Collection and documentation of medicinal mushroom associated *Trichoderma* species

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Abstract

In the present investigation, the *Ganoderma lucidum* mushroom were collected from eighteen different places in and around Thanjavur District, Tamilnadu, India *viz.*, Kavarappattu, Ukkanadu, Pappanadu, Orathanadu, Thennamadu, Uloor, Nellupattu, Paruthiyappar Kovil, Mariyamman Kovil, Poondi, Koviloor, Ammapettai, Thirukarukkavur, Karanthai, Srinivasapuram, Kodimarathumoolai, Tamil University and Nanjikkottai and from different substrates of *Tamarindus indica, Bambusa vulgaris, Borassus flabellifer, Azadirachta indica, Cassia fistula* and *Musa paradisiaca* and *Ganoderma lucidum*. Ten *Ganoderma* associated *Trichoderma* strains were isolated from *Ganoderma lucidum* mushroom and documented.

Key words: Ganoderma, fungal isolates, macro fungi, medicinal mushroom, *Trichoderma.*

INTRODUCTION

Ganoderma lucidum, an oriental fungus, has a long history of use for promoting health and longevity in China, Japan, and other Asian countries. It is a large, dark mushroom with a glossy exterior and a woody texture. The Latin word *lucidus* means "shiny" or "brilliant" and refers to the varnished appearance of the surface of the mushroom. In China, *G. lucidum* is called *lingzhi*, whereas in Japan the name for the Ganodermataceae family is *reishi* or *mannentake*.

In Chinese, the name *lingzhi* represents a combination of spiritual potency and essence of immortality and is regarded as the "herb of spiritual potency," symbolizing success, well-being, divine power, and longevity. Among cultivated mushrooms, *G. lucidum* is unique in that its pharmaceutical rather than nutritional value is paramount. A variety of commercial *G. lucidum* products are available in various forms such as powders, dietary supplements and tea. These are produced from different parts of the mushroom, including mycelia, spores and fruit body. The specific applications and attributed health benefits of *lingzhi* include control of blood glucose levels, modulation of the immune system, hepatoprotection and bacteriostasis,. The various beliefs regarding the health benefits of *G. lucidum* are based largely on anecdotal evidence, traditional use, and cultural mores. However, recent reports provide scientific support and some of the ancient claims of the health benefits of *lingzhi*.

Lingzhi has been recognized as a medicinal mushroom for over 2000 years, and its powerful effects have been documented in ancient scripts (Wasser 2005). The proliferation of *G. lucidum* images in art began in 1400 AD, and they are associated with Taoism (McMeekin 2004). However, *G. lucidum* images extended beyond religion and appeared in paintings, carvings, furniture and even in women's accessories (Wasser 2005). The first book wholly devoted to the description of herbs and their medicinal value was *Shen Nong Ben Cao Jing*, written in the Eastern Han dynasty of China (25-220 AD). This book is also known as "Classic of the Materia Medica" or "Shen-nong's Herbal Classics." It describes botanical, zoological, and mineral substances, and was composed in the second century under the pseudonym of Shen-nong ("the holy farmer"; Zhu, 1998). The book, which has been continually updated and extended, describes the beneficial effects of several mushrooms with particular reference to the medicinal mushroom *G. lucidum* (Zhu, 1998; Upton 2000; Sanodiya *et al.* 2009). In the *Supplement to Classic of Materia Medica* (502-536 AD) and the *Ben Cao Gang Mu* by Li Shin-Zhen, which is considered to be the first pharmacopoeia in China (1590 AD; Ming dynasty), the mushroom was attributed with therapeutic properties, such as tonifying effects, enhancing vital energy, strengthening cardiac function, increasing memory, and antiaging effects. According to the *State Pharmacopoeia of the People's Republic of*

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China (2000), *G. lucidum* acts to replenish Qi, ease the mind, and relieve cough and asthma, and it is recommended for dizziness, insomnia, palpitation, and shortness of breath.

MATERIALS AND METHODS

Description of sample sites

Fruit bodies of *G.lucidum* were collected from various places of Thanjavur districts in Tamil Nadu, India *viz.,* Kavarappattu, Ukkanadu, Pappanadu, Orathanadu, Thennamadu, Uloor, Nellupattu, Paruthiyappar Kovil, Mariyamman Kovil, Poondi, Koviloor, Ammapettai, Thirukarukkavur, Karanthai, Srinivasapuram, Kodimarathumoolai, Tamil University and Nanjikkottai and from different substrates of *Tamarindus indica, Bambusa vulgaris, Borassus flabellifer, Azadirachta indica, Cassia fistula* and *Musa paradisiaca* and *Ganoderma lucidum.*

Sample collection

This study has been undertaken by collection for one year from Nov -2019 and Dec - 2019. At the same time, isolation of tissue cultures from the basidiomes was carried out for further studies. The study of the basidiomes was made on macro (size, colour, number pores/mm, length of tubes) and microscopic characters (somatic and reproductive structures). Colours are according to Munsell (1975) and Herbaria abbreviations following Holmgren *et al*. (1990). The collected fruitbodies were carefully stored in polythene bags and transported to the laboratory for mycological examination.

Isolation of *G. lucidum* (Ryvarden, 1991)

Tissues of *Ganoderma* fructification were surface sterilized with tap water and 0.1% mercuric chloride solution, then rinsed for 2min in sterile distilled water. Fruit bodies of *G.lucidum* were inoculated on to the Potato Dextrose Agar (PDA) medium. After inoculation the fungal cultures were purified using pure culture technique and stock culture was maintained in PDA slants for further investigation.

Identification

Colony colour and morphology were noted besides hyphal structure, spore size, shapes and spore bearing structure. Identification has been done by referring the standard manuals of soil fungi by Gilman, (1957) and Ainsworth *et al.,* (1973). Spore identification was achieved by reference to Spore atlase of Gregory and Walling (1973).

Lactophenol Cotton blue mounting

A drop of lactophenol cotton blue stain was placed on the clean glass slide, a small tuft of the fungus, preferable with spores and spore bearing structures were transferred into the drop, using a flamed, cooled needle and gently tested using mounted needle. A cover glass was placed over the preparation and care was taken to avoid trapping of air bubbles in the stain. A thin layer of DPX mount was placed around the edge of the coverslip. The slide was observed under the microscope (400x). Microphotography of the individual fungal species was also taken using Nikon phase contrast microscope (Nikan, Japan).

Table 1. Diversity of *Ganoderma* sp. in diffeent places of Thanjavur District

S.No	Different places of Thanjavur District.	Substrates	Isolate code
1	*Kavarappattu*	*Tamarindus indica*	APGI
2	Ukkanadu	*Tamarindus indica*	APG II
3	Pappanadu	*Bambusa vulgaris*	APGIII
4	Orathanadu	*Tamarindus indica*	APGIV
5	Thennamadu	*Tamarindus indica*	APGV
6	Uloor	*Borassus flabellifer.*	APGVI
7	Nellupattu	*Azadirachta indica*	APGVII
8	Paruthiyappar Kovil	*Bambusa vulgaris*	APGVIII
9	Mariyamman Kovil	*Tamarindus indica*	APGIX
10	Poondi	*Tamarindus indica*	APGX
11	Koviloor	*Tamarindus indica*	APGXI
12	Ammapettai	*Tamarindus indica*	APGXII
13	Thirukarukkavur	*Tamarindus indica*	APGXIII
14	Karanthai	*Bambusa vulgaris*	APGXIV
15	Srinivasapuram	*Tamarindus indica*	APGXV
16	Kodimarathumullai	*Tamarindus indica*	APGXVI
17	Tamil University	*Cassia fistula*	APGXVII
18	Nanjikkottai	*Musa paradisiaca*	APGXVIII

A= *Ambikapathy* P = *Prakash* G = *Ganoderma*

RESULTS AND DISCUSSION

Diversity of *Ganoderma* sp. in Thanjavur district was recorded and presented in Table 1. The *Ganoderma* were collected from *Kavarappattu*, Ukkanadu, Pappanadu, Orathanadu, Thennamadu, Uloor, Nellupattu, Paruthiyappar Kovil, Mariyamman Kovil, Poondi, Koviloor, Ammapettai, Thirukarukkavur, Karanthai, Srinivasapuram, Kodimarathumoolai, Tamil University and Nanjikkottai and from different substrates like *Tamarindus indica, Bambusa vulgaris, Borassus flabellifer, Azadirachta indica, Bambusa vulgaris, Cassia fistula* and *Musa paradisiacal.V*arious isolates code were created *viz.,* APGI, APG II, APGIII, APGIV, APGV, APGVI, APGVII, APGVIII, APGIX, APGX, APGXI, APGXII, APGXIII, APGXIV, APGXV, APGXVI, APGXVII and APGXVIII formed to be recorded respectively (Table 1). The macro fungi like *Ganoderma*

Table 2. Isolation of *Trichoderma* from *Ganoderma*

S.No	Different strains of *Ganoderma* sp.	*Trichoderma* strains
1	APG I	GAT I
2	APG II	GAT II
3	APG III	GAT III
4	APG IV	N
5	APG V	GAT IV
6	APG VI	GAT V
7	APG VII	N
8	APG VIII	GAT VI
9	APG IX	N
10	APG X	N
11	APG XI	GAT VII
12	APG XII	N
13	APG XIII	GAT VIII
14	APG XIV	N
15	APG XV	N
16	APG XVI	GAT IX
17	APG XVII	GAT X
18	APG XVIII	N

G = *Ganoderma* A = Association T = *Trichoderma* N = No growth

sp. are important components of diversity which has major role in global ecological processes. They were found to be dependent on the nature of substrates and regions that favours their colonization and growth (Table-1).

From the eighteen isolates of *Ganoderma viz*., APG I, APG II, APG III, APG IV, APG V, APG VI, APG VII, APG VIII, APG IX, APG X, APG XI, APG XII, APG XIII, APG XIV, APG XV, APG XVI, APG XVII and APG. different strains of *Trichoderma viz*., GAT I, GAT II, GAT III, GAT IV, GAT V, GAT VI, GAT VII, GAT VIII, GAT IX and GAT X were recorded (Table-2).

High variability was observed in macroscopic characters of the Chinese '*G.lucidum*' (Ling-zhi), especially in those cultivated samples. Seven characters of basidiomata were examined for their variability. The results showed that the shape of pileus varied from reniform to subcircular and also from convex to concave; the length of stipe was from very short to long; attachment of the stipe to the pileus varied from lateral to nearly central; the surface of pileus was either with radial furrows or with concentrically sulcate zones; the thickness of pileus appeared from one layer (thinner than 1 cm) to several layers; the colour of pore surface varied from whitish to yellowish, the length of tube layer was from short (less than one third of the pileus thickness) to long. The morphological variability was found not only in different specimens but also often seen in a single collection. Attachment of stipe to the pileus varied from nearly central to lateral and pileus thickness varied from very thin to considerably thickness (Xin-cun *et al* 2012). To help the potent choice of sanitary compounds that could be used for mushroom cultivation facility as preventive measures, Jun *et al*.(2019) assessed the sensitivity of four *Trichoderma* species against twelve antifungal agents *viz*., tebuconazole, benzimidazol, difenoconazole, hexaconazole, tricyclazole, fluquinoconazole, fludioxonil, fenarimol, dichloran, benzalkonium chloride, and benomyl. *T. harzianum* and found that the strain DUCC4018 could grow in all concentrations of test antifungal agents, except every concentration of benomyl (10 μg/ml, 100 μg/ml, and 1,000 μg/ml).

In the present study different strains of *Trichoderma* were isolated from *Ganoderma* sp. and each strain had high potential longevity and remarkable functions in the physiological parameters.

The systematic diversity and taxonomy of *Ganoderma* species need to be confirmed. The *Trichoderma* showed excellent suppression of the growth of the pathogens and its population in the rhizosphere thus reduce disease development through competition. Since it produces antibiotics and toxins such as trichothecin and trichodermin, it has direct effect on the pathogens. *Trichoderma* sp. provides natural long term immunity for crops and soil. As such, the *Trichoderma* sp.is a potential candidature for biocontrol agent in the sustainable agricultural practice.

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