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Studies on microscopic and cultural characteristics of *Ganoderma lucidum*

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The microscopic and cultural characteristics of three strains of *G. lucidum* were studied following prescribed methods. The hyphal system was observed to be trimitic with presence of generative, skeletal and binding hyphae. The basidiospores of all isolates were similar in morphology but varied in size with 9.5-11.0 x 6-7.6 µm for G-1, 8.0-10.0 x 5.9-8.0 µm for G-2 and 9.1-10.8 x 6.8-7.9 µm for G-3. All the isolates produced abundant chlamydospores in culture. The colony colour was observed to be dull white in G-1 and white in G-2 and G-3. The colony margin was wavy in G-1, nearly smooth in G-2 and smooth in G-3. The growth habit of mycelium in G-1 appeared trailing; mycelium was thin aerial towards periphery with concentric ridges. The strain G-2 showed fluffy growth, mycelial growth was thick and aerial at centre but thinner towards periphery. The third strain G-3 produced smooth mycelial growth. The oxidase reaction of test G. *lucidum* strains indicated a strong oxidase reaction on tannic acid medium and weak reaction on gallic acid medium.

Key words: Microscopic and cultural characteristics, *Ganoderma lucidum,* oxidase reaction

INTRODUCTION

Ganoderma lucidum is a cosmopolitan polypore known to cause white rot of hardwoods of many tree species (Hepting, 1971; Adaskaveg and Ogawa, 1990). Its fruiting bodies have been used in traditional Chinese medicines for treatment of various human ailments such as hepatitis, chronic bronchitis, gastritis, tumor growth and various immunological disorders (Ling Zhu *et al.*, 2007).

Murril (1902) has considered primary taxonomic characters to be host specificity, geographical distribution and macro morphology of fruiting body while Nobles (1958) has suggested use of cultural characters in developing a taxonomy of Polyporaceae that reflects natural relationships and phylogeny. Cultural studies provide additional information on the biology of wood decaying basidiomycetes. Corner (1983) has maintained that taxonomy of Ganodermataceae should be simplified to base a new classification on basidiocarp developmental studies in the field. Gilbertson and Ryvarden (1986) have reported microscopic characters as of major taxonomic importance in polypores. According to Furtado (1962; 1965) the basidiospores of Ganodermoid polypores are the most dependable character because of their distinctive wall structure. In India, Tiwari *et al.* (2005) have reported utility of different classical and modern taxonomic aspects of genus *Ganoderma* including macro and micro morphology, cultural characters and phylogeny in relation to Indian species. Thus considering the taxonomic significance an attempt has been made to study the microscopic and cultural characteristics of *G. lucidum* which could be of help in its taxonomical clarification.

MATERIALS AND METHODS

A laboratory experiment was carried out during 2009-2010 in the Division of Plant Pathology, Sher-e- Kashmir University of Agricultural Sciences and Technology, Jammu (J&K).

Three strains of *G. lucidum* used in present investigation were coded as G-1, G-2 and G-3. G-1(Israeli isolate or NRCM - OE- 62) and G-2 (NRCM -OE-52) were procured from mushroom centre of Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan and Directorate of Mushroom Research, Solan, H.P, respectively,

while G-3 was a local isolate, the pure culture of which was made through tissue culture.

The pure cultures of *G. lucidum* were maintained by repeated sub culturing on malt extract agar (MEA) at 10 days interval and incubated at 25±1ºC.

Microscopic studies

Microscopic studies were done under light microscope for studying hyphal system, basidiospores and resistant structures, if any.

In order to study hyphal system, the technique of hyphal analysis of polypores developed by Corner (1932, 1953) and also described by Teixeira (1962) was followed. The hyphae from various parts of fruit body; the growing margin, the adult flesh, the context tissue immediately above the tubes and the dissepiments i.e. the tissues separating the tubes were taken. The thin sections of flesh from these parts were individually teased out with fine needles under a dissecting microscope. For studying generative hyphae mycelia were taken from fresh cultures in Petriplates and immature sporophores, while for studying binding and skeletal hyphae mycelia were taken from immature and mature sporophores. *In vitro* cultures of *G. lucidum* were also studied for hyphal structures.

For morphological studies of basidiospores the methodology given by Adaskaveg and Gilbertson (1986) was followed. Basidiospores were extracted from fruiting body tubes by aseptically removing several portions of inner tubes above the pore space. The sections of tubes were then placed in 5 ml sterile distilled water in a small glass beaker and shaken thrice on a mechanical shaker for 30 seconds at an interval of 5 minutes. The suspension was poured through three layers of cheese cloth to remove the tube tissues and the basidiospores were counted using a haemocytometer. Spore length and width were determined for 25 spores of each specimen.

Cultural studies

Cultural morphology was studied according to Nobles (1958). The cultural studies were done by growing the test strains of *G. lucidum* on malt extract agar medium. The malt extract agar medium was prepared by adding 25 g of malt extract and 15 g of agar agar to 1000 ml of distilled water. Then this media was autoclaved at 121ºC for 20 minutes. The prepared media was poured aseptically under laminar flow in Petriplates and kept for solidification. Mycelial discs (5 mm) were cut with sterilised cork borer from actively growing mycelium of each strain and inoculated aseptically in centre of these solidified Petriplates. The inoculated Petriplates were incubated at 25±1ºC and observed periodically for various characteristics till full growth of fungal colony.

Oxidase reaction on tannic and gallic acid medium

To determine the lignin degrading ability of *G. lucidum* strains oxidase reaction was assessed, using the methodology adopted by Davidson *et. al.,* (1938). The oxidase reaction was studied by growing the test *G. lucidum* strains on gallic and tannic acid media.

Preparation of gallic and tannic acid media

Tannic acid medium was prepared by adding 0.5 per cent of tannic acid to malt extract agar medium. Twenty grams of powdered agar and 15 grams of malt extract were dissolved in 850 ml of water in a 2 litre conical flask. 150 ml of water was placed in a separate flask. The dissolved malt agar and water in the flask were autoclaved for 20 minutes at 121ºC. Five grams of tannic acid was dissolved in the flask containing sterilized water after removal from the autoclave. Because heating tannic or gallic acid with agar causes hydrolysis of agar, therefore, the two were not autoclaved together. After malt agar medium had cooled slightly so that it could be handled easily, the tannic acid solution was added and thoroughly mixed. The resulting tannic acid medium was then aseptically poured (about 35 ml) into each sterile 90 mm Petridish. Gallic acid was also prepared in a similar way. Here tannic acid was replaced by gallic acid. On solidification the plates were inoculated in the centre with 7 mm mycelia discs of actively growing test strains of *G. lucidum* and incubated at 30±1 ºC for a week. Three replications were maintained for each treatment. The oxidase reaction in the form of dark brown diffusion zone under the fungal mat was observed in the individual Petriplates for interpretation of results.

Scale for assessing the oxidase reaction

+++ = strong reaction, dark and extending well beyond mycelial margins.
++ = moderate reaction, dark and extending just beyond mycelial margins.
+ = weak reaction, light in colour and remaining under mycelial mat.
- = negative reaction.

RESULTS AND DISCUSSION

The data on the microscopic and cultural characteristics studied are presented in Table 1.

Microscopic characteristics

From the data it is clear that the hyphal system is trimitic, with presence of three basic types of hyphae, the generative hyphae, the skeletal hyphae and binding hyphae. The generative and skeletal hyphae were found in growing margins and tissues of dissepiments while binding hyphae were found only in adult flesh some distance behind the hyphal tip. The generative hyphae were also produced by *in vitro* cultures of *G. lucidum.* The generative hyphae were hyaline and branched with presence of clamp connections.

The skeletal hyphae were unbranched and also with terminal dendritic branching thick walled with narrow lumen and pigmented. The binding hyphae were colourless, much branched and thick walled with terminal branching.

The basidiospores of all the test strains of G. *lucidum* were brown coloured, ovoid shaped, truncate at the apex and double walled with inter wall pillars separating the two. The size of basidiospores differed among the strains and it was 9.5-11.0 × 6-7.6 µm for G-1, 8.0-10.0 × 5.9-8.0 µm for G-2 and 9.1-10.8 × 6.8-7.9 µm for G-3.

Cultural characteristics

The macroscopic cultural characteristics varied among the strains. The colony colour was observed to be dull white in G-1 and white in G-2 and G-3. The colony margins also varied among the strains. The colony margin was wavy in G-1, nearly smooth in G-2 and smooth in G-3. The growth habit of mycelium in G-1 appeared trailing; mycelium was thin aerial towards periphery with concentric ridges. The strain G-2 showed fluffy growth, mycelial growth was thick and aerial at centre but thinner towards periphery. The third strain G-3 produced smooth mycelial growth.

All *G. lucidum* strains studied produced abundant terminal and intercalary chlamydospores in culture. The shape of chlamydospores was globose to fusiform with smooth and thick walls.

Oxidase reaction on tannic and gallic acid media

The oxidase reaction of test *G. lucidum* strains indicated a strong and moderate reactions on tannic acid medium and weak reaction on gallic acid medium.

On tannic acid medium strain G-1 gave a strong oxidase reaction while strains G-2 and G-3 gave a moderate reaction. On gallic acid medium all the test strains gave a weak reaction.

The observations about trimitic hyphal system in *G. lucidum* are similar to those observed by Cunningham (1954). The hyphal characters are often useful in species identification (Zhao, 1989). Adaskaveg and Gilbertson (1986) while studying microscopic characteristics of *G. lucidum* got similar observations about basidiospore morphology. According to Furtado (1962) the basidiospores of ganodermoid polypores are the most dependable character because of their distinctive wall structure. Ganodermataceae have a unique double walled basidiospore and this family was created specially to include polypore fungi characterized by double walled basidiospores. The difference in size of basidiospores could be due to some strain variability.

In vitro morphogenesis and cultural characteristics of basidiomycetes are affected by various environmental factors as light, aeration, temperature, humidity and nutritional conditions (Manachere, 1980). Critical studies on cultural characteristics are very important in species identification of higher basidiomycetes.

The production of abundant chlamydospores in *G. lucidum* cultures has also been reported by Adaskaveg and Gilbertson (1986).

Positive oxidase reaction indicate that *G. lucidum* belong to white rot group of fungi while strong and moderate oxidase reaction on tannic acid medium and weak oxidase reaction on gallic acid medium

Table 1 : Microscopic and cultural characteristics of *Ganoderma lucidum*

Microscopic characteristics	Strain		
	G1 (OE-62)	G2 (OE-52)	G3 (local strain)
1. Hyphal system	Trimitic	Trimitic	Trimitic
a) Generative hyphae	Hyaline, Branched	Hyaline, Branched	Hyaline, Branched
b) Skeletal hyphae	Unbranched thick- walled with a narrow lumen and pigmented	Unbranched thick-walled with a narrow lumen and pigmented	Unbranched thick-walled with a narrow lumen and pigmented
c) Binding hyphae	Colorless, much branched, thick walled	Colorless, much branched, thick walled	Colorless, much branched, thick walled
2.Chlamydospores	Present	Present	Present
3. Basidiospores			
a) Color	Brown	Brown	Brown
b) Shape	Ovoid and truncate at the apex	Ovoid and truncate at the apex	Ovoid and truncate at the apex
c) Walls	Double-walled with inter-wall pillars separating the two	Double-walled with inter-wall pillars separating the two	Double-walled with inter-wall pillars separating the two
d) Size	9.5 - 11.0 x 6 - 7.6µm	8.0 – 10.6 x 5.9 - 8µm	9.1-10.8 x 6.8 – 7.9µm
Cultural characteristics			
1). Colony Color	Dull white	White	White
2) Colony Margins	Wavy	Nearly smooth	Smooth
3).Growth Habit	Trailing, mycelium thin aerial towards periphery with concentric rings	Fluffy, mycelium growth thicker and thinner at periphery, 1-2 concentric rings, aerial mycelium towards centre	Smooth mycelium growth
4) Oxidase reaction			
a) On Tannic acid Medium	+++	++	++
b) On Gallic acid Medium	+	+	+

indicate that test *G. lucidum* strains are predominantly lignin degrading. The ability to degrade lignin varied among the strains tested as strain G-1 proved to be more lignin degrading by giving strong oxidase reaction on tannic acid medium while strains G-2 and G-3 proved to be moderate lignin degraders.

Present observations are in line with those of Davidson *et. al.,* (1938) who reported formation of a dark diffusion zone under the fungus mats in case of white rot fungi and no such reaction in brown rot fungi, when test fungi were grown on media containing small amount of gallic and tannic acid. The findings about lignin degrading ability are supported by Nobles (1958) who found a correlation between lignin degradation by a fungus and production of extracellular oxidase enzyme in pure culture which could be detected by oxidation of gallic or tannic acid to a brown colour.

The information generated on microscopic and cultural characteristics can be of help in distinguishing species with the *G. lucidum* complex.

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