2008 年 1 月 7 日受理

Address reprints requests to: Dr. Tetsuya Ookura, National Food Research Institute, 2−1−12 Kan−nondai, Tsukuba, Ibaraki 305−8642, Japan. Fax; + 81−29−838−7319, email; ookura@affrc.go.jp. The DDBJ accession number for the D1/D2 region sequence of the 26S rDNA of *T. megachiliensis* SN G−42 was AB304086.

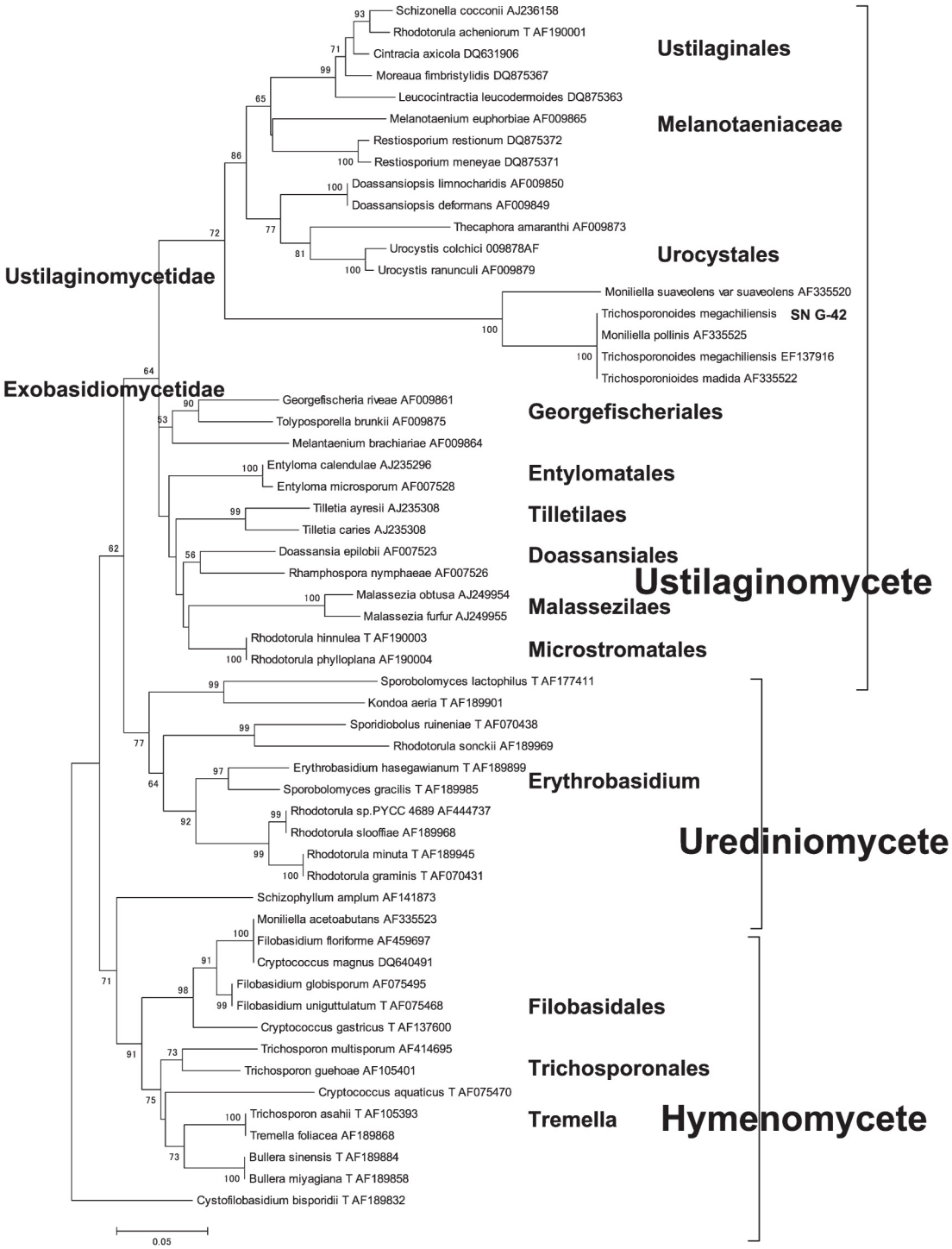

Figure 1 Neighbor-joining analysis on the basis of the D1/D2 rDNA region sequence data of the genera *Trichosporonoides* and *Moniliella*. Numbers on the branches represent bootstrap values (10,000 replicates). Values of ＜50 % are were not reported. Bars represents 0.02 substitutions per site.

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*.

Acknowledgement

We appreciated Dr. Makiko Hamamoto for her critical reading of the manuscript and Ms. Yumiko Ito for the DNA sequencing.

References

1) Ishizuka, H., Wako, H., Kasumi, T., and Sasaki, T., Breeding of a mutant of *Aureobasidium* sp. with high erythritol production. *J. Ferment. Bioeng*, **68**, 310-314, (1989)
2) Wako, K., Kawaguchi, G., Kubo, N., Kasumi, T., Hayashi, K. and Iino, K., Production of polyols by yeasts, *Hakkoukougaku*, **63**, 209-215. in Japanese, (1988)
3) Inglis, G. Douglas N. S. and Sigler, L., *Trichosporonoides megachiliensis*, a new hyphomycete as-

sociated with alfalfa leafcutter bees with notes on *Trichosporonoides* and *Monileilla*. *Mycologia*, **84**, 555–570, (1992)
4) Fell, J. W., Boekhout, T., Fonseca, A., Scorzetti, G., and Statzell-Tallman, A., 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, (2000)
5) Scorzetti, G., Fell, J. W., Fonseca, A., and Statzell-Tallman, A., Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. *FEMS Yeast Res*., **2**, 495–517, (2002)
6) Cryer, D. R., Eccleshall, R., and Marmur, J. Isolation of yeast DNA. In Methods in Cell Biology; Vol. XII; eds. Prescot, D. M., Academic Press, pp. 39–44, (1975)
7) Kurtzman, C. P., and Robnett, C. J., Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5'end of the large-subunit (26S) ribosomal DNA gene. *J. Clin. Microbiol*., **35**, 1216–1223, (1997)
8) Kumar S., Tamura K., and Nei M., MEGA3: Integrated Software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. *Brief. Bioinfor*., **5**, 150–163, (2004)
9) Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G., The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res*., **25**, 4876–4882, (1997)
10) Saitou, N., and Nei, N., A neighbor-joining method: a new method for constructing phylogenetic tree. *Mol. Biol. Evol*., **44**, 406–425, (1987)
11) Altschal, S. F., Gish, W., Miller, W., E. W. Myers, and Lipman, D. L., A basic local alignment search tool. *J. Mol. Bio*l. **215**, 403–410, (1990)
12) De Hoog, G. S., Zalar, P., van de Ende, B. G., and Gunde-Cimerman, N., Relation of Halotolerance to human-pathogenicity in the fungal tree of life: An overview of ecology and evolution under stress. In Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya. eds. Gunde-Cimerman N., Oren A., and Plemenitas, A., Springer-Verlag, pp. 371–395, (2005)

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

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．