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

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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 μL reaction mixture containing 1 μL 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'-CCGGTGACAGCGCATGGGTACC-3') and 10 pM β-tubulin_R (5'-TGAGACCGGGGAAGGAAAC-3') for the partial β-tubulin gene sequence. DNA was amplified in a

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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-µL aliquot of each product was mixed with 1 µL 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 \( \beta \)-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 \( \beta \)-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 \( \beta \)-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 \( \beta \)-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 1. \( Ganoderma \) species used in the present study

| No. | Species                  | Collection sites                          | Collection ID     | Origin         |
|-----|-------------------------|------------------------------------------|-------------------|----------------|
| 1   | \( G. annulare \)        | Korean Collection for Type Culture        | KCTC 16803        | Brazil         |
| 2   | \( G. carnosum \)        | The Centraalbureau voor Schimmecultures  | CBS 516.96        | Netherlands    |
| 3   | \( G. lucidum \)         | Incheon University                       | IUM-4303          | Bangladesh     |
| 4   | \( G. lucidum \)         | Incheon University                       | IUM-4304          | Bangladesh     |
| 5   | \( G. lucidum \)         | Incheon University                       | IUM-4310          | Bangladesh     |
| 6   | \( G. lucidum \)         | The American Type Culture Collection      | ATCC46755         | Canada         |
| 7   | \( G. lucidum \)         | Incheon University                       | IUM-4242          | China          |
| 8   | \( G. lucidum \)         | Mushroom Division at RDA                  | RDA (cultivar Yeongji 1) | Korea         |
| 9   | \( G. lucidum \)         | Konkuk University                        | KU-4011           | Korea          |
| 10  | \( G. lucidum \)         | Mushroom Division at RDA                  | RDA (cultivar Yeongji 2) | Korea         |
| 11  | \( G. lucidum \)         | Konkuk University                        | KU-4015           | Korea          |
| 12  | \( G. lucidum \)         | Konkuk University                        | KU-4006           | Korea          |
| 13  | \( G. lucidum \)         | Konkuk University                        | KU-4009           | Korea          |
| 14  | \( G. lucidum \)         | Mushroom Division at RDA                  | ASI-7152          | Korea          |
| 15  | \( G. lucidum \)         | Konkuk University                        | KU-4035           | Korea          |
| 16  | \( G. lucidum \)         | Incheon University                       | IUM-0938          | Korea          |
| 17  | \( G. lucidum \)         | The American Type Culture Collection      | ATCC 64251        | Taiwan         |
| 18  | \( G. mirabile \)        | The Centraalbureau voor Schimmecultures  | CBS 218.36        | Philippines    |
| 19  | \( G. neo-japonicum \)   | Mushroom Division at RDA                  | ASI-7032          | Korea          |
| 20  | \( G. pfefferi \)        | The Centraalbureau voor Schimmecultures  | CBS 747.84        | Netherlands    |
| 21  | \( G. resinaeatum \)     | The Centraalbureau voor Schimmecultures  | CBS 152.27        | UK             |
| 22  | \( G. resinaeatum \)     | The Centraalbureau voor Schimmecultures  | CBS 220.36        | USA            |
| 23  | \( G. resinaceum \)      | Mushroom Division at RDA                  | ASI-7142          | Korea          |
| 24  | \( G. resinaceum \)      | Mushroom Division at RDA                  | ASI-7143          | Korea          |
| 25  | \( G. resinaceum \)      | Incheon University                       | IUM-3651          | Czech          |
| 26  | \( G. sabamboiense \)    | The American Type Culture Collection      | ATCC 52420        | Argentina      |
| 27  | \( G. tsugae \)          | The American Type Culture Collection      | ATCC 64795        | Canada         |
| 28  | \( G. tsugae \)          | Konkuk University                        | KU-4018           | USA            |
| 29  | \( G. tsugae \)          | The American Type Culture Collection      | ATCC 64794        | USA            |
| 30  | \( G. tornatum \)        | The Centraalbureau voor Schimmecultures  | CBS 109679        | Netherlands    |
| 31  | \( G. valseiicum \)      | The Centraalbureau voor Schimmecultures  | CBS 428.84        | USA            |
| 32  | \( G. weberia \)         | The Centraalbureau voor Schimmecultures  | CBS 219.36        | Philippines    |
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)                      | Accession No.           |
|     |                         |                          | A+T content (%)                  | G+C content (%)         |
|     |                         |                          |                                 | Accession No.           |
|     |                         |                          |                                 | Length (bp)             |
|     |                         |                          |                                 | A+T content (%)         |
|     |                         |                          |                                 | G+C content (%)         |
|     |                         |                          |                                 | Accession No.           |
| 1   | *G. annulare*           | KCTC 16803               | 648                              | 51.54                   |
|     |                         |                          |                                  | 48.46                  |
|     |                         |                          |                                  | JQ520160               |
| 2   | *G. carnosum*          | CBS 516.96               | 653                              | 51.30                   |
|     |                         |                          |                                  | 48.70                  |
|     |                         |                          |                                  | JQ520163               |
| 3   | *G. lucidum*            | IUM-4303                 | 636                              | 51.42                   |
|     |                         |                          |                                  | 48.58                  |
|     |                         |                          |                                  | JQ520182               |
| 4   | *G. lucidum*            | IUM-4304                 | 636                              | 51.42                   |
|     |                         |                          |                                  | 48.58                  |
|     |                         |                          |                                  | JQ520183               |
| 5   | *G. lucidum*            | IUM-4310                 | 636                              | 51.42                   |
|     |                         |                          |                                  | 48.58                  |
|     |                         |                          |                                  | JQ520184               |
| 6   | *G. lucidum*            | ATCC-46755               | 644                              | 50.62                   |
|     |                         |                          |                                  | 49.38                  |
|     |                         |                          |                                  | JQ520185               |
| 7   | *G. lucidum*            | IUM-4242                 | 643                              | 50.54                   |
|     |                         |                          |                                  | 49.46                  |
|     |                         |                          |                                  | JQ520186               |
| 8   | *G. lucidum*            | RDA (cultivar Yeongji 1) | 636                              | 51.89                   |
|     |                         |                          |                                  | 48.11                  |
|     |                         |                          |                                  | JQ520167               |
| 9   | *G. lucidum*            | KU-4011                  | 636                              | 51.73                   |
|     |                         |                          |                                  | 48.27                  |
|     |                         |                          |                                  | JQ520168               |
| 10  | *G. lucidum*            | RDA (cultivar Yeongji 2) | 636                              | 51.73                   |
|     |                         |                          |                                  | 48.27                  |
|     |                         |                          |                                  | JQ520169               |
| 11  | *G. lucidum*            | KU-4015                  | 636                              | 52.04                   |
|     |                         |                          |                                  | 47.96                  |
|     |                         |                          |                                  | JQ520171               |
| 12  | *G. lucidum*            | KU-4006                  | 636                              | 51.73                   |
|     |                         |                          |                                  | 48.27                  |
|     |                         |                          |                                  | JQ520172               |
| 13  | *G. lucidum*            | KU-4009                  | 636                              | 51.73                   |
|     |                         |                          |                                  | 48.27                  |
|     |                         |                          |                                  | JQ520173               |
| 14  | *G. lucidum*            | ASI-7152                 | 636                              | 51.89                   |
|     |                         |                          |                                  | 48.11                  |
|     |                         |                          |                                  | JQ520214               |
| 15  | *G. lucidum*            | KU-4035                  | 636                              | 51.73                   |
|     |                         |                          |                                  | 48.27                  |
|     |                         |                          |                                  | JQ520207               |
| 16  | *G. lucidum*            | IUM-0938                 | 636                              | 51.57                   |
|     |                         |                          |                                  | 48.43                  |
|     |                         |                          |                                  | JQ520176               |
| 17  | *G. lucidum*            | ATCC-64251               | 650                              | 51.38                   |
|     |                         |                          |                                  | 48.62                  |
|     |                         |                          |                                  | JQ520187               |
| 18  | *G. mirabile*           | CBS 218.36               | 673                              | 55.57                   |
|     |                         |                          |                                  | 44.43                  |
|     |                         |                          |                                  | JQ520192               |
| 19  | *G. neo-japonicum*      | ASI-7032                 | 645                              | 51.47                   |
|     |                         |                          |                                  | 48.53                  |
|     |                         |                          |                                  | JQ520193               |
| 20  | *G. pfeifferi*          | CBS 747.84               | 650                              | 51.38                   |
|     |                         |                          |                                  | 48.62                  |
|     |                         |                          |                                  | JQ520198               |
| 21  | *G. resinaceum*         | CBS 152.27               | 650                              | 51.38                   |
|     |                         |                          |                                  | 48.62                  |
|     |                         |                          |                                  | JQ520200               |
| 22  | *G. resinaceum*         | CBS 220.36               | 645                              | 51.32                   |
|     |                         |                          |                                  | 48.68                  |
|     |                         |                          |                                  | JQ520201               |
| 23  | *G. resinaceum*         | ASI-7142                 | 651                              | 51.32                   |
|     |                         |                          |                                  | 48.68                  |
|     |                         |                          |                                  | JQ520202               |
| 24  | *G. resinaceum*         | ASI-7143                 | 650                              | 51.38                   |
|     |                         |                          |                                  | 48.62                  |
|     |                         |                          |                                  | JQ520203               |
| 25  | *G. resinaceum*         | IUM-3651                 | 650                              | 51.23                   |
|     |                         |                          |                                  | 48.77                  |
|     |                         |                          |                                  | JQ520204               |
| 26  | *G. subamboinense*      | ATCC-52420               | 644                              | 50.62                   |
|     |                         |                          |                                  | 49.38                  |
|     |                         |                          |                                  | JQ520205               |
| 27  | *G. tsugae*             | ATCC-64795               | 644                              | 50.00                   |
|     |                         |                          |                                  | 50.00                  |
|     |                         |                          |                                  | JQ520215               |
| 28  | *G. tsugae*             | KU-4018                  | 644                              | 50.62                   |
|     |                         |                          |                                  | 49.38                  |
|     |                         |                          |                                  | JQ520216               |
| 29  | *G. tsugae*             | ATCC-64794               | 650                              | 58.46                   |
|     |                         |                          |                                  | 41.54                  |
|     |                         |                          |                                  | JQ675674               |
| 30  | *G. tornatum*           | CBS 109679               | 642                              | 52.96                   |
|     |                         |                          |                                  | 47.04                  |
|     |                         |                          |                                  | JQ520217               |
| 31  | *G. valesiacum*         | CBS 428.84               | 645                              | 51.32                   |
|     |                         |                          |                                  | 48.68                  |
|     |                         |                          |                                  | JQ520218               |
| 32  | *G. webeianum*          | CBS 219.36               | 646                              | 52.01                   |
|     |                         |                          |                                  | 47.99                  |
|     |                         |                          |                                  | JQ520219               |

ITS, internal transcribed spacer.

![Genetic Diversity of Ganoderma lucidum](image)

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).
G. lucidum (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.

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