

RESEARCH NOTE

Mycobiology 40(1) : 71-75 (2012)
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<http://dx.doi.org/10.5941/MYCO.2012.40.1.071>
pISSN 1229-8093
eISSN 2092-9323

Taxonomy of *Ganoderma lucidum* from Korea Based on rDNA and Partial β -Tubulin Gene Sequence Analysis

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(Received December 31, 2011. Revised January 27, 2012. Accepted February 17, 2012)

In the present study, a phylogenetic analysis was undertaken based on the internal transcribed spacer (ITS) rDNA and partial β -tubulin gene sequence of the *Ganoderma* species. The size of the ITS rDNA regions from different *Ganoderma* species varied from 625 to 673 bp, and those of the partial β -tubulin gene sequence were 419 bp. Based on the results, a phylogenetic tree was prepared which revealed that Korean *Ganoderma lucidum* strains belong in a single group along with a *G. lucidum* strain from Bangladesh.

KEYWORDS : β -Tubulin, *Ganoderma lucidum*, Internal transcribed spacer

Due to perceived health benefits, the *Ganoderma* species is popularly used as a dietary supplement in Korea, China, Japan and other regions of the world. It has also been used to prevent and treat immunological diseases, hypertension and tumorigenesis [1]. However, species identification and circumscription have often been unclear in studies of the *Ganoderma* species, and taxonomic segregation of the genus has remained controversial [2]. Moreover, a number of *Ganoderma* isolates have been misnamed [3]. In addition, taxonomic classification of *Ganoderma lucidum* and its allied species has often been confusing. Here in Korea, the import of *G. lucidum* of low price from other countries is a factor limiting the domestic cultivation of *G. lucidum*. Many of these imported products are of inferior quality. Therefore, the precise identification and classification of commercial lines of *G. lucidum* is important in order to safeguard both public health and industry.

Ribosomal DNA (rDNA) sequences have been widely used to discriminate fungal taxa at the family [4], generic and sub-generic levels [5-8]. Bae *et al.* [9] and Moncalvo *et al.* [2, 10] used rDNA internal transcribed spacer (ITS) sequences to distinguish the taxa between isolates of *Ganodermataceae*. Of the genes coding for proteins with basic metabolic or structural functions, those coding for β -tubulin are receiving increasing attention. Studies have

made use of β -tubulin genes to investigate the relationships between fungi at all levels of taxa, and has also been found to be useful in deep-level phylogenetic studies [11].

This study aimed to investigate the genetic diversity of *G. lucidum* strains isolated in Korea from other *Ganoderma* species by analyzing their ITS rDNA and partial β -tubulin gene sequences.

The *Ganoderma* species used were obtained from the Korean Collection for Type Cultures, the American Type Culture Collection, Incheon University, Konkuk University, the Centraalbureau voor Schimmelcultures, and the Mushroom Division of the Korean Rural Development Administration (Table 1). The *Ganoderma* species were cultured at 25°C on mushroom complete medium (0.46 g KH₂PO₄, 0.5 g MgSO₄, 1 g K₂HPO₄, 2 g yeast extract, 2 g bacto peptone, 20 g glucose, and with or without 20 g/L agar). Fungal DNA was extracted using the CTAB method [12]. PCR reactions were performed with a premixed polymerase kit (Taq PreMix; TNT Research, Seoul, Korea) in a 20 μ L reaction mixture containing 1 μ L of DNA (ca. 10 ng), 10 pM ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and 10 pM ITS4 (5'-TCCTCCGCTTATTGATATGC-3') for the ITS region, and 10 pM β -tubulin_F (5'-CCGGTGCAGGCATGGGTACC-3') and 10 pM β -tubulin_R (5'-TGAAGACGGGGGAAGGGAAC-3') for the partial β -tubulin gene sequence. DNA was amplified in a

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Table 1. *Ganoderma* species used in the present study

No.	Species	Collection sites	Collection ID	Origin
1	<i>G. annulare</i>	Korean Collection for Type Culture	KCTC 16803	Brazil
2	<i>G. carnosum</i>	The Centraalbureau voor Schimmelcultures	CBS 516.96	Netherlands
3	<i>G. lucidum</i>	Incheon University	IUM-4303	Bangladesh
4	<i>G. lucidum</i>	Incheon University	IUM-4304	Bangladesh
5	<i>G. lucidum</i>	Incheon University	IUM-4310	Bangladesh
6	<i>G. lucidum</i>	The American Type Culture Collection	ATCC46755	Canada
7	<i>G. lucidum</i>	Incheon University	IUM-4242	China
8	<i>G. lucidum</i>	Mushroom Division at RDA	RDA (cultivar Yeongji 1)	Korea
9	<i>G. lucidum</i>	Konkuk University	KU-4011	Korea
10	<i>G. lucidum</i>	Mushroom Division at RDA	RDA (cultivar Yeongji 2)	Korea
11	<i>G. lucidum</i>	Konkuk University	KU-4015	Korea
12	<i>G. lucidum</i>	Konkuk University	KU-4006	Korea
13	<i>G. lucidum</i>	Konkuk University	KU-4009	Korea
14	<i>G. lucidum</i>	Mushroom Division at RDA	ASI-7152	Korea
15	<i>G. lucidum</i>	Konkuk University	KU-4035	Korea
16	<i>G. lucidum</i>	Incheon University	IUM-0938	Korea
17	<i>G. lucidum</i>	The American Type Culture Collection	ATCC 64251	Taiwan
18	<i>G. mirabile</i>	The Centraalbureau voor Schimmelcultures	CBS 218.36	Philippines
19	<i>G. neo-japonicum</i>	Mushroom Division at RDA	ASI-7032	Korea
20	<i>G. pfeifferi</i>	The Centraalbureau voor Schimmelcultures	CBS 747.84	Netherlands
21	<i>G. resinaceum</i>	The Centraalbureau voor Schimmelcultures	CBS 152.27	UK
22	<i>G. resinaceum</i>	The Centraalbureau voor Schimmelcultures	CBS 220.36	USA
23	<i>G. resinaceum</i>	Mushroom Division at RDA	ASI-7142	Korea
24	<i>G. resinaceum</i>	Mushroom Division at RDA	ASI-7143	Korea
25	<i>G. resinaceum</i>	Incheon University	IUM-3651	Czech
26	<i>G. subamboinense</i>	The American Type Culture Collection	ATCC 52420	Argentina
27	<i>G. tsugae</i>	The American Type Culture Collection	ATCC 64795	Canada
28	<i>G. tsugae</i>	Konkuk University	KU-4018	USA
29	<i>G. tsugae</i>	The American Type Culture Collection	ATCC 64794	USA
30	<i>G. tornatum</i>	The Centraalbureau voor Schimmelcultures	CBS 109679	Netherlands
31	<i>G. valesiacum</i>	The Centraalbureau voor Schimmelcultures	CBS 428.84	USA
32	<i>G. webeianum</i>	The Centraalbureau voor Schimmelcultures	CBS 219.36	Philippines

RDA, Rural Development Administration.

MyCycler (Bio-Rad, Hercules, CA, USA) according to the following protocol: initial denaturation duration of 5 min at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 62°C and 1 min at 72°C, with final extension for 5 min at 72°C. A 5- μ L aliquot of each product was mixed with 1 μ L of Dyne LoadingStar loading dye (DyneBio, Seoul, Korea), electrophoresed on a 1.2% agarose gel, and visualized with a UV transilluminator. The PCR product sizes for the ITS region were of variable lengths, from 636 to 673 bp. The nucleotide sequences were deposited into the National Center for Biotechnology Information (NCBI) GenBank data base (Table 2). Of those organisms assessed, the PCR product from *G. mirabile* produced the longest ITS region (673 bp). However, the PCR product sizes from the partial β -tubulin genes were identical (419 bp) to the others.

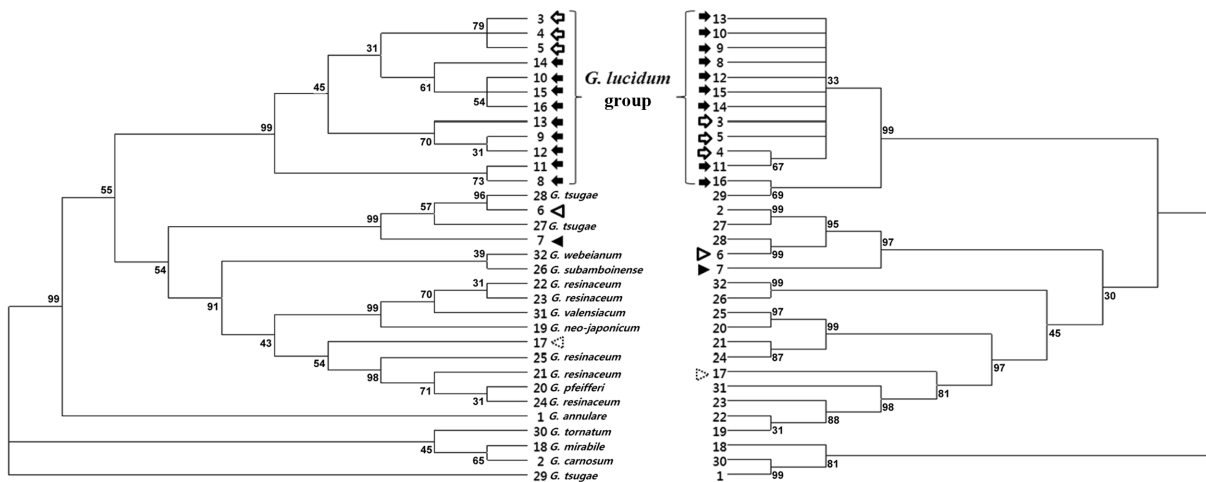
The sequences were aligned for phylogenetic analysis using the program BioEdit (<http://www.mbio.ncsu.edu/bioedit/bioedit.html>). The phylogenetic tree was constructed by a neighbor-joining method using the MEGA5 program [13]. Table 2 lists the sequence information for the ITS

region and the partial β -tubulin gene. Total G + C and A + T content in the ITS region varied from 41.54~50% and 50~58.46%, respectively. The 5.8S gene located between the ITS 1 and 2 regions was, as expected, very well conserved (158 bp in length). Moncalvo *et al.* [14] reported that the 5.8S rDNA sequences of the basidiomycetes isolates were identical, a result agreeing with our findings. The nucleotide composition of the partial β -tubulin gene sequence varied little, with G + C content and A + T content ranging from 54.89~56.56% and 43.44~44.87%, respectively. The phylogenetic trees constructed from the ITS region sequences and partial β -tubulin gene sequences depicted a similar pattern (Fig. 1). The resulting phylogenetic tree suggested a greater level of genetic diversity of *Ganoderma* species originating from different regions. Interestingly, *G. lucidum* strains from Korea and Bangladesh maybe clustered into a single group. However, the *G. lucidum* strains from China, Taiwan and Canada were clustered into other groups. The aligned rDNA sequences of *G. lucidum* strains from Korea (Yeongji 2), China (IUM-4242), Taiwan (ATCC64251) and Canada

Table 2. Sequence information of ITS and the partial β -tubulin gene sequence of *Ganoderma* species

No.	Species	Collection ID	Internal transcribed spacer rDNA				Partial β -tubulin gene			
			Length (bp)	A + T content (%)	G + C content (%)	Accession No.	Length (bp)	A + T content (%)	G + C content (%)	Accession No.
1	<i>G. annulare</i>	KCTC 16803	648	51.54	48.46	JQ520160	419	44.63	55.37	JQ675613
2	<i>G. carnosum</i>	CBS 516.96	653	51.30	48.70	JQ520163	419	44.63	55.37	JQ675616
3	<i>G. lucidum</i>	IUM-4303	636	51.42	48.58	JQ520182	419	43.68	56.32	JQ675635
4	<i>G. lucidum</i>	IUM-4304	636	51.42	48.58	JQ520183	419	43.91	56.09	JQ675636
5	<i>G. lucidum</i>	IUM-4310	636	51.42	48.58	JQ520184	419	43.44	56.56	JQ675637
6	<i>G. lucidum</i>	ATCC46755	644	50.62	49.38	JQ520185	419	44.15	55.85	JQ675638
7	<i>G. lucidum</i>	IUM-4242	643	50.54	49.46	JQ520186	419	44.15	55.85	JQ675639
8	<i>G. lucidum</i>	RDA (cultivar Yeongji 1)	636	51.89	48.11	JQ520167	419	43.68	56.32	JQ675620
9	<i>G. lucidum</i>	KU-4011	636	51.73	48.27	JQ520168	419	43.68	56.32	JQ675621
10	<i>G. lucidum</i>	RDA (cultivar Yeongji 2)	636	51.73	48.27	JQ520169	419	43.68	56.32	JQ675622
11	<i>G. lucidum</i>	KU-4015	636	52.04	47.96	JQ520171	419	43.91	56.09	JQ675624
12	<i>G. lucidum</i>	KU-4006	636	51.73	48.27	JQ520172	419	43.68	56.32	JQ675625
13	<i>G. lucidum</i>	KU-4009	636	51.73	48.27	JQ520173	419	43.68	56.32	JQ675626
14	<i>G. lucidum</i>	ASI-7152	636	51.89	48.11	JQ520214	419	43.68	56.32	JQ675667
15	<i>G. lucidum</i>	KU-4035	636	51.73	48.27	JQ520207	419	43.68	56.32	JQ675660
16	<i>G. lucidum</i>	IUM-0938	636	51.57	48.43	JQ520176	419	43.91	56.09	JQ675629
17	<i>G. lucidum</i>	ATCC 64251	650	51.38	48.62	JQ520187	419	44.87	55.13	JQ675640
18	<i>G. mirabile</i>	CBS 218.36	673	55.57	44.43	JQ520192	419	44.63	55.37	JQ675645
19	<i>G. neo-japonicum</i>	ASI-7032	645	51.47	48.53	JQ520193	419	44.63	55.37	JQ675646
20	<i>G. pfeifferi</i>	CBS 747.84	650	51.38	48.62	JQ520198	419	44.87	55.13	JQ675651
21	<i>G. resinaceum</i>	CBS 152.27	650	51.38	48.62	JQ520200	419	44.87	55.13	JQ675653
22	<i>G. resinaceum</i>	CBS 220.36	645	51.32	48.68	JQ520201	419	44.63	55.37	JQ675654
23	<i>G. resinaceum</i>	ASI-7142	645	51.32	48.68	JQ520202	419	44.63	55.37	JQ675655
24	<i>G. resinaceum</i>	ASI-7143	650	51.38	48.62	JQ520203	419	45.11	54.89	JQ675656
25	<i>G. resinaceum</i>	IUM-3651	650	51.23	48.77	JQ520204	419	44.87	55.13	JQ675657
26	<i>G. subamboinense</i>	ATCC 52420	644	50.62	49.38	JQ520205	419	44.39	55.61	JQ675658
27	<i>G. tsugae</i>	ATCC 64795	644	50.00	50.00	JQ520215	419	44.63	55.37	JQ675668
28	<i>G. tsugae</i>	KU-4018	644	50.62	49.38	JQ520216	419	44.15	55.85	JQ675669
29	<i>G. tsugae</i>	ATCC 64794	650	58.46	41.54	JQ675674	419	43.91	56.09	JQ675673
30	<i>G. tomatum</i>	CBS 109679	642	52.96	47.04	JQ520217	419	44.63	55.37	JQ675670
31	<i>G. valesiacum</i>	CBS 428.84	645	51.32	48.68	JQ520218	419	44.63	55.37	JQ675671
32	<i>G. webeianum</i>	CBS 219.36	646	52.01	47.99	JQ520219	419	44.63	55.37	JQ675672

ITS, internal transcribed spacer.

**Fig. 1.** Phylogenetic trees constructed from the internal transcribed spacer (ITS) rDNA region sequence (left) and partial β -tubulin gene sequence (right) of the *Ganoderma* species. ◀, *G. lucidum* (Korea); ◻, *G. lucidum* (Bangladesh); ▲, *G. lucidum* (China); ◁, *G. lucidum* (Canada); ◆, *G. lucidum* (Taiwan).

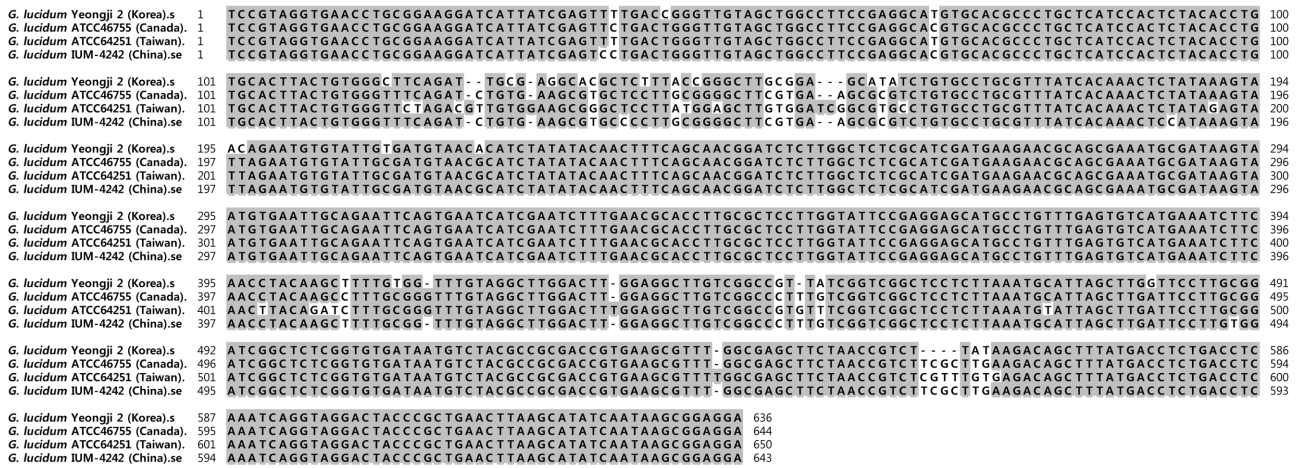


Fig. 2. Aligned rDNA sequences of the internal transcribed spacer (ITS)1, 5.8s and ITS2 regions. Alignment gaps are indicated by dots and conserved bases are indicated by dark shading. The DNA sequence from left to right reads from 5 to 3.

(ATCC46755) are shown in Fig. 2. Wu *et al.* [15] reported that *G. lucidum* had undergone certain variations after being introduced from its original locations to Korea. These variations were related to differences in ecological habitats, and lead to subtle discriminations in morphological traits as well as resulting medical efficacy. The *G. tsugae* ATCC 64794 strain demonstrated a different phylogenetic pattern, as observed from its ITS rDNA region, and was not related to any of the other strains used. However it closely clustered in terms of the phylogeny with *G. lucidum* strains, based on its partial β -tubulin gene sequences. In order to satisfy controversial questions regarding the different phylogeny of *G. tsugae* ATCC 64794 as constructed from the rDNA region and partial β -tubulin gene sequences, additional integrated phylogenetic analyses using other molecular techniques such as random amplification of polymorphic DNA, amplified fragment length polymorphism and sequence characterized regions, may be necessary.

In the present study, we analyzed the ITS rDNA region and partial β -tubulin gene sequences of *Ganoderma* species in order to clarify their genetic relationships. Of the *Ganoderma* species, Korean *G. lucidum* strains, including cultivar Yeongji 1 and 2, were specifically identified as differing from those from China, Taiwan and Canada. The taxonomy of the genus is traditionally based on morphological characteristics. However, difficulty remains in distinguishing between these close groups, such as populations or strains of the same species. Zheng *et al.* [16] reported that environmental factors, variability, interhybridization and morphological propensity can lead to inaccurate identification of *Ganoderma* species.

Acknowledgements

This study was supported by a National Joint Agricultural

Research Project of the RDA (Project No. 20110401-302-586-001-08-00), Republic of Korea.

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