Recent Advances in Molecular Systematics of the *Ganoderma lucidum* complex

Department of Agricultural Chemistry National Taiwan University
Rucy-Shyang Hseu

The genus Ganoderma was established by Karsten (1881) for the laccate and stipitate white rot fungus *Polyporus lucidus* W. Curt. Ganoderma is a cosmopolitan genus of wood decaying polypore fungi of economic importance. Several species cause severe diseases in plantations or in forests. In the Orient Ganoderma is merely regarded as an herb of longevity. The fungus has been used in folk medicine for hundreds of years and strains are commercially cultivated for preparation of health products. Isolates used in pharmaceutical and medicinal studies, and consequently commercially cultivated isolates, largely refer to G. lucidum. However, as used in the pharmaceutical literature, this name encompasses several laccate Ganoderma species that might differ in their bioactive compounds.

The G. lucidum species complex includes *G. tsugae* Murr., *G. valesiacum* Boud., *G. oregonense* Murr., *G. resinaceum* Boud., *G. pfeifferi Bres., G. oerstedii (Fr.)* Torr., G. ahmadii Stey, and several other taxa that are restricted to tropical areas. The use of traditional taxonomic methods was inconclusive in systematic of the group, and these methods are useless to characterize individual strains. However, an accurate identification system and a phylogenetically-based classification of Ganoderma taxa, together with development of genetic markers for individual strains, would have practical implications in epidemiology studies, and pharmacology.

We examined 45 isolates representing several genera, subgenera, sections and species of Ganodermataceae to infer natural relationships in the group. Phylogenetic characters were produced from nucleotide sequence data from the internal transcribed spacer regions (ITS 1 and ITS 2) of the ribosomal gene (rDNA) and from divergent region D2 of the large ribosomal subunit gene (LSU-D2). The ITS dataset provided phylogenetic information at lower taxonomic levels while the LSU dataset was more useful at higher levels. Results of parsimony analysis support Ganoderma, and Amauroderma as distinct genera. Results indicated that subgenus Elfvingia is monophyletic, while sections Characoderma and Phaeonema are not. (Ref. 1)

A gene phylogeny of 29 isolates of the G. lucidum complex collected in temperate and subtropical areas was produced by parsimony analysis from nucleotide sequence data of the ITS and LSU-D2. Results were compared with morphological, ecological, cultural and mating data. Phylogenetically related isolates have similar culture characteristics, but they may share these characteristics with distant taxa. Therefore, culture characters are less polymorphic than morphological characters between recently diverged taxa, but are useless in recognizing monophyletic groups. A species concept based on monophyly and potential evidence of genetic isolation is proposed, and taxonomy of the G. lucidum complex is revised. Collections named *G. lucidum* in North America and in Asia are not conspecific with European G. lucidum. The sister group of European G. lucidum is an Argentinean taxon labelled *G. oerstedii*. North American G. lucidum is related to a Formosan isolate identified as *G.* boninense. (Ref. 2)

Parsimony analysis of nueleotide sequences produced from the internal transcribed spacers (ITS) of the ribosomal gene (rDNA) distinguished six phyla in G. lucidum complex. Each phylum represented one or more putative species. While some isolates have identical ITS sequence, all of them could be clearly differentiated by genetic fingerprinting using random amplified polymorphic DNA.

To investigate the suitability of RAPD markers for taxonomic identification and grouping of isolates of the *G.* lucidum complex, RAPD fragments (RAPDs) were used as phenotypic characters in numerical and parsimony analyses. Results show those data from RAPDs do not distinguish the same clades as ITS data do. Groupings based on analysis of RAPD data were very sensitive to the choice of the grouping method used, and no consistent grouping of isolates could be proposed. However, analysis with RAPDs did resolve several robust terminal clades containing putatively conspecific isolates, suggesting that RAPDs might be helpful for systematics at the lower taxonomic levels that are unresolved by ITS sequence data. The conclusion is that ITS sequence can be used to identify isolates of the *G.* lucidum complex, whereas RAPDs can be used to differentiate between isolates having identical ITS sequences. (Ref. 3)

Last year (1996) the nucleotide sequence of a cDNA encoding manganese superoxide dismutase (SOD; EC 1.15.1.1) from Ganoderma microsporum Hseu has been determined. It is the first time that the nueleotide sequence of MN-SOD gene from Basidiomyeetes was reported. By using PCR primers designed from the cDNA nucleotide sequence of G. microsporum, an internal fragment of MN-SOD genes possesses two introns that interrupt the coding region from 28 Ganoderma isolates amplified. Phylogenetic analysis of the nueleotide sequence data from the internal fragment of MN-SOD gene support Ganoderma, Amauroderma and Fomitopsis are distinct genera. This 28 Ganoderma isolates were evidenced to 5 groups, similar to the result from the internal transcribed spacers (ITS) of the ribosomal gene (rDNA), and MN-SOD gene nueleotide sequence could even differentiate the basal relationships within these groups that were not resolved by ITS nueleotide sequence. We found the MN-SOD gene nucleotide sequence of Ganoderma is genus-specific, species-specific, and strain-specific. (Ref. 4)

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Ruey-Shyang Hseu

Associate Professor in Department of Agricultural Chemistry, National Taiwan University, Born on 26th, June, 1958

Education

1981 : B. Sc. in Agriculture, National Taiwan University, Taipei, Taiwan, ROC.

1984 : M. Sc. in Agriculture, National Taiwan University

1990 : Ph. D. in Agriculture, National Taiwan University

Work Experience : Teaching Assistant (1981), Lecture(1986), Associate Professor(1990) in Department of Agricultural Chemistry, National Taiwan University

Memberships : Chinese Agricultural Chemical Society. Mycological Society of Republic of China, America, British, Japan and Korean

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CANITED **Reishi**