

## 5S rRNA Sequence of *Trimorphomyces papilionaceus*

Her, Yong, Young-Won Kang, Yong-Ha Park\* and Hack Sung Jung

Department of Microbiology, College of Natural Sciences, and Research Center for Molecular Microbiology, Seoul National University, Seoul 151-742, Korea

\*Korean Collection for Type Cultures, Genetic Engineering Center, Korea Institute of Science and Technology, Daejeon 305-606, Korea

The sequence of the cytoplasmic 5S rRNA from *Trimorphomyces papilionaceus*, a basidiomycetous yeast, was determined by the direct chemical method for sequencing RNA and compared to the known 5S rRNA sequences of 19 basidiomycetous fungi. There were 26 nucleotide differences between *T. papilionaceus* and *Tremella mesenterica* both of which belong to the Tremellaceae of the Tremellales. Based on Knuc values, the closest fungus was *Tilletiaria anomala*, another basidiomycetous yeast which belongs to the Sporobolomycetales of the Sporobolomycetales. *T. papilionaceus* did not show any significant phylogenetic relationship with other fungi.

**KEY WORDS** □ *Trimorphomyces papilionaceus*, 5S rRNA sequence

Recently, the natural classification of yeasts and yeast-like fungi has been of increasing interest. Many fungi of ontogenic budding stages are now being recognized as phylogenetically diverse representatives as opposed to the currently adopted classification system of yeasts (15). When some dimorphic basidiomycetous fungi were listed by Bandoni (1) and accepted as yeast-like fungi by Kreger-van Rij (9), taxonomic implications of basidiomycetous yeasts became a topic of great concerns because many of their taxonomic or phylogenetic positions still remain to be clarified. In the viewpoint of the systematics of some basidiomycetous fungi, *Trimorphomyces papilionaceus*, a basidiomycetous yeast, was examined in this study.

*T. papilionaceus*, a monotypic species, was described under the Tremellaceae by Oberwinkler and Bandoni (11) because of its cruciately septate basidia. Like other tremellas, this fungus is mycoparasitic and develops a monokaryotic yeast, but after the conjugation of compatible cells, it is characterized by a dikaryotic yeast phase of paired conidia from which a dikaryotic hyphal phase is produced to give a fruitbody (2). The dikaryotic paired conidia are uniquely butterfly-shaped and are produced repeatedly. This kind of conjugated conidia are absent in ordinary tremellas and are readily recognizable on the basis of morphology. The taxonomic relationship of *T. papilionaceus* with tremellas remained unclear in respect of structures of its conidia and

septal pore and possible difference of its basidiocarpic development (11). To discuss its uncertain affiliation with tremellas and other basidiomycetous fungi, *T. papilionaceus* was tested on the basis of its 5S rRNA sequence and other published sequence data.

### MATERIALS AND METHODS

The strain number of *Trimorphomyces papilionaceus* used in this study was UBC No. 75-7237-d as a dikaryotic haploid yeast cultured on PDA. The organism was grown in a shaking culture of PD broth at 23°C and was harvested by centrifugation at 6000 rpm when O.D.600 arrived 10. Harvested cells were broken with glass beads and the supernatant was extracted three to five times with LETS-saturated phenol and then precipitated with ethanol. 5S rRNA was separated on a 10% polyacrylamide-8M urea gel (13) and eluted from the gel. 3'-End was labelled with [5'-<sup>32</sup>P]pCp (Amersham) and T4 RNA ligase (Pharmacia) (5) and partial digests of 3'-labelled material was sequenced by the direct chemical method and analyzed on a 12% polyacrylamide-8M urea gel (12). The primary sequence was determined and compared to the sequence data of 19 basidiomycetous fungi. The number of different bases between each two compared sequences of 20 basidiomycetous fungi was evaluated by calculating their corresponding Knuc values according to the formula of Kimura (7, 8). The secondary



Table 1. 1/2 KnuC values between each two species of 20 basidiomycetous fungi

	<i>T.p.</i>	<i>T.a.</i>	<i>U.v.</i>	<i>R.c.</i>	<i>R.h.</i>	<i>R.t.</i>	<i>E.v.</i>	<i>T.o.</i>	<i>T.c.</i>	<i>A.e.</i>	<i>C.r.</i>	<i>E.a.</i>	<i>E.g.</i>	<i>F.c.</i>	<i>P.o.</i>	<i>R.c.</i>	<i>S.c.</i>	<i>T.m.</i>	<i>D.d.</i>	<i>A.a.</i>
<i>Trimorphomyces papilionaceus</i>	—	0.128	0.285	0.263	0.246	0.259	0.168	0.163	0.144	0.160	0.157	0.157	0.158	0.151	0.157	0.169	0.151	0.131	0.153	0.158
<i>Tilletiaria anomala</i> <sup>A</sup>	0.128	—	0.257	0.195	0.167	0.232	0.111	0.096	0.103	0.104	0.102	0.102	0.115	0.108	0.097	0.095	0.114	0.113	0.108	0.097
<i>Ustilago violacea</i> *	0.285	0.257	—	0.209	0.180	0.040	0.187	0.206	0.208	0.270	0.241	0.232	0.258	0.259	0.250	0.246	0.241	0.240	0.242	0.249
<i>Rhizoctonia croconum</i> <sup>A</sup>	0.263	0.195	0.209	—	0.041	0.175	0.135	0.140	0.154	0.189	0.180	0.180	0.217	0.180	0.195	0.178	0.187	0.209	0.174	0.194
<i>Rhizoctonia hiemalis</i> <sup>A</sup>	0.246	0.167	0.180	0.041	—	0.135	0.101	0.106	0.124	0.160	0.160	0.160	0.180	0.160	0.171	0.167	0.173	0.141	0.160	0.160
<i>Rhodosporiidium toruloides</i> <sup>A</sup>	0.259	0.232	0.040	0.175	0.135	—	0.161	0.165	0.167	0.257	0.217	0.209	0.227	0.217	0.226	0.222	0.217	0.218	0.204	0.210
<i>Exobasidium vaccinii</i> <sup>A</sup>	0.168	0.111	0.187	0.135	0.101	0.161	—	0.031	0.045	0.109	0.094	0.100	0.120	0.094	0.112	0.110	0.106	0.083	0.093	0.094
<i>Trichosporon oryzae</i> <sup>A</sup>	0.163	0.096	0.206	0.140	0.106	0.165	0.031	—	0.031	0.111	0.088	0.094	0.113	0.088	0.106	0.094	0.100	0.113	0.087	0.088
<i>Tilletia controversa</i> <sup>A</sup>	0.144	0.103	0.208	0.154	0.124	0.167	0.045	0.031	—	0.112	0.084	0.078	0.085	0.096	0.078	0.088	0.073	0.095	0.072	0.073
<i>Agaricus edulis</i> <sup>A</sup>	0.160	0.104	0.270	0.189	0.160	0.257	0.109	0.111	0.112	—	0.031	0.036	0.060	0.041	0.036	0.045	0.041	0.066	0.060	0.060
<i>Coprinus radiatus</i> <sup>A</sup>	0.157	0.102	0.241	0.180	0.160	0.217	0.094	0.088	0.084	0.031	—	0.004	0.036	0.009	0.013	0.013	0.009	0.045	0.026	0.027
<i>Efibulobasidium albescens</i> <sup>A</sup>	0.157	0.102	0.232	0.180	0.160	0.209	0.100	0.094	0.078	0.036	0.004	—	0.031	0.013	0.009	0.009	0.004	0.041	0.022	0.022
<i>Exidia glandulosa</i> <sup>A</sup>	0.158	0.115	0.258	0.217	0.180	0.227	0.120	0.113	0.085	0.060	0.036	0.031	—	0.046	0.031	0.040	0.027	0.046	0.046	0.018
<i>Filobasidium capsuligenum</i> <sup>A</sup>	0.151	0.108	0.259	0.180	0.160	0.217	0.094	0.088	0.096	0.041	0.009	0.013	0.046	—	0.022	0.022	0.018	0.045	0.036	0.036
<i>Pleurotus ostreatus</i> <sup>A</sup>	0.157	0.114	0.250	0.195	0.160	0.226	0.112	0.106	0.078	0.036	0.013	0.009	0.031	0.022	—	0.017	0.004	0.041	0.031	0.031
<i>Russula cyanoxantha</i> <sup>A</sup>	0.169	0.113	0.246	0.178	0.171	0.222	0.110	0.094	0.088	0.045	0.013	0.009	0.040	0.022	0.017	—	0.013	0.050	0.031	0.031
<i>Schizophyllum commune</i> <sup>A</sup>	0.151	0.108	0.241	0.187	0.167	0.217	0.106	0.100	0.073	0.041	0.009	0.004	0.027	0.018	0.004	0.013	—	0.036	0.026	0.027
<i>Tremella mesenterica</i> <sup>A</sup>	0.131	0.098	0.240	0.209	0.173	0.218	0.083	0.113	0.095	0.066	0.045	0.041	0.046	0.045	0.041	0.050	0.036	—	0.055	0.046
<i>Dearymyces deliquescens</i> <sup>*</sup>	0.153	0.108	0.242	0.174	0.141	0.204	0.093	0.087	0.072	0.060	0.026	0.022	0.046	0.036	0.031	0.031	0.026	0.055	—	0.031
<i>Auricularia auricula-judae</i> <sup>*</sup>	0.158	0.097	0.249	0.194	0.160	0.210	0.094	0.088	0.073	0.060	0.027	0.022	0.018	0.036	0.031	0.027	0.026	0.046	0.031	—

The sequence reference symbol is +; Walker and Doolittle (14). ++; Huysmans *et al.* (6), and A; Berlin RNA Databank (16).

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(Received September 19, 1992)

(Accepted October 2, 1992)

### 초 록: *Trimorphomyces papilionaceus*의 5S rRNA 염기서열

허 용 · 강영원 · 박용하<sup>1</sup> · 정화성 (서울대학교 자연과학대학 미생물학과, 서울대학교 분자미생물학 연구센터, <sup>1</sup>한국과학기술연구원 유전공학연구소 유전자원센터)

담자성 효모의 일종인 *Trimorphomyces papilionaceus*의 세포질 5S rRNA의 염기서열을 RNA의 직접화학 분석법에 의하여 결정하고 그 결과를 19종 담자성 균류의 기존의 보고된 5S rRNA 서열들과 비교하였다. 본 종과 같이 형태학적으로 흰목이목의 흰목이과에 분류되어 있는 *Tremella mesenterica*와는 26개의 염기차이가 있었으며 두 종이 계통학적으로는 다른 종임을 시사하였다. K<sub>nu</sub>c 값에 의하여 상호 유연성을 비교한 균류 중에서 본 종과 가장 유사한 종은 Sporobolomycetales의 Sporobolomycetaceae에 속하는 또 다른 담자성 효모의 일종인 *Tilletiaria anomala*였으며, K<sub>nu</sub>c 값에 근거하여 볼때 *T. papilionaceus*는 다른 균류와 비교하여 이렇다할 계통학적 연관성을 보이지 않았다.