Mycobiology 30(2): 61-64 (2002)

Comparison of Characteristics of *Ganoderma lucidum* According to Geographical Origins (III): Classification between Species of Genus *Ganoderma* Using Dikaryon-Monokaryon Mating

Hong Kyu Kim, Myoung Yong Shim, Geon Sik Seo[1] and Hong Gi Kim[1]*

Chungnam Agricultural Research & Extension Services, Daejeon 305-314, Korea
[1]Dept. of Agricultural Biology, Chungnam National University, Daejeon 305-764, Korea
(Received April 11, 2002)

A Monokaryotic strain G8M without clamp connections was isolated from germinated basidiospore that was obtained from cultivated fruit body. Strain G8M was used as a tester isolate for 'dikaryon-monokaryon mating' (di-mon mating) with the strains of *Ganoderma lucidum*, G6 and G35 (Korean wild strains), G3 (Taiwan), G4 (Canada), G15 (America), *G. oregonense* G24, *G. resinaceum* G28, *G. oerstedii* G23, and *G. subamboinense* G29. Isolate G8M was compatible to Korean strains G6 and G35, but was incompatible to foreign strains G3, G4, or G15. Compatible reactions between strains were readily observed macroscopically. Clear barrage lines formed between incompatible strains. These clear lines were not apparent in compatible di-mon matings. The Korean strains were morphologically distinct; they did not form any chlamydospores, and stopped growth at 35°C. The strains of *G. lucidum* from Korea may be considered as different species from Taiwan, Canadian and American cultures.

KEYWORDS: Classification, Compatibility, Di-mon mating, *Ganoderma lucidum*

Traditionally, genus *Ganoderma* has been classified on morphological characteristics of fruit body such as size and color, and stipe attachment patterns (Steyaert, 1972; Corner, 1983). In nature, however, the morphological variation appears to be affected by environmental conditions during basidiocarp development. Furthermore, maturation of basidiocarps requires somewhat long periods that are affected by environmental factors to morphogenesis. Because most samples were generated to singletons which were affected by different environmental factors each other, the taxonomy of these fungi by morphological characteristics is very confuse.

In most basidiomycete, sexual reproduction and intraspecific mating is common. Mating with a standard tester strain is used to confirm whether the dikaryon was produced for species identification. However, it is very difficult to obtain the monokaryotic mycelium of *G. lucidum*, because the frequency of basidiospore germination is very low (Seo, 1995).

Buller (1930) reported that a monokaryotic isolate of *Coprinus lagopus* could fuse with a dikaryotic isolate. The nuclei of dikaryons moved to monokaryons and monokaryotic mycelium developed to dikaryotic mycelium. Quintanilha (1939) proposed that it should be called 'Buller's phenomenon'. Mating between dikaryotic mycelia and monokaryotic mycelia is generally referred to as di-mon mating (Papazian, 1950). Hseu *et al.* (1987) mentioned that classification of *Ganoderma* species was possible by di-mon mating.

*Corresponding author <E-mail: hgkim@cnu.ac.kr>

To elucidation of taxonomical situation of Korean *Ganoderma* species, growth and morphological characteristics of 8 species including foreign and Korean *G. lucidum* collected from different geographical origins were analysed. However, only growth and morphological characteristic data of *Ganoderma* species were insufficient for exact species identification (Kim, 2000). The objectives of this study were to provide a stronger basis for clarifying the taxonomy of *G. lucidum* from Korea using di-mon mating.

Materials and Methods

Strains and cultures. A tester isolate G8M was selected from germinated basidiospore that were obtained from cultivated fruit body of *G. lucidum* isolate G8. Thirteen strains of 8 species, *G. oerstedii*, *G. pfeifferi*, *G. subamboinense*, *G. weberianum*, *G. mirabile*, and *G. lucidum* complex such as *G. lucidum*, *G. oregonense*, and *G. resinaceum* were used in this study (Table 1). Two strains (G6 and G35) were isolated from Korean wild fruit bodies and 11 other strains were obtained from Institute of Agricultural Science and Technology, Suwon (G8 and G15) and Korean Collection for Type Cultures (G3, G4, G23, G24, G26, G28, G29, G33 and G48). All of the isolates were cultured on potato dextrose agar (PDA) at 25 ± 1°C and stored at 4°C until use.

Di-mon mating test. The dikaryotic strains were placed 3 to 4 cm apart from the monokaryotic isolates (G8M) on PDA plate and cultured for 2 weeks at 28 ± 1°C. Compati-

Table 1. Strains of *Ganoderma* species used in this study

Strain	Species	Strain number[a]	Locality	Nuclear type
G8M	*G. lucidum*	–	Korea	Monokaryon
G3	*G. lucidum*	ATCC 64251, KCTC 6283	Taiwan	Dikaryon
G4	*G. lucidum*	ATCC 46755, KCTC 6450	Canada	Dikaryon
G6	*G. lucidum*	ASI 7002	Korea, Wild strain	Dikaryon
G8	*G. lucidum*	ASI 7004	Korea, Cultivated strain	Dikaryon
G15	*G. lucidum*	MRI 5008	U.S.A.	Dikaryon
G23	*G. oerstedii*	ATCC 52411, KCTC 6286	Argentina	Dikaryon
G24	*G. oregonense*	ATCC 64487, KCTC 6287	U.S.A.	Dikaryon
G26	*G. pfeifferi*	CBS 747.84, KCTC 6512	Netherlands	Dikaryon
G28	*G. resinaceum*	ATCC 52413, KCTC 6453	U.S.A.	Dikaryon
G29	*G. subamboinense*	ATCC 52420, KCTC 6289	Argentina	Dikaryon
G33	*G. weberianum*	CBS 219.36, KCTC 6425	Philippines	Dikaryon
G35	*G. lucidum*	CNRDA 14	Korea, Wild strain	Dikaryon
G48	*G. mirabile*	CBS 218.36, KCTC 6424	Philippines	Dikaryon

[a]ASI : Institute of Agricultural Science, Korea, ATCC : American Type Culture Collection, U.S.A., CBS : Centraalbureau voor Schim melcultures, Netherlands, CNRDA : Chungnam Rural Development Administration, Korea, KCTC : Korean Collections for Type Cultures, MRI : Mushro om Research Institute, University of Pennsylvania, U.S.A.

bility between strains was verified microscopically by observance of the clamp connection of the mycelium of isolate G8M.

Results and Discussion

Microscopic morphology of di-mon mating test. Monokaryotic isolate G8M of *G. lucidum* was used as a tester isolate for di-mon mating with dikaryotic strains including Korean wild type strain G6 and G35, Taiwan strain G3, Canadian strain G4, American strain G15 as well as *G. oerstedii* G23, *G. oregonense* G24, *G. pfeifferi* G26, *G. resinaceum* G28, *G. subamboinense* G29, *G. weberianum* G33 and *G. mirabile* G48. Among those combinations, clamp connections were only formed between isolate G8M and Korean strains G6 or G35 (Table 2). Other *G. lucidum* strains including Taiwan (G3), Canadian (G4) and American strain (G15) did not form clamp connections. The isolates of *G. lucidum* complex such as *G. oregonense* and *G. resinaceum* also did not form clamp connections on the mycelia of isolate G8M.

Table 2. Mating reaction between monokaryotic strain of *Ganoderma lucidum* (G8M) and dikaryotic strains of *Ganoderma* species

Strain	Form clamp connection	Strain	Form clamp connection
G3	–	G26	–
G4	–	G28	–
G6	+	G29	–
G15	–	G33	–
G23	–	G35	+
G24	–	G48	–

+ : Formed clamp connection.
– : Not formed clamp connection.

Macroscopic morphology of di-mon mating test. Dikaryotic strains of 8 species, *G. lucidum*, *G. oerstedii*, *G. oregonense*, *G. pfeifferi*, *G. resinaceum*, *G. subamboinense*, *G. weberianum* and *G. mirabile* were incubated with monokaryotic isolates *G. lucidum* G8M by the dual culture on PDA. A monokaryotic isolate G8M of *G. lucidum* was compatibile with the dikaryotic strain G6 and G35, but incompatible with strains G3, G4, G15, *G. oregonense* G24, *G. resinaceum* G28, *G. oerstedii* G23, and *G. subamboinense* G29 (Fig. 1). Contact zone of incompatible combination showed clear brown contact line.

Hseu *et al.* (1989) reported that classification between species in genus *Ganoderma* was possible by di-mon test and suggested that it was the most effective method for classification. We used the di-mon mating test to provide a firm basis for clarifying the taxonomy of *G. lucidum* from Korea. The results of microscopic observations were in good agreement with the macroscopic features. This method is considered as very useful and economical than other methods, because it may save time and labor and do not need to find genetic markers.

In our previous research, Korean *G. lucidum* showed different characteristics in various aspects such as mycelial growth, micro- and macrosopic characters, compared to the Taiwan and North American *G. lucidum.* In addition, Korean *G. lucidum* strains also differed in many traits including growth characteristics of fruit bodies and histo-anatomical characteristics when compared with those for other geographical origins (Kim *et al.*, 2001a and b). Because the Korean *G. lucidum* strain G6, G8 and G35 did not form chlamydospores and not grow at 35°C, they were different from the other foreign strains of *G. lucidum* used in this study which formed chlamydospores and grew at 35°C.

Korean *G. lucidum* strains, therefore, could be consi-

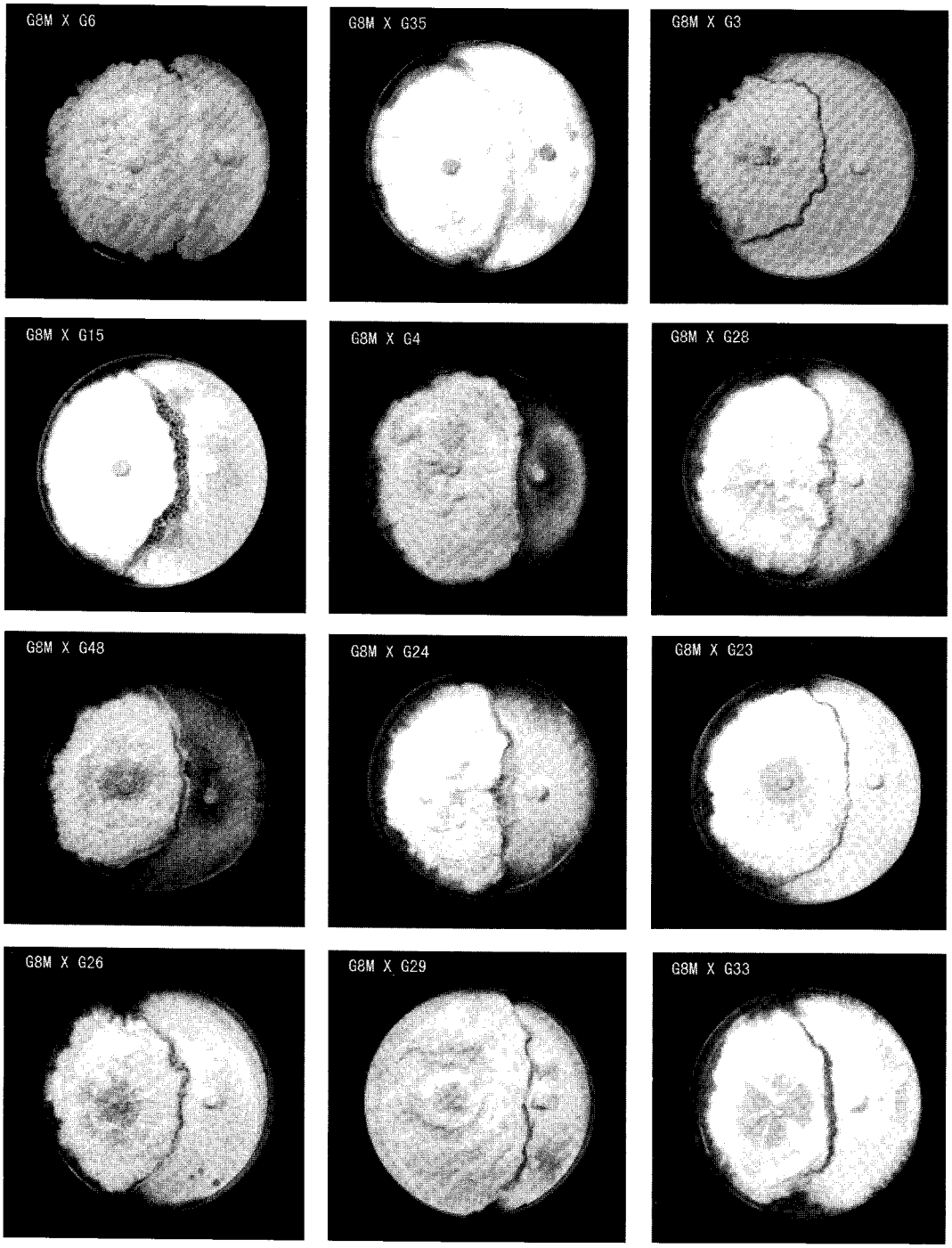

Fig. 1. Di-mon mating interaction between monokaryon of *Ganoderma lucidum* and dikaryon of various *Ganoderma* species after 14 days at 28°C. Monokaryotic strain of *G. lucidum* G8M was compatible with dikaryotic strains *G. lucidum* G6 and G35, but was incompatible with dikaryotic strains *G. lucidum* G3, G4 and G15.

dered to be a different species from Taiwan or North American strains by di-mon mating test in this study as well as previous results.

Acknowledgement

We are grateful to Dr M. P. Challen at Horticulture Research International, Wellesbourne, UK for his kind review of this manuscript.

References

Buller, A. H. R. 1930. The biological significance of conjugate nuclei in *Coprinus lagopus* and other Hymenomycetes. *Nature* **126**: 686-689.

Corner, E. J. H. 1983. Ad Polyporaceas I. *Amauroderma* and *Ganoderma*. *Nova Hedwigia* **75**: 1-182.

Hseu, R. S. and Wang, H. H. 1987. Application of dikaryon-monokaryon mating reaction to the identification of *Ganoderma* species. *J. Chinese Agri. Chem. Soc.* **25**: 118-124.

_____ and _____. 1989. Characterization of *Ganoderma lucidum* di-mon mating. *Sci. Culti. Edible Fungi* **XII**: 217-225.

Kim, H. K. 2000. Studies on taxonomical position of *Ganoderma lucidum* complex in Korea. Ph. D. Thesis. Chungnam National University, Korea (in Korean).

_____, Lee, H. D., Kim, Y. G., Han, G. H. and Kim, H. G. 2001a. Comparison of characteristics of *Ganoderma lucidum* according to geographical origins (I): Consideration of growth characteristics. *Mycobiol.* **29**: 1-6.

_____, Seo, G. S. and Kim, H. G. 2001b. Comparison of characteristics of *Ganoderma lucidum* according to geographical origins (II): Consideration of morphological characteristics. *Mycobiol.* **29**: 80-84.

Papazian, H. P. 1950. Physiology of the incompatibility factors in *Schizophyllum commune*. *Bot. Gaz.* **112**: 143-163.

Quintanilha, A. 1939. Etude genetique du phenomene du Buller. *Bol. Sco. Btoter.* **13**: 425-486.

Seo, G. S. 1995. *In vitro* photomorphogenesis and genetic diversity in the basidiomycete, *Ganoderma lucidum*. Ph. D. Thesis. Tottori University, Japan 1-132.

Steyaert, R. L. 1972. Species of *Ganoderma* and related genera mainly of the Bogor and Lieden herbaria. *Persoonia* **7**: 55-118.