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Full Length Research Paper

Genetic diversity analysis of *Ganoderma* species and development of a specific marker for identification of medicinal mushroom *Ganoderma lucidum*

Young-Jin Park[1], O-Chul Kwon[1], Eun-Suk Son[1], Dae-Eun Yoon[1], Woorijarang Han[1], Jae-Young Nam[1], Young-Bok Yoo[2] and Chang-Soo Lee[1]*

[1]Department of Applied Biochemistry, Konkuk University, Chung-Ju 380-701, Republic of Korea.
[2]Mushroom Research Division, Department of Herbal Crop Research, National Institute of Horticultural and Herbal Science, Rural Development Administration, Suwon 404-707, Korea.

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The genetic diversity of 59 *Ganoderma* species from various regions was determined by different molecular markers, including the internal transcribed spacer (ITS) rRNA and a partial β-tubulin gene, as well as randomly amplified polymorphic DNA (RAPD) analysis. The size of the ITS rRNA gene regions from different *Ganoderma* species varied from 625 to 673 bp, and that of the partial β-tubulin gene sequence was 419 bp. A phylogenetic tree based on the ITS region, the partial β-tubulin gene, and the RAPD profiles revealed that *Ganoderma lucidum* strains could be classified into 1 group together with *G. lucidum* from Bangladesh. One fragment unique to *G. lucidum* was selected from the RAPD profile and then sequenced. One primer pair (designated as GSF and GSR) based on this specific fragment was designed to amplify a 559 bp DNA fragment within the sequenced region. A single band with the expected size of 559 bp was observed from *G. lucidum*, except for *G. lucidum* strains from China, Canada, and Taiwan. This specific marker for *G. lucidum* from RAPD analysis, also supported by the phylogenetic analysis of the ITS and partial β-tubulin gene sequences, will be useful for the PCR-based identification of *G. lucidum* in research applications as well as in the market.

Key words: Internal transcribed spacer, β-tubulin, medicinal mushroom, *Ganoderma lucidum,* randomly amplified polymorphic DNA (RAPD).

INTRODUCTION

Ganoderma lucidum is popularly used as a dietary supplement in Korea, China, and Japan, as well as in other regions of the world. It is known to prevent and treat immunological diseases, including tumorigenesis (Liu et al., 2002). In the case of *Ganoderma* species, the identification of species was often unclear, and the taxonomic segregation of the genus remained controversial (Moncalvo et al., 1995a). In addition, the taxonomic classification of *G. lucidum* and its allied species has always been confusing. Hence, a number of *Ganoderma* isolates have been misidentified and misnamed (Smith and Sivasithamparam, 2000).

*Corresponding author. E-mail: cslee@kku.ac.kr.

Ribosomal RNA (rRNA) gene sequences have widely been used to discriminate fungal taxa at the family (Hibbett and Donoghue, 1995), generic, and sub-generic levels (Vilgalys and Sun, 1994; Crawford et al., 1996; Anderson et al., 1998; Chillali et al., 1998). Bae et al. (1995) and Moncalvo et al. (1995a, b) used rRNA gene internal transcribed spacer (ITS) sequences to distinguish between isolates of Ganodermataceae. Among genes coding for proteins with basic metabolic or structural functions, β-tubulin genes are receiving increasing attention as markers. Studies have made use of β-tubulin genes to investigate relationships among fungi at all levels, and they were also found to be useful in deep-level phylogenetic studies (Thon and Royse, 1999). Aside from the genetic diversity analysis described above, several techniques also have been used in basidiomycetes,

such as Amplified Fragment Length Polymorphism (AFLP) (Qi et al., 2003), Restriction Fragment Length Polymorphism (RFLP) (Park and Ryu, 1996), and Random Amplified Polymorphic DNA (RAPD) (Wang et al., 2003). Among these techniques, RAPD is still one of the cheapest and quickest methods for accessing variability at the DNA level and is especially useful for intraspecies analysis.

This study investigated the genetic diversity of *G. lucidum* strains among selected *Ganoderma* species through analysis of the ITS rRNA and partial β-tubulin gene sequences and developed a specific marker for *G. lucidum* based on RAPD analysis.

MATERIALS AND METHODS

Fungal species and culture conditions

Fungal species 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 Rural Development Administration (Table 1). *Ganoderma* species were cultured on mushroom complete medium (MCM: 0.46 g KH_2PO_4, 0.5 g $MgSO_4$, 1 g K_2HPO_4, 2 g yeast extract, 2 g Bacto peptone, and 20 g glucose with or without 20 g/L agar) at 25°C.

DNA extraction and PCR amplification

Cultured mycelia (filtered through 2 layers of MiraCloth; Calbiochem) were ground in liquid nitrogen, and genomic DNA was extracted using the CTAB method (Cao et al., 1998). All PCR reactions were performed with a premixed polymerase kit (Taq PreMix; TNT Research, Korea) in a 20 µl reaction mixture containing 1 µl DNA (ca. 10 ng) and the primers shown in Table 2. After screening the amplification conditions for each assay, PCR assays were carried out using the same PCR conditions. DNA was amplified in a MyCycler™ (Bio-Rad, Hercules, CA) according to the following program: initial denaturation for 5 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 56°C, and 1 min at 72°C, and a final extension time of 5 min at 72°C. A 5 µl aliquot of each product was mixed with 1 µl Dyne LoadingStar loading dye (DyneBio, Korea), electrophoresed on a 1.2% agarose gel, and visualized with a UV transilluminator. The amplified fragments of the ITS and partial β-tubulin gene, and the specific fragment for *G. lucidum* from RAPD, were eluted and sequenced (Macrogen Inc., Korea). Nucleotide sequences were deposited in the National Center for Biotechnology Information (NCBI) Genbank data base (Table 3). The sequences of the ITS regions and partial β-tubulin genes 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 (Tamura et al., 2011). Confidence values for individual branches of the resulting tree were assessed by the bootstrap analysis in which 1,000 bootstrapped trees were generated from resampled data.

RESULTS AND DISCUSSION

Analysis of ITS and partial β-tubulin gene sequences

Table 3 lists sequence information for the ITS region and the partial β-tubulin gene. The PCR product sizes of the ITS region were of variable length from 636 to 673 bp. Among them, the PCR product from *G. mirabile* had the longest ITS region (673 bp), except for *Laetiporus persicinus* (736 bp). However, the PCR product sizes of the partial β-tubulin gene were identical (419 bp). The total G + C and A + T content of the ITS region was 44.43–50.00% and 41.38~55.57%, respectively. The 5.8S gene located between the ITS 1 and 2 regions was, as expected, very well conserved (158 bp in length). The aligned 16S rRNA gene sequences of *Ganoderma* species are shown in Figure 1. Moncalvo et al. (1995c) reported that the 5.8S rRNA sequences of basidiomycete isolates were identical, which is similar to our findings. The nucleotide composition of the partial β-tubulin gene sequence varied little, with G + C and A + T contents of 54.65–56.8% and 43.2–45.35%, respectively. The phylogenetic trees constructed from ITS region sequences and the partial β-tubulin gene sequences depicted a similar pattern (Figure 2). The phylogenetic tree suggests that *Ganoderma* species from different regions show a high level of genetic diversity. Interestingly, Korean *G. lucidum* strains could be clustered into 1 group together with *G. lucidum* strains from Bangladesh. However, the *G. lucidum* strains from China, Taiwan, and Canada were clustered into different groups. In order to satisfy controversial questions regarding the different phylogeny of *Ganoderma* species as constructed from the 16S rRNA 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.

RAPD analysis and identification of a specific marker for *G. lucidum*

Repeat sequences from Korean weedy rice, originally referred to as universal rice primers (URPs), have been used for the fingerprinting of diverse genomes of plants, animals, and microbes (Kang et al., 2002). In this study, 12 URP primers were used for the molecular analysis of 59 *Ganoderma* species (data not shown). Among them, the URP1 primer revealed a good polymorphic amplification pattern. In addition, amplification with primer URP1 produced a uniform DNA band of 631 bp in all *G. lucidum* strains, except those from China, Canada, and Taiwan. Three unidentified *Ganoderma* species also showed the same or similar patterns with *G. lucidum* from Korea and Bangladesh (Figure 3a). This result corresponds with those of the phylogenetic analysis of the ITS rRNA gene and the partial β-tubulin gene sequences. These results suggest that *G. lucidum* strains have a high level of genetic similarity and some *G. lucidum* strains have been misidentified. The taxonomy of the genus is traditionally based on morphological characteristics. However, there is still difficulty in distinguishing among close groups, such

Table 1. Fungal species used in the present study.

S/N	Species	Collection sites	Collection ID	Origin
1	*Ganoderma annulare*	Korean Collection for Type Culture	KCTC 16803	Brazil
2	*Ganoderma applanatum*	The American Type Culture Collection	ATCC 44053	Japan
3	*Ganoderma applanatum*	Incheon University	IUM-3985	Netherlands
4	*Ganoderma carnosum*	The Centraalbureau voor Schimmelcultures	CBS 516.96	Netherlands
5	*Ganoderma curtisii*	The Centraalbureau voor Schimmelcultures	CBS 100132	Netherlands
6	*Ganoderma lobatum*	The American Type Culture Collection	ATCC 42985	Canada
7	*Ganoderma lobatum*	Mushroom Division at RDA	ASI-7061	U.S.A
8	*Ganoderma lucidum*	Mushroom Division at RDA	ASI-7004	Korea
9	*Ganoderma lucidum*	Mushroom Division at RDA	ASI-7013	Korea
10	*Ganoderma lucidum*	Mushroom Division at RDA	ASI-7071	Korea
11	*Ganoderma lucidum*	Mushroom Division at RDA	ASI-7074	Korea
12	*Ganoderma lucidum*	Mushroom Division at RDA	ASI-7091	Korea
13	*Ganoderma lucidum*	Mushroom Division at RDA	ASI-7094	Korea
14	*Ganoderma lucidum*	Mushroom Division at RDA	ASI-7135	Korea
15	*Ganoderma lucidum*	Incheon University	IUM-0047	Korea
16	*Ganoderma lucidum*	Incheon University	IUM-0757	Korea
17	*Ganoderma lucidum*	Incheon University	IUM-0938	Korea
18	*Ganoderma lucidum*	Incheon University	IUM-3986	Korea
19	*Ganoderma lucidum*	Incheon University	IUM-4002	Korea
20	*Ganoderma lucidum*	Incheon University	IUM-4100	Korea
21	*Ganoderma lucidum*	Mushroom Division at RDA	ASI-7117	Korea
22	*Ganoderma lucidum*	Incheon University	IUM-4303	Bangladesh
23	*Ganoderma lucidum*	Incheon University	IUM-4304	Bangladesh
24	*Ganoderma lucidum*	Incheon University	IUM-4310	Bangladesh
25	*Ganoderma lucidum*	The American Type Culture Collection	ATCC 46755	Canada
26	*Ganoderma lucidum*	Incheon University	IUM-4242	China
27	*Ganoderma lucidum*	Mushroom Division at RDA	ASI-7037	Korea
28	*Ganoderma lucidum*	The American Type Culture Collection	ATCC 64251	Taiwan
29	*Ganoderma lucidum*	Korean Collection for Type Culture	KCTC 16802	Thailand
30	*Ganoderma meredithae*	The American Type Culture Collection	ATCC 64492	U.S.A
31	*Ganoderma meredithae*	Mushroom Division at RDA	ASI-7140	unknown
32	*Ganoderma mirabile*	The Centraalbureau voor Schimmelcultures	CBS 218.36	Philippines
33	*Ganoderma neo-japonicum*	Mushroom Division at RDA	ASI-7032	unknown
34	*Ganoderma oregonense*	Mushroom Division at RDA	ASI-7049	U.S.A
35	*Ganoderma oregonense*	Mushroom Division at RDA	ASI-7062	U.S.A
36	*Ganoderma oregonense*	Mushroom Division at RDA	ASI-7063	U.S.A

Table 1. Contd.

37	*Ganoderma oregonense*	Mushroom Division at RDA	ASI-7067	U.S.A
38	*Ganoderma pfeifferi*	The Centraalbureau voor Schimmelcultures	CBS 747.84	Netherlands
39	*Ganoderma resinaceum*	The American Type Culture Collection	ATCC 52416	Argentina
40	*Ganoderma resinaceum*	The Centraalbureau voor Schimmelcultures	CBS 152.27	UK
41	*Ganoderma resinaceum*	The Centraalbureau voor Schimmelcultures	CBS 220.36	U.S.A
42	*Ganoderma resinaceum*	Mushroom Division at RDA	ASI-7142	Korea
43	*Ganoderma resinaceum*	Mushroom Division at RDA	ASI-7143	Korea
44	*Ganoderma resinaceum*	Incheon University	IUM-3651	Czech
45	*Ganoderma subamboinense* var.*laecisporum*	The American Type Culture Collection	ATCC 52420	Argentina
46	*Ganoderma* species	Konkuk University	KU-4033	Korea
47	*Ganoderma* species	Konkuk University	KU-4035	Korea
48	*Ganoderma* species	Mushroom Division at RDA	ASI-7033	Papuanewguinea
49	*Ganoderma* species	Mushroom Division at RDA	ASI-7034	Papuanewguinea
50	*Ganoderma* species	Mushroom Division at RDA	ASI-7038	Papuanewguinea
51	*Ganoderma* species	Mushroom Division at RDA	ASI-7146	unknown
52	*Ganoderma* species	Mushroom Division at RDA	ASI-7150	unknown
53	*Ganoderma* species	Mushroom Division at RDA	ASI-7151	unknown
54	*Ganoderma* species	Mushroom Division at RDA	ASI-7152	unknown
55	*Ganoderma tsugae*	The American Type Culture Collection	ATCC 64795	Canada
56	*Ganoderma tsugae*	Mushroom Division at RDA	ASI-7064	U.S.A
57	*Ganoderma tornatum*	The Centraalbureau voor Schimmelcultures	CBS 109679	Netherlands
58	*Ganoderma valesiacum*	The Centraalbureau voor Schimmelcultures	CBS 428.84	U.S.A
59	*Ganoderma webeianum*	The Centraalbureau voor Schimmelcultures	CBS 219.36	Philippines
60	*Laetiporus persicinus*	The Centraalbureau voor Schimmelcultures	CBS 274.92	Netherlands

Table 2. The sequences of primers used in the present study.

Primers	**Nucleotide sequence (5'-3')**	**specificity**
ITS1	TCCGTAGGTGAACCTGCGG	ITS rRNA
ITS4	TCCTCCGCTTATTGATATGC	
β-tubulin_F	CCGGTGCAGGCATGGGTACC	partial β-tubulin gene
β-tubulin_R	TGAAGACGGGGGAAGGGAAC	
URP1	ATCCAAGGTCCGAGACAACC	RAPD
GSF	CCCTAAACCTCTCAAAGTCA	*G. lucidum*
GSR	TATCGTACAGGTTCTCGTG	

Table 3. Sequence information of ITS and the partial β-tubulin gene sequence of *Ganoderma* species.

S/N	Species	Collection ID	ITS rRNA				Partial β-tubulin gene			
			Length (bp)	A+T content (%)	G+C content (%)	Acc. No.	Length (bp)	A+T content (%)	G+C content (%)	Acc. No.
1	*Ganoderma annulare*	KCTC16803	648	51.54	48.46	JQ520160	419	44.63	55.37	JQ675613
2	*Ganoderma applanatum*	ATCC44053	650	51.54	48.46	JQ520161	419	44.87	55.13	JQ675614
3	*Ganoderma applanatum*	IUM-3985	642	52.49	47.51	JQ520162	419	44.15	55.85	JQ675615
4	*Ganoderma carnosum*	CBS 516.96	653	51.30	48.7	JQ520163	419	44.63	55.37	JQ675616
5	*Ganoderma curtisii*	CBS 100132	635	51.34	48.66	JQ520164	419	43.68	56.32	JQ675617
6	*Ganoderma lobatum*	ATCC 42985	625	52.8	47.2	JQ520165	419	44.87	55.13	JQ675618
7	*Ganoderma lobatum*	ASI-7061	645	51.63	48.37	JQ520166	419	45.35	54.65	JQ675619
8	*Ganoderma lucidum*	ASI-7004	636	51.89	48.11	JQ520167	419	43.68	56.32	JQ675620
9	*Ganoderma lucidum*	ASI-7013	636	51.73	48.27	JQ520168	419	43.68	56.32	JQ675621
10	*Ganoderma lucidum*	ASI-7071	636	51.73	48.27	JQ520169	419	43.68	56.32	JQ675622
11	*Ganoderma lucidum*	ASI-7074	636	51.26	48.74	JQ520170	419	43.2	56.8	JQ675623
12	*Ganoderma lucidum*	ASI-7091	636	52.04	47.96	JQ520171	419	43.91	56.09	JQ675624
13	*Ganoderma lucidum*	ASI-7094	636	51.73	48.27	JQ520172	419	43.68	56.32	JQ675625
14	*Ganoderma lucidum*	ASI-7135	636	51.73	48.27	JQ520173	419	43.68	56.32	JQ675626
15	*Ganoderma lucidum*	IUM-0047	636	51.73	48.27	JQ520174	419	43.91	56.09	JQ675627
16	*Ganoderma lucidum*	IUM-0757	636	51.42	48.58	JQ520175	419	43.68	56.32	JQ675628
17	*Ganoderma lucidum*	IUM-0938	636	51.57	48.43	JQ520176	419	43.91	56.09	JQ675629
18	*Ganoderma lucidum*	IUM-3986	636	51.73	48.27	JQ520177	419	43.91	56.09	JQ675630
19	*Ganoderma lucidum*	IUM-4002	636	51.42	48.58	JQ520178	419	43.91	56.09	JQ675631
20	*Ganoderma lucidum*	IUM-4100	636	51.89	48.11	JQ520179	419	43.68	56.32	JQ675632
21	*Ganoderma lucidum*	ASI-7117	636	51.73	48.27	JQ520180	419	43.91	56.09	JQ675633
22	*Ganoderma lucidum*	IUM-4303	636	51.42	48.58	JQ520182	419	43.68	56.32	JQ675635
23	*Ganoderma lucidum*	IUM-4304	636	51.42	48.58	JQ520183	419	43.91	56.09	JQ675636
24	*Ganoderma lucidum*	IUM-4310	636	51.42	48.58	JQ520184	419	43.44	56.56	JQ675637
25	*Ganoderma lucidum*	ATCC46755	644	50.62	49.38	JQ520185	419	44.15	55.85	JQ675638
26	*Ganoderma lucidum*	IUM-4242	643	50.54	49.46	JQ520186	419	44.15	55.85	JQ675639
27	*Ganoderma lucidum*	ASI-7037	636	52.04	47.96	JQ520181	419	43.91	56.09	JQ675634
28	*Ganoderma lucidum*	ATCC 64251	650	51.38	48.62	JQ520187	419	44.87	55.13	JQ675640
29	*Ganoderma lucidum*	KCTC 16802	636	51.42	48.58	JQ520188	419	43.91	56.09	JQ675641
30	*Ganoderma meredithae*	ATCC 64492	635	51.34	48.66	JQ520190	419	43.91	56.09	JQ675643
31	*Ganoderma meredithae*	ASI-7140	635	51.34	48.66	JQ520191	419	44.39	55.61	JQ675644
32	*Ganoderma mirabile*	CBS 218.36	673	55.57	44.43	JQ520192	419	44.63	55.37	JQ675645
33	*Ganoderma neo-japonicum*	ASI-7032	645	51.47	48.53	JQ520193	419	44.63	55.37	JQ675646
34	*Ganoderma oregonense*	ASI-7049	645	51.32	48.68	JQ520194	419	45.35	54.65	JQ675647

Table 3. Contd.

35	*Ganoderma oregonense*	ASI-7062	644	50.62	49.38	JQ520195	419	44.15	55.85	JQ675648
36	*Ganoderma oregonense*	ASI-7063	642	52.49	47.51	JQ520196	419	44.39	55.61	JQ675649
37	*Ganoderma oregonense*	ASI-7067	642	52.49	47.51	JQ520197	419	44.87	55.13	JQ675650
38	*Ganoderma pfeifferi*	CBS 747.84	650	51.38	48.62	JQ520198	419	44.87	55.13	JQ675651
39	*Ganoderma resinaceum*	ATCC 52416	645	51.32	48.68	JQ520199	419	44.63	55.37	JQ675652
40	*Ganoderma resinaceum*	CBS 152.27	650	51.38	48.62	JQ520200	419	44.87	55.13	JQ675653
41	*Ganoderma resinaceum*	CBS 220.36	645	51.32	48.68	JQ520201	419	44.63	55.37	JQ675654
42	*Ganoderma resinaceum*	ASI-7142	645	51.32	48.68	JQ520202	419	44.63	55.37	JQ675655
43	*Ganoderma resinaceum*	ASI-7143	650	41.38	48.62	JQ520203	419	45.11	54.89	JQ675656
44	*Ganoderma resinaceum*	IUM-3651	650	51.23	48.77	JQ520204	419	44.87	55.13	JQ675657
45	*Ganoderma subamboinense var. laecisporum*	ATCC52420	644	50.62	49.38	JQ520205	419	44.39	55.61	JQ675658
46	*Ganoderma* Species	KU-4033	642	52.49	47.51	JQ520206	419	44.63	55.37	JQ675659
47	*Ganoderma* Species	KU-4035	636	51.73	48.27	JQ520207	419	43.68	56.32	JQ675660
48	*Ganoderma* Species	ASI-7033	645	51.47	48.53	JQ520208	419	44.87	55.13	JQ675661
49	*Ganoderma* Species	ASI-7034	645	51.32	48.68	JQ520209	419	45.35	54.65	JQ675662
50	*Ganoderma* Species	ASI-7038	636	51.42	48.58	JQ520210	419	43.91	56.09	JQ675663
51	*Ganoderma* Species	ASI-7146	636	51.57	48.43	JQ520211	419	43.68	56.32	JQ675664
52	*Ganoderma* Species	ASI-7150	648	52.01	47.99	JQ520212	419	43.91	56.09	JQ675665
53	*Ganoderma* Species	ASI-7151	648	52.01	47.99	JQ520213	419	43.91	56.09	JQ675666
54	*Ganoderma* Species	ASI-7152	636	51.89	48.11	JQ520214	419	43.68	56.32	JQ675667
55	*Ganoderma tsugae*	ATCC 64795	644	50.0	50.0	JQ520215	419	44.63	55.37	JQ675668
56	*Ganoderma tsugae*	ASI-7064	644	50.62	49.38	JQ520216	419	44.15	55.85	JQ675669
57	*Ganoderma tornatum*	CBS 109679	642	52.96	47.04	JQ520217	419	44.63	55.37	JQ675670
58	*Ganoderma valesiacum*	CBS 428.84	645	51.32	48.68	JQ520218	419	44.63	55.37	JQ675671
59	*Ganoderma webeianum*	CBS 219.36	646	52.01	47.99	JQ520219	419	44.63	55.37	JQ675672
60	*Laetiporus persicinus*	CBS 274.92	736	51.77	48.23	JQ686188	419	44.63	55.37	JQ686189

as populations or strains of the same species. Zheng et al. (2009) reported that environmental factors, variability, interhybridization, and morphological propensity cause the inaccurate identification of *Ganoderma* species. For the specific identification of *G*. *lucidum* strains, the 631 bp DNA fragment was eluted and sequenced to generate primers. Primers consisting of 20 and 19 oligonucleotides (Table 2) were designed from the sequence of the specific DNA fragment for *G*. *lucidum*. A single band with the expected size of 559 bp was observed from *G*. *lucidum*, except for *G*. *lucidum* strains from China, Canada, and Taiwan (Figure 3b). This result indicates that primers GSF and GSR are specific for *G*. *lucidum* and could be used to differentiate *G*. *lucidum* from other *Ganoderma* species.

In the present study, we analyzed the ITS rRNA gene region and the partial β-tubulin gene sequences and RAPD profiles to clarify the genetic relationships among *Ganoderma* species. Among the *Ganoderma* species, Korean *G*. *lucidum* strains were discriminated from strains from China, Taiwan, and Canada. We also developed the specific primers GSF and GSR for *G*. *lucidum* identification. Medicinal mushrooms and their value-added products have a world trade

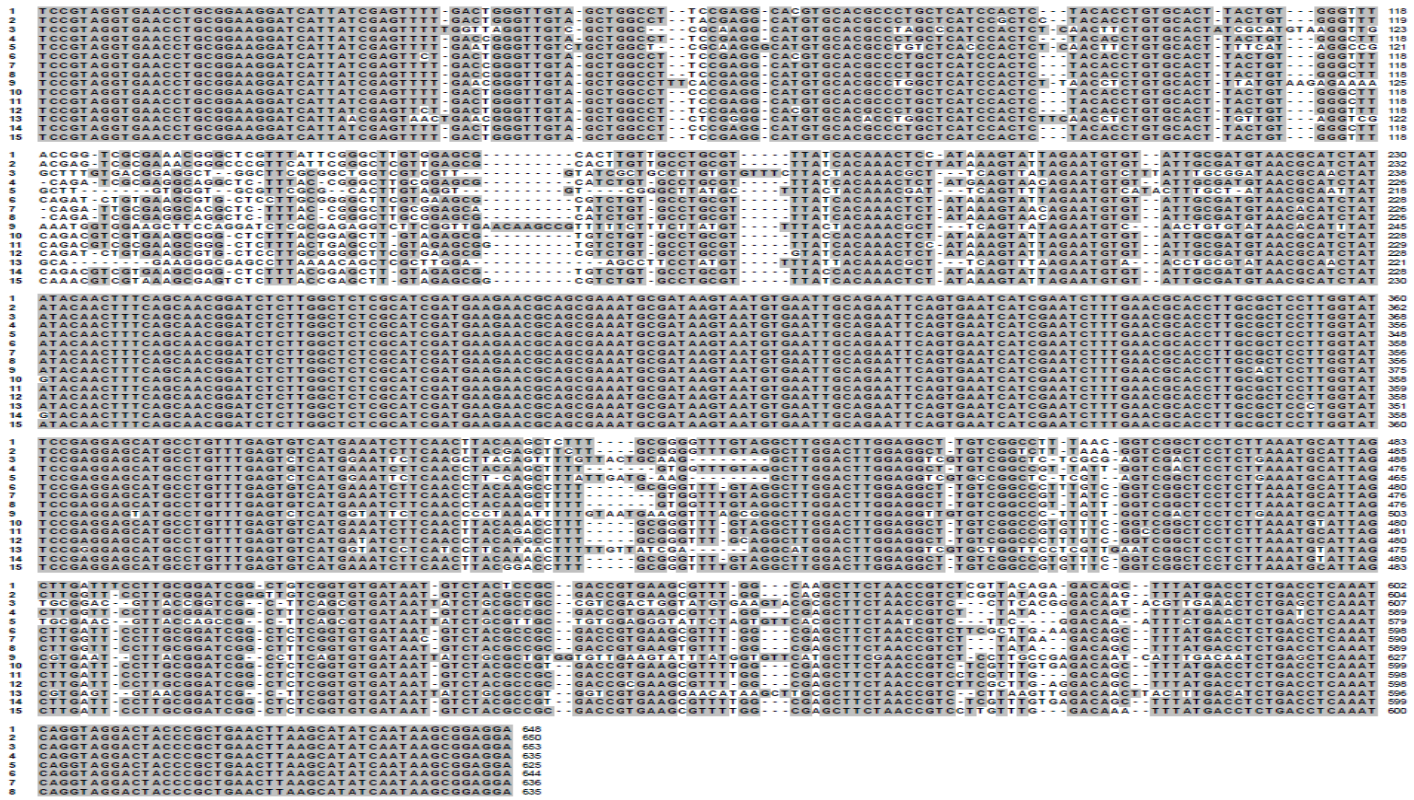

Figure 1. Aligned sequences of the ITS1, 5.8s, and ITS2 regions. Alignment gaps are indicated by dots and conserved bases by dark shade. The DNA sequence from left to right reads from 5' to 3' ends. 1, *G. annulare* KCTC 16803; 2, *G. applanatum* ATCC 44053; 3, *G. carnosum* CBS 516.96; 4, *G. curtisii* CBS 100132; 5, *G. lobatum* ATCC 42985; 6, *G. lucidum* ATCC 46755; 7, *G. lucidum* KCTC 16802; 8, *G. meredithae* ATCC 64492; 9, *G. mirabile* CBS 218.36; 10, *G. resinaceum* ATCC 52416; 11, *G. subamboinense* var. *laecisporum* ATCC 52420; 12, *G. tsugae* ATCC 64795; 13, *G. tornatum* CBS 109679; 14, *G. valesiacum* CBS 428.84; 15, *G. weberianum* CBS 219.36.

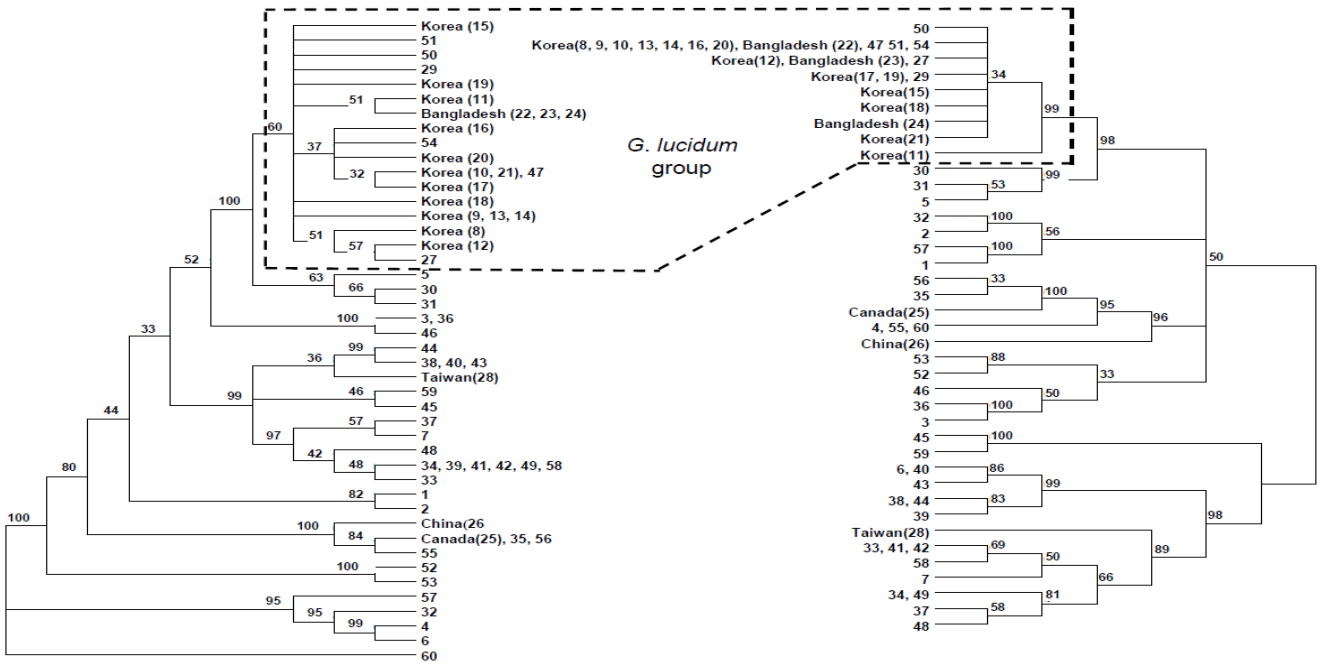

Figure 2. Phylogenetic trees constructed from the ITS rRNA gene region sequences (left) and partial β-tubulin gene sequences (right) of *Ganoderma* species. Korea, Bangladesh, China, Canada and Taiwan (*G. lucidum* strains from different countries).

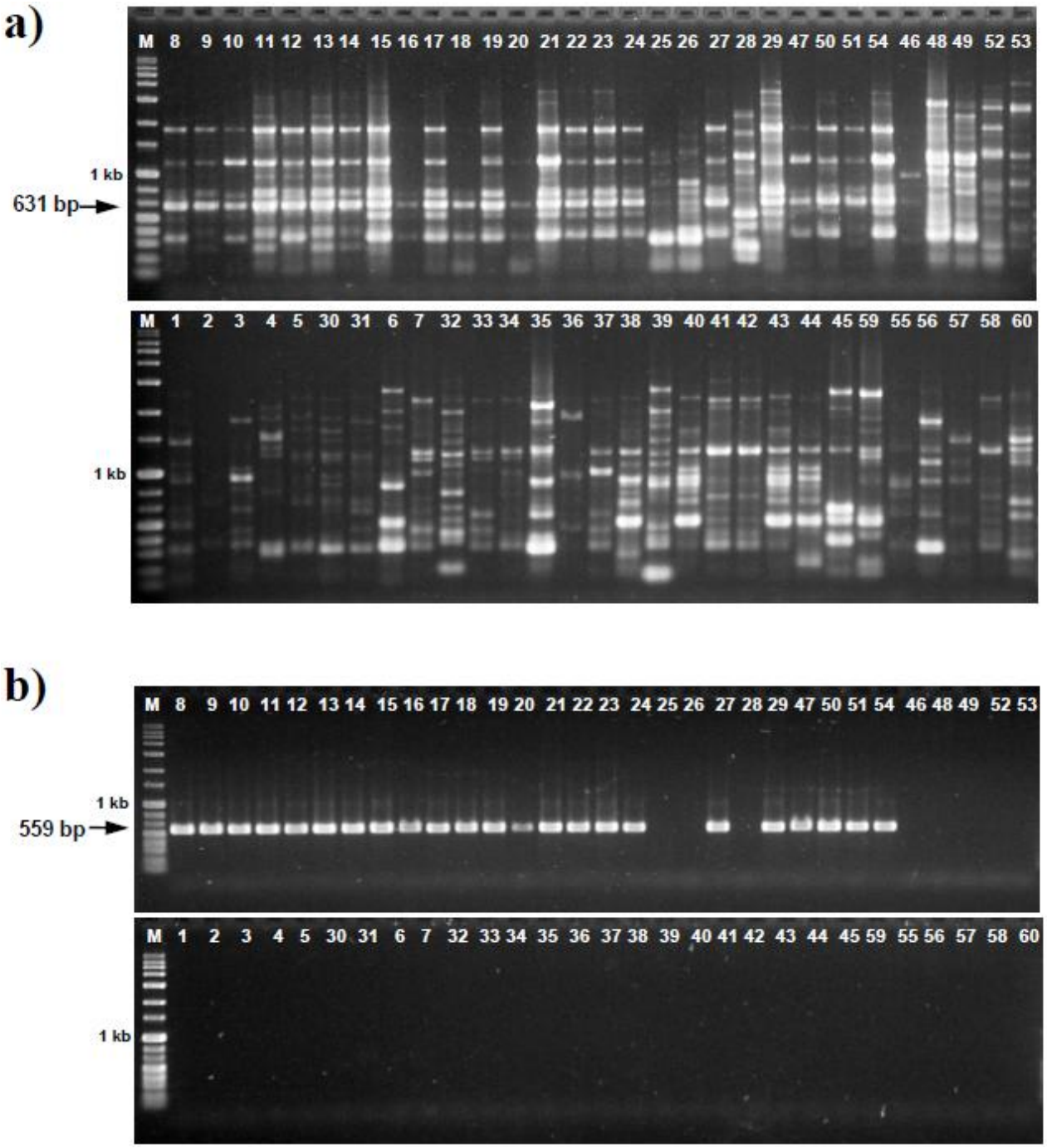

Figure 3. RAPD analysis of 59 *Ganoderma* species using the URP1 primer (a). PCR assay for the specific identification of *G. lucidum* (b). The arrow indicates the *G. lucidum*-specific amplified fragment. M: size markers (1 kb ladder).

of about 4 billion dollars (Perumal, 2009). Currently, the global demand for *G. lucidum* is very high. However, circumstances where identical strains have different names, or different strains have the same name, often lead to confusion in cultivation and incorrect medication. Therefore, the precise identification and classification of commercial lines of *G. lucidum* is important for the protection of both public health and industry.

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