COMPLETE REPORT

Saprobic fungi on dead leaves of *Magnolia liliifera*: Diversity and some biological activities

BY

Asst. Prof. Dr. Ekachai Chukeatirote
Assoc. Prof. Dr. Kevin Hyde
School of Science

This research was made possible by support of
Mae Fah Luang University 2013

Contract number 33/2556
Grant number 56101020033

Saprobic fungi on dead leaves of Magnolia liliifera: Diversity and some biological activities

BY

Asst. Prof. Dr. Ekachai Chukeatirote

Assoc. Prof. Dr. Kevin Hyde

School of Science

This research was made possible by support of

Mae Fah Luang University 2013

EXECUTIVE SUMMARY

Fungi are one of the fascinating organisms. Their diversity remains debatable and is second only to insects in the number of species thought to exist. The most widely accepted numbers of the fungal species is ca. 1.5 million and some researchers believe that the fungal species number is much higher. Presently, 100,000 fungal species (~7%) have been discovered and hence many fungi remain to be further explored. The fungal organisms play a key role in ecological functioning, have an impact in causing disease, and are potential in biotechnological innovations such as in new medicine, nutriceutical, enzyme, food and insecticide discovery and production. This study is therefore undertaken to study a diversity of fungi on dead leaves of *Magnolia liliifera*. This plant species is an important economic plant. Our study reveals 35 fungal taxa including three possible new species. In addition, some interesting isolates were selected to test if they could produce the metabolites exhibiting antimicrobial activity. Based on a disc diffusion assay, it was found that most fungal isolates produced antimicrobial compounds against one or more testing microbes. This present study is expected to provide an insight on biodiversity data of saprobic fungi in Thailand and potential use of these fungal bioresources for production of useful bioactive compounds.

ABSTRACT

In this study, the diversity of fungi on decaying leaves of *Magnolia liliifera* was determined. Thirty-five taxa were identified from *M. liliifera* consisting of 8 ascomycetes and 27 asexual taxa. The most abundant species found were *Sporidesmium* sp. (80%), *Colletotrichum fructicola* (70%) and *Stachybotrys parvispora* (70%). Decaying leaves collected in an early stage of decomposition supported greater fungal diversity than those collected in a later stage of decomposition. Saprobic fungi were also found to be specific to different tissues types (leaf lamina, midrib, petiole). One taxon obtained from the diversity study of saprobic fungi on leaves of *M. liliifera* is probably new to science. This isolate was initially designated as Ascomycete sp. 1 based on its ascus structure. The isolate is now being studied further in terms of both morphology and gene sequence data. It is expected that molecular analysis will help identify these fungal taxa. Some saprobic fungi were selected to screen their antimicrobial activity. The fungal crude extracts were obtained using ethyl acetate. It was showed that these fungal extracts were able to inhibit the growth of some microbial pathogens using a disc diffusion assay.

Keywords: Antimicrobial activity; Fungal diversity; *Magnolia liliifera*; Taxonomy

บทคัดย่อ

ในงานวิจัยนี้ ผู้วิจัยได้ทำการศึกษาความหลากหลายของเชื้อราบนใบไม้ที่เน่าเปื่อยของมณฑาดอย จากการทดลองผู้วิจัยสามารถแยกเชื้อราและจัดจำแนกได้เป็นจำนวน 35 ชนิด ประกอบด้วย เชื้อราในกลุ่มแอสโคมัยซีตจำนวน 8 ชนิด และเชื้อราในกลุ่มที่ไม่พบโครงสร้างแบบอาศัยเพศจำนวน 27 ชนิด สำหรับเชื้อราที่พบเป็นจำนวนมากได้แก่ เชื้อราชนิด *Sporidesmium* (80%), *Colletotrichum fruticola* (70%) และ *Stachybotrys parvispora* (70%) นอกจากนี้ ผู้วิจัยได้ศึกษาถึงการปรากฏของเชื้อราในระยะของการเน่าเปื่อยที่ต่างกัน พบว่า การปรากฏและจำนวนของเชื้อราในระยะขั้นต้นของการเน่าเปื่อยจะมีความหลากหลายที่สูงกว่า และที่สำคัญ ข้อมูลที่ได้จากการทดลองยังพบว่า การปรากฏของกลุ่มเชื้อราจะมีความจำเพาะต่อบริเวณที่แตกต่างกันของใบไม้

นอกจากนี้ ยังพบเชื้อรา 1 ชนิดที่แยกได้มีลักษณะเด่นทางสัณฐานวิทยา และมีแนวโน้มน่าจะเป็นเชื้อราชนิดใหม่ อย่างไรก็ตาม ข้อมูลที่ได้จากลักษณะรูปร่างเพียงอย่างเดียวยังไม่เพียงพอ ดังนั้น การศึกษาทางด้านลำดับพันธุกรรมจะช่วยยืนยันผลการทดลองดังกล่าวนี้ ซึ่งอยู่ระหว่างการศึกษาเพิ่มเติม

ผู้วิจัยได้ทำการคัดเลือกเชื้อราบางสายพันธุ์ เพื่อทำการทดสอบความสารมารถในการสร้างสารที่มีฤทธิ์ยับยั้งเชื้อจุลินทรีย์ก่อโรค จากการทดลองพบว่า สารสกัดหยาบจากเชื้อราที่แยกได้ส่วนใหญ่มีฤทธิ์ยับยั้งการเจริญของเชื้อทดสอบได้อย่างน้อย 1 ชนิด

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

1.1 Background

Fungi are eukaryotic organisms which are a large group and diverse in morphology, physiology, ecology and genetics. They play several key roles that are important in improving human welfare. These include the utilization of fungi in agriculture and biotechnology. Various products from fungal metabolites are widespread such as biofertilizers, mycoherbicides, fermented food products, antibiotics, enzymes and organic acids. In addition, fungi also act as decomposers in the ecosystem; this is an important mechanism of organic matter degradation.

Magnolia liliifera is one of the most valuable ornamental fragrant trees of Asia. This plant species is in the Family Magnoliaceae. The natural range of this species albeit not restricted has a major centre in East and South-East Asia. It is a medium size tree with large velvet leaves and fragrant yellowish flowers (~4 – 5 inches wide). Its leaves are large (7 – 9 inches long and 4 inches wide). In general, the plant in this genus has attracted a lot of horticultural interest due to its impressive colourful flowers. Its scent of flowers is also nice and valued by many people. Besides, some bioactive compounds (i.e., magnolol and honokiol) are believed to exhibit anti-anxiety and anti-angiogenic properties.

During the past decade, studies on fungal diversity have increased. Several fungi have been discovered in plants of the tropical forests. Due to an important role of fungi that can be used in industrial applications, it is therefore not surprising that the study on fungal diversity is currently one of the hottest topics in biological science. *M. liliifera* is widely present in the north of Thailand. There are some studies describing the biodiversity of saprobic fungi on woody litter *M. liliifera* (Promputtha et al., 2004; Kodsueb et al., 2008a, b). Besides, several fungi isolated in these reports are new and interesting as they may play a key role in industry and biotechnology if further work has been performed. This research is thus presented with an expectation to provide an insight into the biodiversity data and to explore the potential use of these fungi in industrial application.

1.2 Objectives of this study

- To study the diversity of saprobic fungi on leaves of *Magnolia liliifera.*
- To determine some biological activities of saprobic fungi including antimicrobial activity.

1.3 Scope of work

The project was carried out as follows:

Part I: Sample collection and fungal isolation

This study will collect samples of decaying leaves of *Magnolia liliifera* in northern Thailand. The fungal isolates will then be screened and identified using morphological characteristics and possibly by DNA sequencing for some potential isolates.

Part II: Biological activity assay

The fungal isolates will be selected based on the data previously described. This study will focus on some fungi and use them as representatives for this study. Those with previously described data on their biological activity or those possibly identified as new species will be emphasised. The antimicrobial activity of some interesting fungal isolates will be determined.

CHAPTER 2
LITERATURE REVIEWS

How many fungal species on earth is an interesting question among mycologists? At present, the classical number of fungi has been estimated at 1.5 million species. This number is based on an average number of fungal species associated with the plant species. So far, there are approximately 100,000 species (ca. 7%) being described. It is therefore important to search for these undescribed fungi which occur in unexplored habitats, hosts or poorly studied countries especially in tropical regions (Hawksworth and Rossman, 1997). Fungal diversity in temperate forests have been relatively studied a longer history than in the tropical forests, although the tropics and subtropics are generally thought to be greater fungal diversity than at higher latitudes. The reasons for higher diversity at low latitudes are not always clear, but this might be because of relevant factors such as host diversity, resource abundance and habitat diversity (Lodge and Cantrell, 1995).

2.1 Saprobic ascomycetes: diversity and importance

The number of fungi worldwide was estimated at 1.5 million species by Hawksworth (1991, 2001b). The numbers of fungi occurring on a single host and in particular whether they are specific to that host are a significant indicator for assessing global number of fungal species (Hyde et al., 2007). Host-specificity and host-recurrence may be an appropriate terms for saprobic fungi. Host-specificity is a term where a species is restricted to a particular host or group of related species, but does not occur on other unrelated plants in the same habitat. Host-recurrence is the frequent or predominant occurrence of a saprobic fungus on a particular host or range of hosts, but the fungus also occurs infrequently on other host plants in the same habitat (Zhou and Hyde, 2001). Many fungi which are saprobes on palms appear to be unique (e.g. Astrosphaeriella, Manokwaria, Myelosperma, Oxydothis, Palmicola, and Pemphidium). There are examples of Oxydothis species which are known from only a single host, and others from more than one host (Hyde, 1994) Saprobic fungi may be specific at the host genus level (Hyde et al., 2007). The example study was done by McKenzie et al. (1999, 2000, 2002) which the fungal species from Agathis, Metrosideros, Nothofagus (southern beech) in Southern Hemiphere in New Zealand were low overlap. Saprobic fungi were more specific at the host family level such as fungal composition in three hosts in Magnoliaceae are varied (Kodsueb et al., 2008). Leaf litter fungi also specific to tissue types (Duong et al.,

2008; Photita et al., 2001; 2003; Pinnoi et al., 2006; Yanna and Hyde, 2001). Yanna and Hyde (2001) found that different frond parts of palms supported distinct fungal communities on most samples.

Estimates of global fungal numbers also depend on relevant factors such as the impact of methodology, of geography, and of decomposition stages. The diversity of saprobic fungi on various hosts have been studied in many countries (Hyde et al., 2001; Kannangara et al., 2007; Photita et al., 2001; Polishook et al., 1996; Santana et al., 2005). Most studies of saprobic fungi have been carried out by using direct observation (moist chamber method), particle filtration or surface washing isolation (Duong et al., 2004; Paulus et al., 2006; Polishook et al, 1996; Promputtha et al., 2004). Polishook et al. (1996) used particle filtration and moist chambers incubation to isolate fungal species on leaf litter from two tree species in Puerto Rico. Many more species have been isolated using particle filtration more than by using moist chambers. However, this does not mean that all fungi isolated from particle filtration method are saprobic fungi (Duong et al., 2004).

Lodge (1997) hypothesized that the higher diversity of tree species in less disturbed forest contributed to greater ascomycete diversity. Only few studies on fungal diversity of saprobic fungi in Thailand have been carried out in recent years (Table 2.1). In northern Thailand, forests have greater plant diversity (Gardner et al., 2000). There have been several studies concerning fungal diversity in the forests of northern Thailand in recent years including studies of fungi on monocotyledons (Bhilabutra et al., 2010; Bussaban et al., 2004; Photita et al., 2003; Thongkantha et al., 2008), on *Shorea obtusa* (Dipterocarpaceae) (Osono et al., 2009), on *Ficus* species (Wang et al., 2008a), on leaf and woody litter of Magnoliaceae (Promputtha et al., 2004; Kodsueb et al., 2008), on *Castanopsis diversifolia* (Duong et al., 2008). Most fungi from all tree species were host-specific. Similarly, fungal diversity on leaf litter of five tree species in Doi Suthep-Pui National Park, Thailand were studied by Duong et al. (2008). Each host species also had different fungal communities. Castanopsis diversifolia had the highest diversity of fungi. Studies of saprobic fungi have provided more biodiversity data. Many new taxa have been also described.

Table 2.1 Recent studies on diversity of saprobic fungi in Thailand

Hosts	Litter types	Sites	Seasons	References
Musa acuminata	Petioles, pseudostems and leaves	Doi Suthep-Pui, Chiang Mai	Wet and dry	Photita et al. (2003)
Dracaena lourieri, *Pandanus* spp.	Leaves, leaf sheaths, prop-roots and seeds	Chiang Dao, Doi Suthep-Pui, Chiang Mai and Phayao	Wet and dry	Thongkantha et al. (2008)
Shorea obtusa	Leaves	Naresuan University, Phayao	Wet and dry	Osono et al. (2009)
Ficus species	Leaves	Mae Taeng, Chiang Mai	N/A*	Wang et al. (2008a)
Magnolia liliifera	Leaves lamina, vein, petioles	Doi Suthep-Pui, Chiang Mai	Wet	Promputtha et al. (2004)
Magnolia liliifera, *Manglietia garrettii*, *Michelia baillonii*	Wood	Doi Suthep-Pui, Chiang Mai	Wet and dry	Kodsueb et al. (2008)
Castanopsis diversifolia	Leaves lamina, vein, petioles	Doi Suthep-Pui, Chiang Mai	Wet	Duong et al. (2008)
Thysanolaena latifolia, *Saccharum spontaneum*	Leaves and stems	Doi Suthep-Pui, Chiang Dao, Mae Rim, Chiang Mai	Wet and dry	Bhilabutra et al. (2010)
Eleiodoxa conferta	Leaves, rachides and petioles	Sirindhorn peat swamp forest, Narathiwat	Wet and dry	Pinnoi et al. (2006)
Hevea brasiliensis	Leaves, branches	Para rubber plantations, Nakhon Si Thammarat and Songkhla	Wet and dry	Seephueak et al. (2010, 2011)
Delonix regia	Pods	Boundary areas of Khao Yai National Park	Wet	Somrithipol et al. (2002)

2.2 *Magnolia liliifera*

Magnolia liliifera (L.) Baill., family Magnoliaceae, is in evergreen forest distributed in India, Nepal, Sikkim, Bhutan, Tibet, South China, Thailand, Myanmar, Malaysia, Malay Peninsula and Borneo. In northern Thailand, this tree is commonly found in Doi Suthep-Pui National Park. The tree is up to 15 m high with open irregular crown and smooth, pale brown bark. Leaves are 20-50 × 7-15 cm, narrowly obovate with blunt tip and tapering at the base. Mature leaves are dark green, smooth or with scattered hairs on mid vein below. 9-15 pairs of widely spaced side veins. Stalks are 5-7 cm, swollen at base, stipule scar more than ¾ of total length (Gardner et al., 2000). The characteristic of *M. liliifera* are shown in Figure 2.1. *M. liliifera* leaves are large and thick, which may provide a large number of fungal species. Promputtha et al. (2004) investigated saprobic fungi from *M. liliifera* leaves on the forest of Doi Suthep-Pui National Park in wet season. Many diverse fungal communities and undescribed species were discovered. Therefore, this host is a good source for study saprobic fungi. Saprobic fungi on decaying woody litter of *M. liliifera* in the forest of Doi Suthep-Pui National Park during the wet and dry seasons were studied by Kodsueb et al. (2008). The results showed that samples collected in the dry season provided greater species richness than samples collected in the wet season.

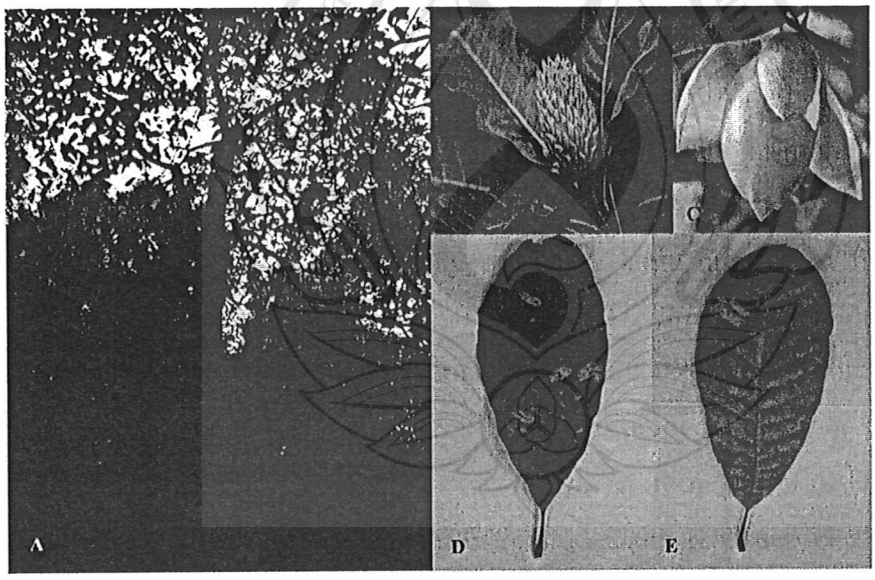


Figure 2.1 *Magnolia liliifera*. A, tree; B, fruit; C, flower; D, recent fallen leaf; E, dead leaf. (B and C from http://www.rspg.or.th/plants_data/rare_plants/rare_plants19.htm, and http://toptropicals.com/catalog/uid/Magnolia_liliifera.htm).

2.3 Secondary metabolites of saprobic fungi

Fungi are well-known as natural resources utilizing for medicinal and biotechnological applications (Hawksworth, 2001a). The economically important products produced by fungi are diverse such as antibiotics, enzymes, vitamins, pharmaceutical compounds, fungicides, plant growth regulators, hormones and proteins. There are two classes of products during fungal life cycle, namely primary and secondary metabolites. Primary metabolites are essential for growth of fungi includes enzymes, fats, alcohol and organic acids (Murphy and Horgan, 2005). On the other hands, secondary metabolites are not essential for vegetative growth but to resist unfavorable environments, finish cell proliferation, differentiation and entire life cycle to reach the purpose of self-defense and survival (Zhong and Xiao, 2009). In addition, secondary metabolites possess many bioactivities of antimicrobial, anti-inflammatory, antioxidant, anticancer, antiparasitic and immunomodulating etc. (Zhong and Xiao, 2009).

During the past two decades, there are many important fungi derived compounds such as penicillins, cephalosporins, cyclosporin, griseofulvin, and lovastatin mevinolin. Up to now, fungi have been recognized to produce relatively a high number of bioactive products about 38% of all microbial products. Among fungal species, the ascomycetes and basidiomycetes are the most frequent producers of bioactive secondary metabolites with about 6400 compounds (Berdy, 2005). Most species of which namely to from Aspergillus, Penicillium, Fusarium, Trichoderma, Phoma, Alternaria, Acremonium and Stachybotrys, Ganoderma, Lactarius and Aureobasidium are good producers to produce several hundreds of compounds (Berdy, 2005; Zhong and Xiao, 2009). Higher fungi are exhibited diverse bioactive compounds and the drug-like characteristics of chemical structure, which present as a major natural compounds library for new drug discovery (Konig et al., 2006; Strobel, 2003). Fungal secondary metabolites are in the classes of heterocyclics (e.g. camptothecin, decaturins), polyketides (e.g. aflatoxin, fumonisins), sterols (e.g. ergosterol, phycomysterol), non-ribosomal peptides (e.g. sirodesmin, siderophores), terpenes (e.g. deoxynivalenol, Paclitaxel) (Fox and Howlett, 2008; Zhong and Xiao, 2009). Some secondary metabolites and their bioactivities are listed in Table 2.2.

Table 2.2 Some secondary metabolites from Ascomycetes and their bioactivities

Species	Metabolites	Activities	References
Acremonium zeae	pyrrocidine A	antibacterial antifungal	Wicklow et al. (2005)
Alternaria brassicicola	alternariol altechromone A herbarin A	antimicrobial antifungal xanthine oxidase inhibitory	Gu (2009)
Aspergillus fumigatus	fumigaclavine C	antifungal mycotoxin	Liu et al. (2004)
Beltrania rhombica	rhombidiol (1) rhombitriol (2)	antibacterial antifungal	Rukachaisirikul et al. (2005)
Camposporium quercicola	quercilolin tenellic acid A 2',4'-dihydroxyacetophenone	antibacterial	Wang et al. (2008b)
Cephalosporium sp. IFB-E001	graphislactone A	antioxidant	Song et al. (2005)
Chaetomium globosum	globosumone A	cytotoxic	Bashyal et al. (2005)
Cladosporium herbarum	aspernigrin A	cytotoxic xanthine oxidase inhibitor	Ye et al. (2005)
Diaporthe sp. CR 146	cytosporone B	antifungal cytotoxic	Brady et al. (2000)
Fusarium oxysporum strain	vincristine	anticancer	Zhang et al. (2000)

CHAPTER 3
METHODOLOGY

3.1 Study site and sample collection

The study site was located in an evergreen forest in Doi Suthep-Pui, Chiang Mai, northern Thailand (N 18° 48' 18.73", E 98 ° 54' 47.28", elev., 107 m) and samples were collected in the dry season, when there was low humidity; the forest floor was damp but not wet. During March – November 2013, decaying leaves were randomly collected from each individual tree of *M. liliifera* and returned to the laboratory.

3.2 Fungal isolation and examination

Leaves were divided into two stages of decomposition (five leaves for each stage); stage I were recently green or yellow fallen leaves and stage II were mostly decaying brown leaves (Figure 3.1). Five leaves of each stage were observed. Samples were incubated in 15 cm diameter sterile petri dishes with a tissue paper moistened with sterile distilled water at room temperature. Leaves were examined for fungi after one week, three weeks and six weeks of incubation and fungi occurring on leaf lamina, midrib or petiole were recorded. Fungi were identified based on morphological characters using the references of Carmichael et al. (1980), Ellis (1976), Hanlin (1998), Nag Raj (1993), Seifert et al. (2011), Sivanesan (1984), and Sutton (1980). These characters generally include shape and size of ascomata, papilla, paraphyses, asci, ascospores, and conidia. Single spore isolation method was used in fungal isolation (Chomnunti et al., 2011). All cultures were grown on potato dextrose agar (PDA) and malt extract agar (MEA) and deposited in MFLU Culture Collection (MFLUCC).

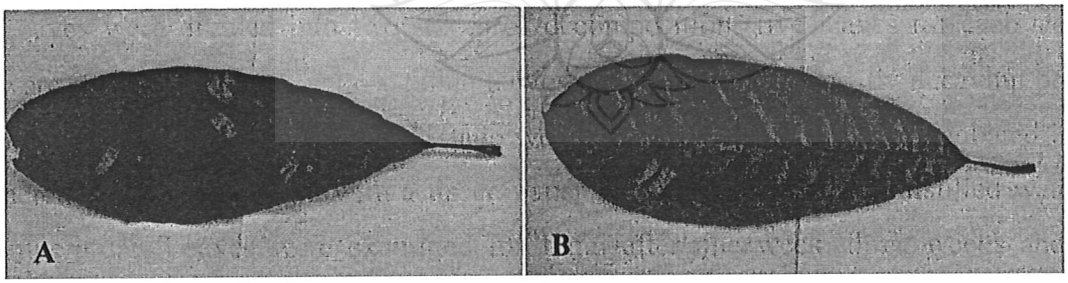


Figure 3.1 *Magnolia liliifera* leaves at each stage of decomposition. (A) and (B) Stage of decomposition I and II.

3.3 Data analysis

The percentage of occurrence of fungi was calculated and fungal taxa with a percentage occurrence higher than 10 are regarded as 'common species' in this study. The fungal percentage occurrence was calculated. Species diversity were compared in each stage of decomposition (stage I and II), three incubation periods (week 1, 3 and 6) and different parts of leaf (leaf lamina, midrib and petiole) using diversity indices to demonstrate the result of species diversity of a community (Shannon and Weaver, 1949). Species richness means the number of fungal species in a community and species evenness means the contribution (relative equability) of individuals (McCune and Grace, 2002). The Shannon index (H') refers to the abundance of species diversity of a community and the Shannon evenness (E') refers to the equability of species diversity, which ranges from 0 to 1. If the Shannon evenness is equal to 1, then every species in the community has the same frequency of occurrence.

3.4 Preparation of fungal extracts

Ten fungal isolates were selected for antimicrobial activity assay. These fungal isolates were selected since they are genera which have been reported on producing many bioactivities and/or were marked as probably new species.

Inoculum was prepared by cutting agar of pure culture plate into discs, 4 mm. diameter, from the margin of a colony. The inoculum disc was inverted onto the centre of an 85 mm petri dish containing 20 ml of sabouraud dextrose agar (SDA). Inoculated plates were incubated for 30 days at 28 °C. Solid phase extraction method was adapted for fungal metabolites production. For each isolate, five replicate experiments were carried out. Fungal cultures with agar from each isolate were macerated with 30 ml ethyl acetate (EtOAc) and blended with a sterile stainless blender. This extraction process was carried out three times. The agar slurry was left sitting overnight for the first EtOAc extraction, and eight hours each for the second and third extractions. All three EtOAc were combined and transferred to a pre-weighed vial and air dried to yield a crude extract. Crude extracts were extracts prepared to a concentration of 1 g/ml in methanol and stored in 4 °C in airtight bottles. The process of the solid phase extraction is showed in Figure 3.2.

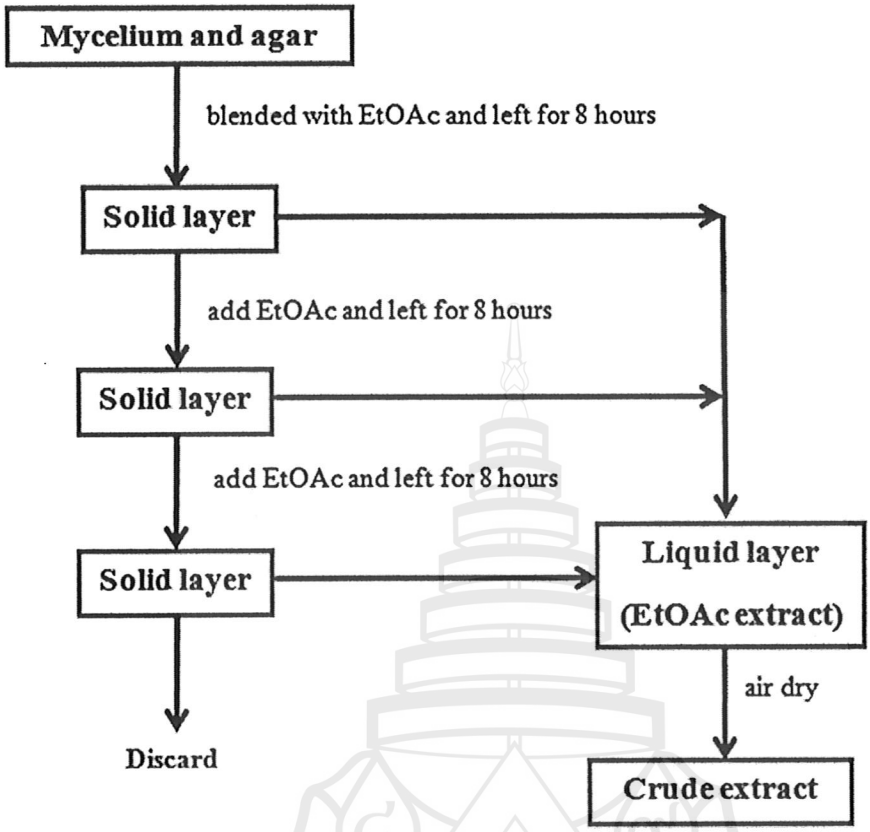


Figure 3.2 Overall procedure of extracts preparation

3.5 Antimicrobial activity assay

Five pathogenic bacteria and 4 pathogenic fungi were used as test microorganisms for antimicrobial activities assay of non-fractioned extracts. *Bacillus cereus* (TISTR 687), *Escherichia coli* (TISTR 780), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (TISTR 292), *Staphylococcus aureus* (TISTR 1466) and *Candida albicans* (TISTR 5779) were obtained from STIC, Mae Fah Luang University, Chiang Rai. *Colletotrichum fructicola* (MFLUCC10-0202), *Fusarium* sp. (MFUCC11-0219) and *Alternaria* sp. (MFUCC11-0123) were obtained from Mae Fah Luang University Culture Collection (MFUCC), Mae Fah Luang University, Chiang Rai.

A modified paper disk method was used for antimicrobial activities assay (Gu, 2009; Wang et al, 2008b). Bacteria were grown in nutrient broth at 37 °C for 18 hr and yeast was grown in potato dextrose broth at 30 °C for 18 hr. After that, they were adjusted to

approximately 108 colony-forming unit per milliliter (CFU/ml). Agar plates were swabbed uniformly by test bacteria on nutrient agar (NA) and yeast on potato dextrose agar (PDA). Whereas, other fungi were grown in PDA at 28 °C for 2-3 days until radius of colony are reached to approximately 20 mm. Sterile paper disks (6 mm diameter) each containing 20 µl of sample solution (0.1 g/ml) were dried thoroughly and placed on the surface of medium. The test plates were then incubated at 37 °C for 24 hr for bacteria and 28 °C for 1-4 days for fungi. Methanol was used as negative controls. Streptomycin sulphate (10 µg/ml) were used for bacteria as a positive control. For each test, five replicates were performed. The diameter (mm) of the growth inhibition are examined and measured.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Diversity of fungi on *Magnolia liliifera*

In this study, the fungal diversity on decaying leaves of *M. liliifera* in the dry season in northern Thailand was investigated. In total, 35 taxa were identified from decaying leaves of *M. liliifera* (Table 4.1). This comprised of 8 ascomycetes (representing 23% of all taxa) and 27 anamorphic fungi (77%) including 11 coelomycetes (31%) and 16 hyphomycetes (46%). It should also be noted that some isolates were observed during an isolation procedure and were not able to isolate as a pure culture. Their percentage of occurrence is also shown in Table 4.1. The most abundant species were *Sporidesmium* sp. (80%), *Colletotrichum fructicola* (70%), *Stachybotrys parvispora* (70%), *Dicyma pulvinata* (60%), *Lasiosphaeria*-like sp. 1 (60%) and *Volutella consors* (60%). Diversity indices of fungi on decaying leaves of *M. liliifera* are shown in Table 4.2.

Fungal diversity of *M. liliifera* in the dry season can be compared with previous studies in the wet season (Promputtha et al., 2004). The fungal number recorded on a succession study was 22 taxa from 110 leaf samples (Promputtha et al., 2004). In the present study, 35 fungal taxa were identified from 10 leaf samples. This sample number was shown to be a threshold for studying fungal diversity in *M. liliifera*. It seems that leaves in the dry season support more fungal taxa than in the wet season. Similar results were reported by Kodsueb et al. (2008), Seephueak et al. (2010) and Seephueak et al. (2011) who found that samples collected in the dry season had greater species richness and higher Shannon diversity index than samples collected in the wet season. There were no seasonal effects of fungal communities on palms in Hong Kong (Yanna and Hyde, 2001) or on *Pandanus penetrans* in Thailand (Thongkantha et al., 2008). Pinnoi et al. (2006) showed that the spore germination and reproduction of fungi required quite high humidity. Fungal composition and dominant species reported by Promputtha et al. (2004) were different as there were only a few overlapping genera occurred in both studies (*Colletotrichum*, *Phaeosphaeria*, *Phomopsis*, *Stachybotrys* and *Volutella*). This is possibly because the given name of taxa may be different following sexual-asexual states which were presented on samples. For example, *Clonostachys rosea* was found in this study, whereas *Bionectria ochroleuca*, which is linked to be sexual states of this fungus (Schroers, 2001), was reported by Promputtha et al. (2004). However,

differences in the number of samples and the years of collecting, which varied in temperature, humidity and rainfall make comparisons difficult.

The picture of some fungal representatives including their ascomata, ascus, and conidia was given in Appendix.

Table 4.1 List of saprobic fungi found in this study and their percentage of occurrence occurring on two stages (I and II) of decomposition

Fungal species	Isolate Nos.	Stage I	Stage II	Overall
Anamorph of *Eutypa* sp. 1	0		60	30
Ascomycetes sp. 1*	1		20	10
Ascomycetes sp. 2	0		20	10
Beltrania rhombica	1	40		20
Botryosphaeria sp.	0	20	20	20
Canalisporium caribense	0		20	10
Ceolomycetes sp. 1	0	20		10
Ceolomycetes sp. 2	1		20	10
Ceolomycetes sp. 3	0		20	10
Cladosporium sp. 1	1	20		10
Cladosporium sp. 2	1		20	10
Clonostachys compactiuscula	0	20		10
Clonostachys rogersoniana	1	20		10
Clonostachys rosea	1	20		10
Colletotrichum fructicola	2	80	60	70
Dicyma pulvinata	0	100	20	60
Fusicoccum aesculi	2	40	20	30
Hyphomycetes sp. 1	0	20		10
Lasiodiplodia theobromae	1	60		30
Lasiosphaeria-like sp.	1	80	40	60
Montagnula sp.	0		40	20
Nodulisporium sp.	0		20	10
Ophioceras cf. *leptosporum*	1	20	60	40
Pestalotiopsis sp.	0	20		10
Phaeosphaeria sp.*	1	40		20
Phialophora sp.	1		20	10
Phoma sp. 1	0	60	40	50
Phoma sp. 2	1	20		10
Phomatospora sp.	0	20		10
Phomopsis sp.	0	20		10
Sporidesmium sp.	1	100	60	80
Stachybotrys parvispora	1	80	60	70
Stachylidium bicolor	0		20	10
Volutella consors	1	100	20	60
Zygosporium sp.	0		20	10

Table 4.2 Diversity indices of fungi from *M. liliifera* at two stages of decomposition

	Stage I	Stage II
Species richness	23	21
Species evenness	88	44
Shannon indices	2.84	2.9
Shannon evenness	0.74	0.84

4.2 Effect of decomposing stages on fungal communities

The decomposition process by saprobic fungi follows three succession stages: the pioneer (early) stage, mature (middle) stage and the impoverished (later) stage (Yanna and Hyde, 2002). Pioneer communities are generally of low fungal diversity and have few species occurring at high percentage occurrence. Mature communities have high fungal diversity and have many species occurring at high percentage occurrence and some species become obviously dominant (Promputtha et al., 2004). This hypothesis is supported by our results showing different fungal communities between each stage of decomposition. The fungi present at each stage of decomposition of *M. liliifera* are listed in Table 4.3. On *M. liliifera*, dominant species at stage I of decomposition were *D. pulvinata* (100%), *Sporidesmium* (100%) and *V. consors* (100%). The most abundant species at stage II of decomposition were anamorph of *Eutypa* sp. 1 (60%), *C. fructicola* (60%), *Sporidesmium* (60%), *Ophioceras* cf. *leptosporum* (60%) and *S. parvispora* (60%).

The replacement of fungal species composition sequentially throughout decomposing process relies on the capability of decomposers to utilize organic matter and nutrients, which are particular to each substrate or host (Frankland, 1992; Tang et al., 2005). In early stage, decomposer fungi might switch their roles from endophyte and pathogen (Lodge and Cantrell, 1995; Duong et al., 2008). The evidence that endophytic fungi change to be saprobes has been reported in many previous studies (Koide et al., 2005; Osono et al., 2004; 2009; Promputtha et al., 2010; Purahong and Hyde, 2011; Tang et al., 2005). Promputtha et al. (2010) also proved that endophytes can produce various degrading enzymes in succession process, which is an important activity for their adaptation to saprobic lifestyle. The primary enzymes degrade small soluble carbon-based molecules, such as hemicelluloses and the most complex cellulose and lignin are then degraded at the late stage of decomposition (Promputtha et al., 2010). In addition, soilborne and airborne fungi can colonize fallen leaves (Duong et al., 2008) so that leaves which have been on the forest floor for a long time might

have higher fungal diversity than leaves which have recently fallen. In this study, decaying leaves of *M. liliifera* in stage I of decomposition supported greater fungal diversity than in stage II of decomposition suggesting that endophytic fungi might be play an important role in the decay of dead leaves and these fungi are different at different stages of decay. Seephueak et al. (2010, 2011) studied fungi on different stages of decaying leaf and branch litter of the rubber tree. They showed that the number of taxa on middle stage decaying branches was higher than new and old decaying fallen branches

Table 4.3 Fungal species on each stage of decomposition on *M. liliifera* leaves

Fungal species appearing on both stages of decomposition	**Fungal species appearing on stage I of decomposition**	**Fungal species appearing on stage II of decomposition**
Botryosphaeria sp.	*Beltrania rhombic*	*Phialophora* sp.
Colletotrichum fructicola	Ceolomycetes sp. 1	Anamorph of *Eutypa* sp. 1
Dicyma pulvinata	*Phoma* sp. 2	Ascomycetes sp. 1
Fusicoccum aesculi	*Cladosporium* sp. 1	Ascomycetes sp. 2
Lasiosphaeria-like sp.	*Clonostachys rogersoniana*	*Canalisporium caribense*
Ophioceras cf. *leptosporum*	*Lasiodiplodia theobromae*	Ceolomycetes sp. 2
Phoma sp. 1	Hyphomycetes sp. 1	Ceolomycetes sp. 3
Sporidesmium sp.	*Clanostachys compactiuscula*	*Nodulisporium* sp.
Stachybotrys parvispora	*Pestalotiopsis* sp.	*Cladosporium* sp. 2
Volutella consors	*Phaeosphaeria* sp.	*Montagnula* sp.
	Phomatospora sp.	*Stachylidium bicolor*
	Phomopsis sp.	*Zygosporium* sp.
	Clonostachys rosea	
Number species = 10	**Number species = 13**	**Number species = 12**

4.3 Effect of leaf tissue types on fungal communities

The most abundant species on the leaf lamina were *S. parvispora* (70%), *D. pulvinata* (60%), *Lasiosphaeria*-like sp. 1 (60%), *Sporidesmium* (40%) and *Phoma* sp. 1 (40%). The most abundant species in the midribs were *Sporidesmium* (60%), *C. fructicola* (50%), *V. consors* (40%), anamorph of *Eutypa* sp. 1 (30%) and *S. parvispora* (30%). The most abundant species on the petioles were *Sporidesmium* (40%), *Fusicoccum aesculi* (30%) and *Ophioceras* cf. *leptosporum* (30%).

Leaf lamina provides the largest surface area and may support a greater number of fungal species than other parts of leaf (Promputtha et al., 2004). In early week of incubation, leaf lamina provided the higher number of fungi than midribs and petioles. Then, number of fungi on the leaf lamina decreased, while number of fungi on midribs and petioles increased in later week of incubation of *M. liliifera* (Figure 4.1). It may be assumed that primary fungal saprobes prefer to colonize leaf lamina, and later fungi are able to colonize midrib and petiole. Structure and moisture content of different tissue types may influence the presence of saprobic fungi. The leaf lamina contains thin-walled parenchymatous cells and little moisture. Midrib and petiole have more sclerenchyma cells that are thick-walled supporting more nutrients for fungal growth (Pinnoi et al., 2006). Petioles contain vascular bundles, which may keep moisture for a longer time (Fisher et al., 2002). This may result in more fungi on midribs and petioles in the later period.

Figure 4.1 Percentage of fungi occurring on different parts of leaf of *M. liliifera* at three incubation periods. P = Petioles, M = Midrib, L = Leaf lamina.

Some fungi can grow in every part of the leaf, some grow only on leaf lamina and/or midrib and/or petiole. Some fungi are restricted to a single tissue type. For example, on *M. liliifera*, *Ceolomycetes* sp. 3 and *Cladosporium* sp. 2 were found only on midrib. *Coelomycetes* sp. 2, *Nodulisporium* sp., Phomopsis sp. and Stachylidium bicolor were found only on petiole. The recurrence of saprobic fungi on certain tissues has been also observed with other hosts (Duong et al., 2008; Photita et al., 2001, 2003; Pinnoi et al., 2006; Promputtha et al., 2004; Yanna and Hyde, 2001). Yanna and Hyde (2001) found that different parts of palms supported distinct fungal communities on most samples. The tissue specificity

of saprobic fungi may be due to differing nutritional requirements and enzymatic capabilities to utilize different substrates (Adaskaveg et al., 1991; Photita et al., 2003; Yanna and Hyde, 2001).

4.4 Antimicrobial activity of fungal extracts

The antimicrobial assay of ten fungal metabolites was processed in vitro by modified disc diffusion methods against nine pathogenic microorganisms. The result of microbial growth inhibition testing by fungal extracts was shown in Table 4.4. For antibacterial test, it was found that four fungal species inhibited the growth of at least one test bacteria. Among them, *Ophioceras* cf. *leptosporum* (JM18) showed the best antibacterial activity against *S. aureus* and followed by *B. cereus* (Figure 4.2A). *Fusicoccum aesculi* (JM19-2) and *Chaetomium* sp. (CI18) also exhibited antimicrobial activity against *Ps. aeruginosa*. Another fungi, *Clonostachys rosea* (JM13) inhibited the growth of *B. cereus*. In antifungal test, only two fungal species *Chaetomium* sp. (CI18) and *Clonostachys rosea* (JM13) showed the inhibition of growth to *C. fructicola* (Figure 4.2B), respectively.

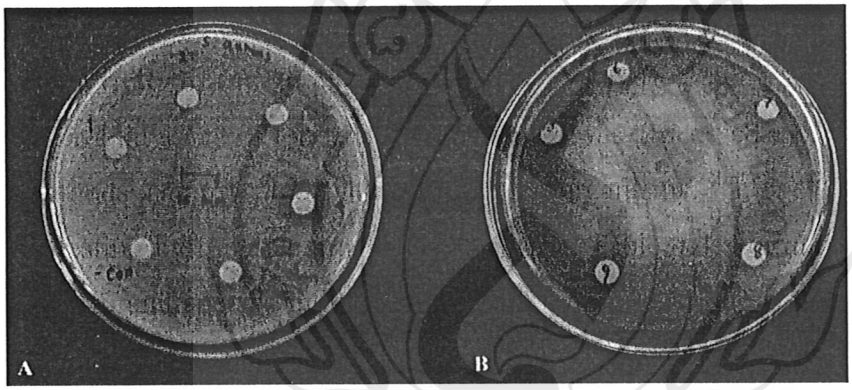


Figure 4.2 Disc diffusion assay showing antimicrobial activity of fungal extracts. (A) Inhibition zone of *Ophioceras* cf. *leptosporum* (JM18) on S. aureus plate (arrow point). (B) Inhibition zone of *Chaetomium* sp. 1 (CI18) with *C. fructicola*.

Table 4.4 Antimicrobial activities of fungal extracts (Mean ± SD)

Fungal extracts	Zone of inhibition (mm)								
	BC	EC	PA	ST	SA	CA	CF	F	A
Stachybotrys parvispora JM09	0	0	0	0	0	0	0	0	0
Clonostachys rosea JM13	0	0	0	0	0	0	0	0	0
Beltrania rhombica JM14	0	0	8±0.6	0	0	0	11±1.3	0	0
Ophioceras cf. *leptosporum* JM18	8±0	0	0	0	0	0	8±1	0	0
Fusicoccum aesculi JM19-1	0	0	9±0.7	0	0	11±1.6	0	0	0
Fusicoccum aesculi JM19-2	0	0	0	0	0	10±1	0	0	0
Ascomycetes sp. 1 JM25	0	0	0	0	0	0	0	0	0
Hyphomycetes sp. 2 CI03	0	0	0	0	0	0	0	0	0
Hyphomycetes sp. 3 CI09	8±0.3	0	0	0	15±1	0	0	0	0
Chaetomium sp. CI18	0	0	0	0	0	0	0	0	0
Methanol	0	0	0	0	0	0	0	0	0
Streptomycin sulphate	20	20	15	20	14	0	0	0	0

Note. BC = *Bacillus cereus*, EC = *Escherichia coli*, PA = *Pseudomonas aeruginosa*, ST = *Salmonella typhimurium*, SA = *Staphylococcus aureus*, CA = *Candida albicans*, CF = *Colletotrichum fructicola*, F = *Fusarium* sp. and A = *Alternaria* sp.

CHAPTER 5
CONCLUSION

Leaf litter is an excellent source of fungi to study biodiversity and their bioactive compounds. Previous studies have focused mainly on the diversity and taxonomy of saprobic fungi. The biotechnological application of these fungi is rather poor. This study was initiated in order to establish data for fungal saprobes on both of biodiversity and biotechnological applications. The fungal diversity on leaf litter of two selected hosts in northern Thailand was investigated and possible new taxa isolated from these hosts were studied. In addition, the morphological and molecular systematic of fungi in family Planistromellaceae were studied via type specimens. The potential of saprobic fungi in producing antimicrobial substances were tested and analyzed for the group of active compounds.

5.1 Diversity of saprobic fungi on *Magnolia liliifera*

In the current study, saprobic fungi on dead leaves of *Magnolia liliifera* collected in dry season were studied and compared. Thirty-five fungi were found from *M. liliifera*. The stages of decomposition, incubation periods and parts of leaf are important factors that also influence fungal diversity on each host. Saprobic fungi were specific to different tissues types (leaf lamina, midrib, petiole). Furthermore, this work contributes more biodiversity data to global fungal estimates and new information of fungi in Thailand.

5.2 Possible new fungi discovered from this study

The study of diversity of fungi on leaf litter of *Magnolia liliifera* showed that three taxa are probably new to science. These taxa are being described and illustrated in terms of morphology. The nucleotide sequences of ITS and LSU region once obtained will be blasted in GenBank to reveal the closest matches. From the results, three unidentified fungi show different morphological characters from other fungal genera and it is difficult to identify these fungi to the genus level. Molecular phylogenetic needs to be done to help identify these fungal taxa in the future.

5.3 Active compounds of saprobic fungi

The extracts of nine saprobic fungi were assayed for the in vitro antimicrobial activity against nine pathogenic microorganisms. The results showed that most of fungal isolates

produced antimicrobial compounds. *Clonostachys rosea* (JM13) showed the highest antimicrobial activity with a broad spectrum of activity against several bacteria (gram negative and positive) and fungi. This study showed that saprobic fungi have a promising prospect for production of useful bioactive compounds.

REFERENCES

Adaskaveg JE, Blanchette RA and Gilbertson RL. 1991. Decay of date palm wood by white-rot and brown-rot fungi. Can. J. Bot. 69, 615–629.

Bashyal BP,Wijeratne EMK, Faeth SH and Gunatilaka AA. 2005. Globosumones A-C, cytotoxic orsellinic acid esters from the Sonoran desert endophytic fungus Chaetomium globosum. J. Nat. Prod. 68, 724–728.

Berdy J. 2005. Bioactive microbial metabolites: a personal view. J. Antibiot. 58, 1–26.

Bhilabutra W, McKenzie EHC, Hyde KD and Lumyong S. 2010. Fungi on the grasses, Thysanolaena latifolia and Saccharum spontaneum, in northern Thailand. Mycosphere 1, 301–314.

Brady SF, Singh MP, Janso JE and Clardy J. 2000. Cytoskyrins A and B, new BIA active bisanthraquinones isolated from an endophytic fungus. Org. Lett., 2, 4047–4049.

Bussaban B, Lumyong P, McKenzie EHC, Hyde KD and Lumyong S. 2004. Fungi on Zingiberaceae (ginger). In EBG Jones, M Tanticharoen and KD Hyde (Eds.), Thai Fungal Diversity (pp.189–195). Bangkok: BIOTEC.

Carmichael JW, Kendrick WB, Conners IL and Sigler L. 1980. Genera of Hyphomycetes. Edmonton: University of Alberta Press.

Chomnunti P, Schoch CL, Aguirre-Hudson B, Ko-Ko TW, Hongsanan S, Jones EBG, Kodsueb R, Phookamsak R, Chukeatirote E, Bahkali AH and Hyde KD. 2011. Capnodiaceae. Fungal Divers. 51, 103–134.

Duong ML, Lumyong S and Hyde KD. 2004. Fungi on leaf litter. In EBG Jones, M Tanticharoen and KD Hyde (Eds.), Thai Fungal Diversity (pp.163–171). Bangkok: BIOTEC.

Duong ML, McKenzie EHC, Lumyong S and Hyde KD. 2008. Fungal succession on senescent leaves of Castanopsis diversifolia in Doi Suthep-Pui National Park, Thailand. Fungal Divers. 30, 23–36.

Ellis MB. 1976. More Dematiaceous Hyphomycetes. Surrey: Commonwealth Mycological Institute.

Fisher JB, Tan HTW and Toh LPL. 2002. Xylem of rattans: vessel dimensions in climbing palms. Am. J. Bot. 89, 196–202.

Fox EM and Howlett BJ. 2008. Secondary metabolism: regulation and role in fungal biology. Curr. Opin. Microbiol. 11(6), 481–487.

Frankland JE. 1992. Mechanisms in fungal succession. In DT Wicklow and G Carroll (Eds.) The Fungal Community (2nd ed). New York: Marcel Dekker.

Gardner S, Sidisunthorn P and Anusarnsunthorn V. 2000. A field guide to forest trees of Northern Thailand. Bangkok: Kobfai Publishing Project.

Gu W. 2009. Bioactive metabolites from Alternaria brassicicola ML-P08, an endophytic fungus residing in Malus halliana. World J. Microbiol. Biotechnol. 25, 1677–1683.

Hanlin RT. 1998. Combined keys to illustrated genera of ascomycetes, volumes I & II. Minnesota: American Phytopathological Society.

Hawksworth DL. 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycol. Res. 95, 641–655.

Hawksworth DL. 2001a. Horizons in Exploiting Filamentous Fungi. In SB Pointing and KD Hyde (Eds.), Bio-exploitation of Filamentous fungi, Fungal Diversity Research Series 6 (pp 1–12). Hong Kong: Fungal Diversity Press.

Hawksworth DL. 2001b. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol. Res. 105, 1422–1432.

Hawksworth DL and Rossman AY. 1997. Where are all the undescribed fungi? Phytopathology 87, 888–891.

Hyde KD. 1994. Fungi from palms. XIII. The genus Oxydothis, a revision. Sydowia 46, 265–314.

Hyde KD, Bussaban B, Paulus B, Crous PW, Lee S, McKenzie EHC, Photita W and Lumyong S. 2007. Diversity of saprobic microfungi. Biodiv. Conserv. 16, 7–35.

Hyde KD, Ho WH, McKenzie EHC and Dalisay T. 2001. Saprobic fungi on bamboo culms. Fungal Divers. 7, 35–48.

Kannangara S, Parkinson D and Deshappriya N. 2007. Fungal biomass changes during the decomposition of leaves of Michelia nilagirica and Semecarpus coriaceae in an upper montane rainforest in Sri Lanka. Pedobiologia 51, 159–168.

Kodsueb R, McKenzie EHC, Lumyong S and Hyde KD. 2008. Diversity of saprobic fungi on Magnoliaceae. Fungal Divers. 30, 37–53.

Koide K, Osono T and Takeda H. 2005. Fungal succession and decomposition of Camellia japonica leaf litter. Ecol. Res. 20, 599–609.

Konig GM, Kehraus S, Seibert SF, Abdel-Lateff A and Muller D. 2006. Natural products from marine organisms and their associated microbes. Chembiochem 7, 229–238.

Liu JY, Song YC, Zhang Z, Wang L, Guo ZJ, Zou WX and Tan RX. 2004. Aspergillus fumigates CY018, an endophytic fungus in Cynodon dactylon as a versatile producer of new and bioactive metabolites. J. Biotechnol. 114, 279–287.

Lodge DJ. 1997. Factors related to diversity of decomposer fungi in tropical forests. Biodiv. Conserv. 6, 681–688.

Lodge DJ and Cantrell S. 1995. Fungal communities in wet tropical forest variation in time and space. Can. J. Bot. 73, S1391–S1398.

McCune B and Grace JB. 2002. Analysis of ecological communities. Oregon: MjM Software Design.

McKenzie EHC, Buchanan PK and Johnston PR. 1999. Fungi on pohutukawa and other Metrosideros species in New Zealand. New Zealand J. Bot. 37, 335–354.

McKenzie EHC, Buchanan PK and Johnston PR. 2000. Checklist of fungi on Nothofagus species in New Zealand. New Zealand J. Bot. 38, 635–720.

McKenzie EHC, Buchanan PK and Johnston PR. 2002. Checklist of fungi on kauri (Agathis australis) in New Zealand. New Zealand J. Bot. 40, 269–296.

Murphy RA and Horgan KA. 2005. Antibiotics, Enzymes and Chemical Commodities from fungi. In K Kavanagh (Eds.), Fungi: biology and applications (pp.113-143). London: John Wiley and Sons.

Nag Raj TR. 1993. Coelomycetous anamorphs with appendage-bearing conidia. Ontario: Mycologue Publications.

Osono T, Bhatta BK and Takeda H. 2004. Phyllosphere fungi on living and decomposing leaves of giant dogwood. Mycoscience 45, 35–41.

Osono T, Ishii Y, Takeda H, Seramethakun T, Khamyong S, To-Anun C, Hirose D, Tokumasu S and Kakishima M. 2009. Fungal succession and lignin decomposition on Shorea obtusa leaves in a tropical seasonal forest in northern Thailand. Fungal Divers. 36, 101–119.

Paulus B, Kanowski J, Gadek PA and Hyde KD. 2006. Diversity and distribution of saprobic microfungi in leaf litter of an Australian tropical rainforest. Mycol. Res. 110, 1441–1454.

Photita W, Lumyong P, McKenzie EHC, Hyde KD and Lumyong S. 2003. Saprobic fungi on dead wild banana. Mycotaxon 80, 345–356.

Photita W, Lumyong S, Lumyong P, Ho WH, McKenzie EHC and Hyde KD. 2001. Fungi on Musa acuminata in Hong Kong. Fungal Divers. 6, 99–106.

Pinnoi A, Lumyong S, Hyde KD and Jones EBG. 2006. Biodiversity of fungi on the palm Eleiodoxa conferta in Sirindhorn peat swamp forest, Narathiwat, Thailand. Fungal Divers. 22, 205–218.

Polishook JD, Bills GF and Lodge DJ. 1996. Microfungi from decaying leaves of two rain forest trees in Puerto Rico. J. Ind. Microbiol. 17, 284–294.

Promputtha I, Hyde KD, McKenzie EHC, Peberdy JF and Lumyong S. 2010. Can leaf degrading enzymes provide evidence that endophytic fungi becoming saprobes? Fungal Divers. 41, 89–99.

Promputtha I, Lumyong S, Lumyong P, McKenzie EHC and Hyde KD. 2004. Fungal saprobes on dead leaves of Magnolia lillifera (Magnoliaceae) in Thailand. Cryptogam. Mycol. 25, 43–47.

Purahong W and Hyde KD. 2011. Effects of fungal endophytes on grass and non-grass litter decomposition rates. Fungal Divers. 47, 1–7.

Rukachaisirikul V, Kaewbumrung C, Phongpaichi S and Hajiwangoh Z. 2005. Eudesmane sesquiterpenes from the aquatic fungus Beltrania rhombica. Chem. Pharm. Bull. 53, 238–240.

Santana M, Lodge D and Lebow P. 2005. Relationship of host recurrence in fungi to rates of tropical leaf decomposition. Pedobiologia 49, 549–564.

Schroers HJ. 2001. A monograph of Bionectria (Ascomycota, Hypocreales, Bionectriaceae) and its Clonostachys anamorphs. Stud. Mycol. 46, 73–74.

Seephueak P, Petcharat P and Phongpaichit S. 2010. Fungi associated with degrading leaf litter of para rubber (Hevea brasiliensis). Mycology 1, 213–227.

Seephueak P, Phongpaichit S, Hyde KD and Petcharat V. 2011. Diversity of saprobic fungi on decaying branch litter of the rubber tree (Hevea brasiliensis). Mycosphere 2, 307–330.

Seifert K, Morgan-Jones G, Gams W and Kendrick B. 2011. The genera of hyphomycetes. Utrecht: CBS-KNAW Fungal Biodiversity Centre.

Shannon CE and Weaver W. 1949. The mathematical theory of communication. Urbana,:University of Illinois Press.

Sivanesan A. 1984. The bitunicate ascomycetes and their anamorphs. Vaduz: J. Cramer.

Somrithipol S, Jones EBG and Hywel-Jones NL. 2002. Fungal diversity and succession on pods of Delonix regia (Leguminosae) exposed in a tropical forest in Thailand. In KD Hyde and EBG Jones (Eds.) Fungal Succession, Fungal Divers, 10 (pp. 131–139). Hong Kong: Fungal Diversity Press.

Song YC, Huang WY, Sun C, Wang FW and Tan RX. 2005. Characterization of Graphislactone A as the antioxidant and free radical–scavenging substance from the culture of Cephalosporium sp. IFB-E001, an endophytic fungus in Trachelospermum jasminoides. Biol. Pharm. Bull. 28, 506–509.

Strobel GA. 2003. Endophytes as sources of bioactive products. Microbes. Infect. 5, 535–544.

Sutton BC. 1980. The Coelomycetes. Surrey: Commonwealth Mycological Institute.

Tang AMC., Jeewon R and Hyde KD. 2005. Succession of microfungal communities on decaying leaves of Castanopsis fissa. Can. J. Bot. 51, 967–974.

Thongkantha S, Lumyong S, McKenzie EHC and Hyde KD. 2008. Fungal saprobes and pathogens occurring on tissues of Dracaena lourieri and Pandanus spp. in Thailand. Fungal Divers. 30, 149–169.

Wang HK, Hyde KD, Soytong K and Lin FC. 2008a. Fungal diversity on fallen leaves of Ficus in northern Thailand. J. Zhejiang Univ-SC. B. 9, 835–841.

Wang L, Ding JY, Song HC, Shen KZ, Wang LM, Sun R, Wang CR, Li GH, Li L and Zhang KQ. 2008b. Screening and isolation of antibacterial activities of the fermentative extracts of freshwater fungi from Yunnan Province, China. Ann. Microbiol. 58, 579–584.

Wicklow DT, Roth S, Deyrup ST and Gloer JB. 2005. A protective endophyte of maize: Acremonium zeae antibiotics inhibitory to Aspergillus flavus and Fusarium verticillioides. Mycol. Res. 109, 610–618.

Yanna HWH and Hyde KD. 2001. Fungal communities on decaying palm fronds in Australia, Brunei, and Hong Kong. Mycol. Res. 105, 1458–1471.

Ye YH, Zhu HL, Song YC, Liu JY and Tan RX. 2005. Structural revision of Aspernigrin A, reisolated from Cladosporium herbarum IFB-E002. J. Nat. Prod. 68, 1106–1108.

Zhang L, Guo B, Li H, Zeng S, Shao H, Gu S and Wei R. 2000. Preliminary study on the isolation of endophytic fungus of Catharanthus roseus and its fermentation to produce products of therapeutic value. Chinese Trad. Herbal Drugs 31, 805–807.

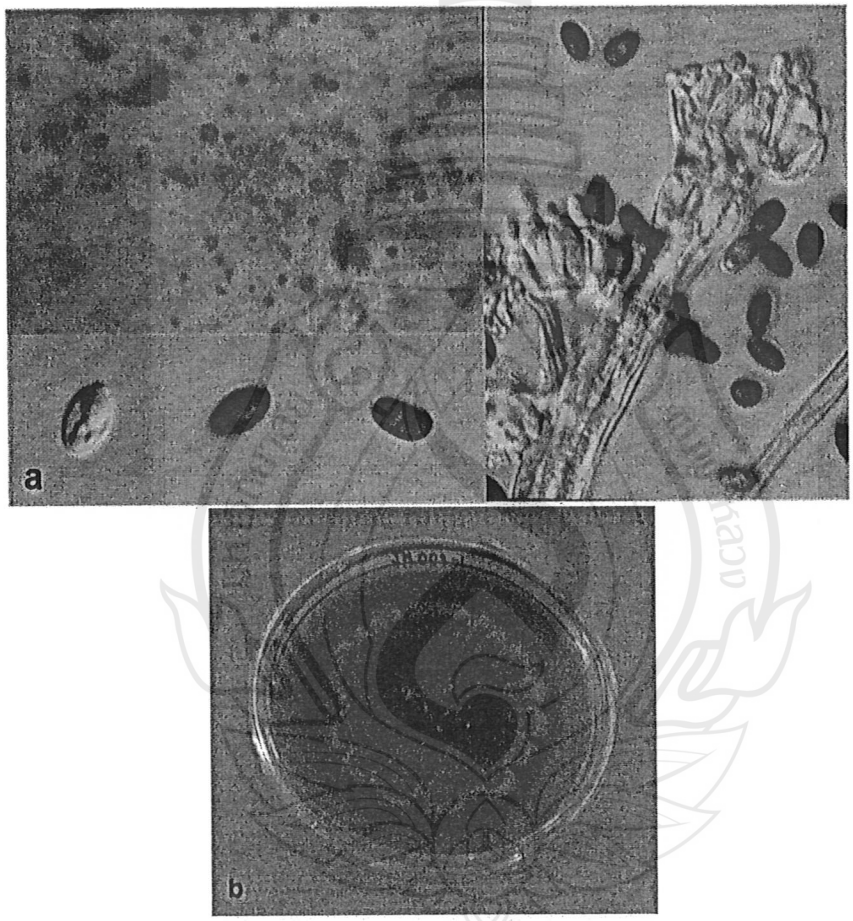
Zhong JJ and Xiao JH. 2009. Secondary Metabolites from Higher Fungi: Discovery, Bioactivity, and Bioproduction. Adv. Biochem. Eng. Biotechnol. 113, 79–150.

Zhou D and Hyde KD. 2001. Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi. Mycol. Res. 105, 1449–1457.

APPENDIX

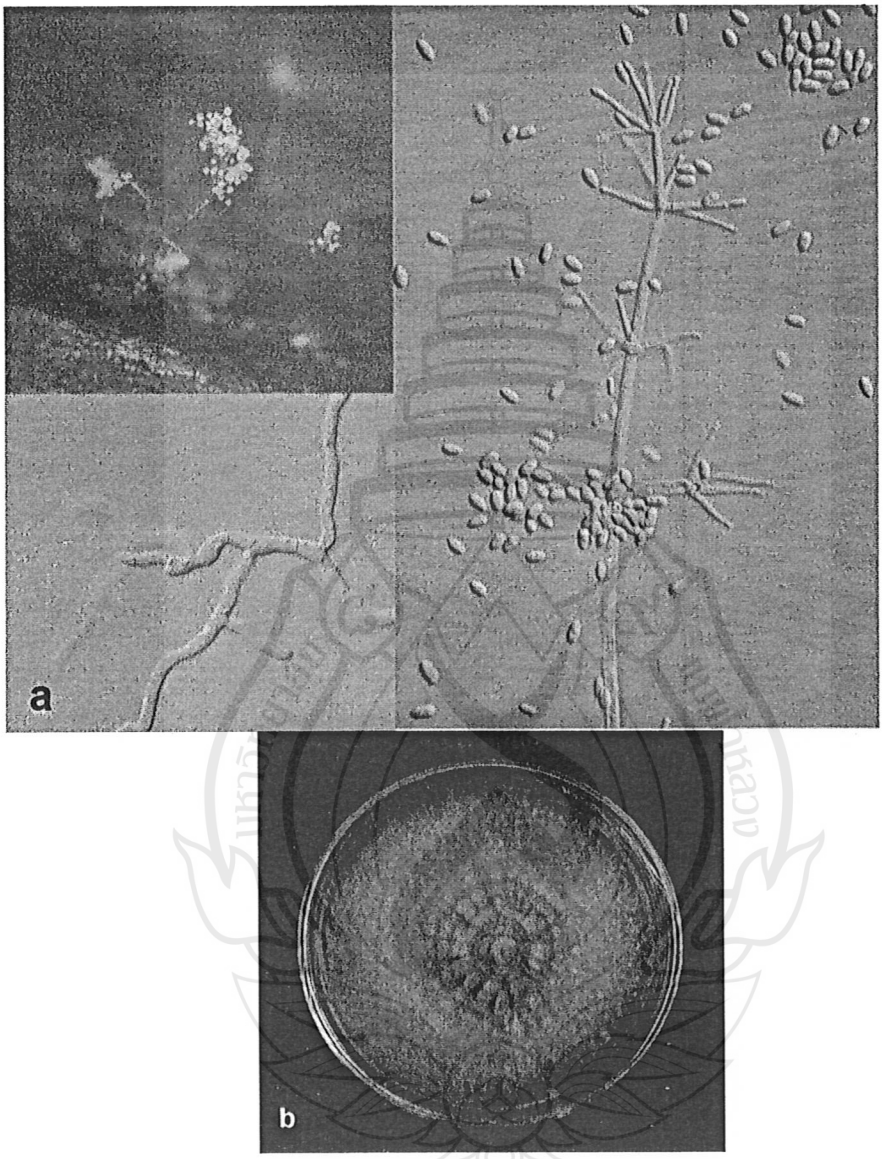
Morphological characteristics of some fungal representatives

1. *Stachybotrys parvispora* (JM09)



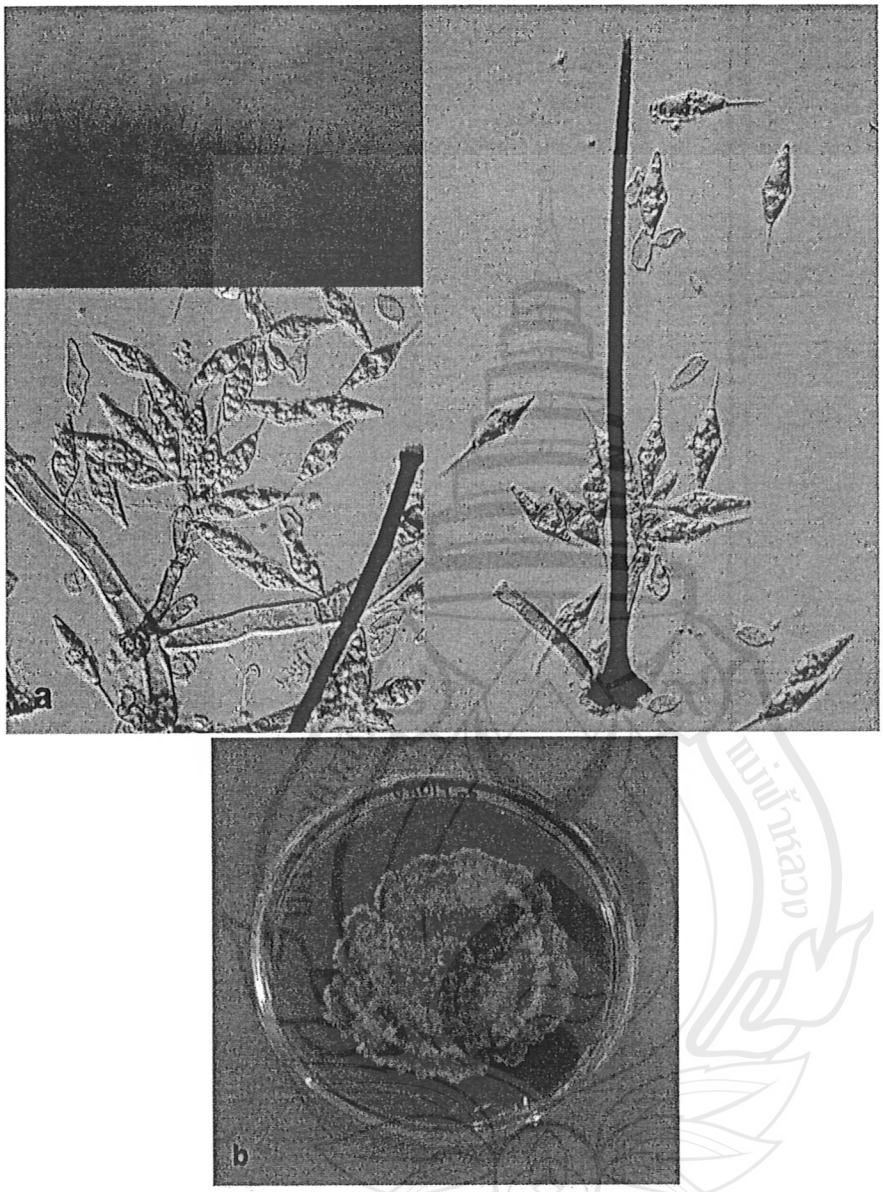
Note. (a) Morphological character on *M. liliifera* leaf. (b) Colony characteristic on SDA. at 28 °C, 30 days.

2. *Clonostachys rosea* (JM13)



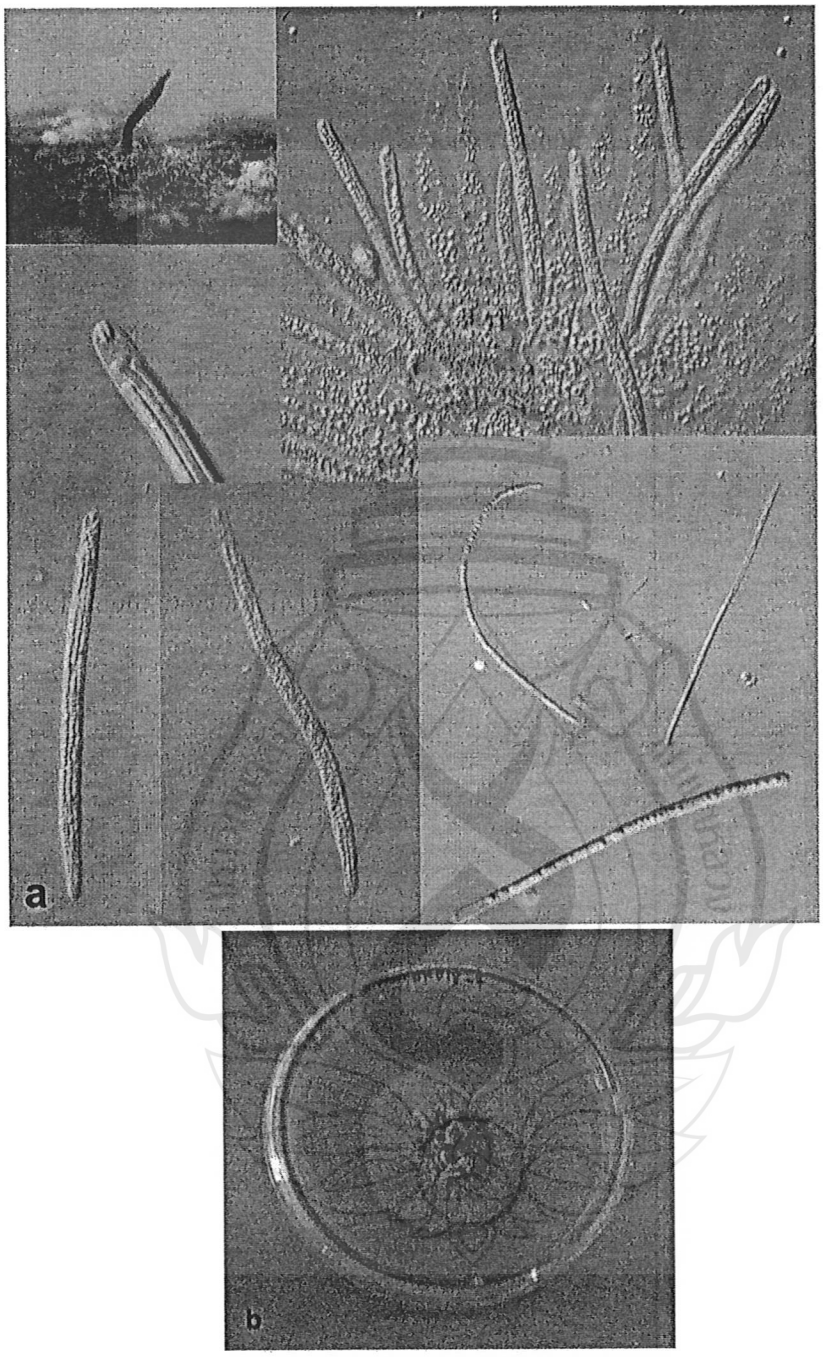
Note. (a) Morphological character on *M. liliifera* leaf. (b) Colony characteristic on SDA. at 28 °C, 30 days.

3. *Beltrania rhombica* (JM14)



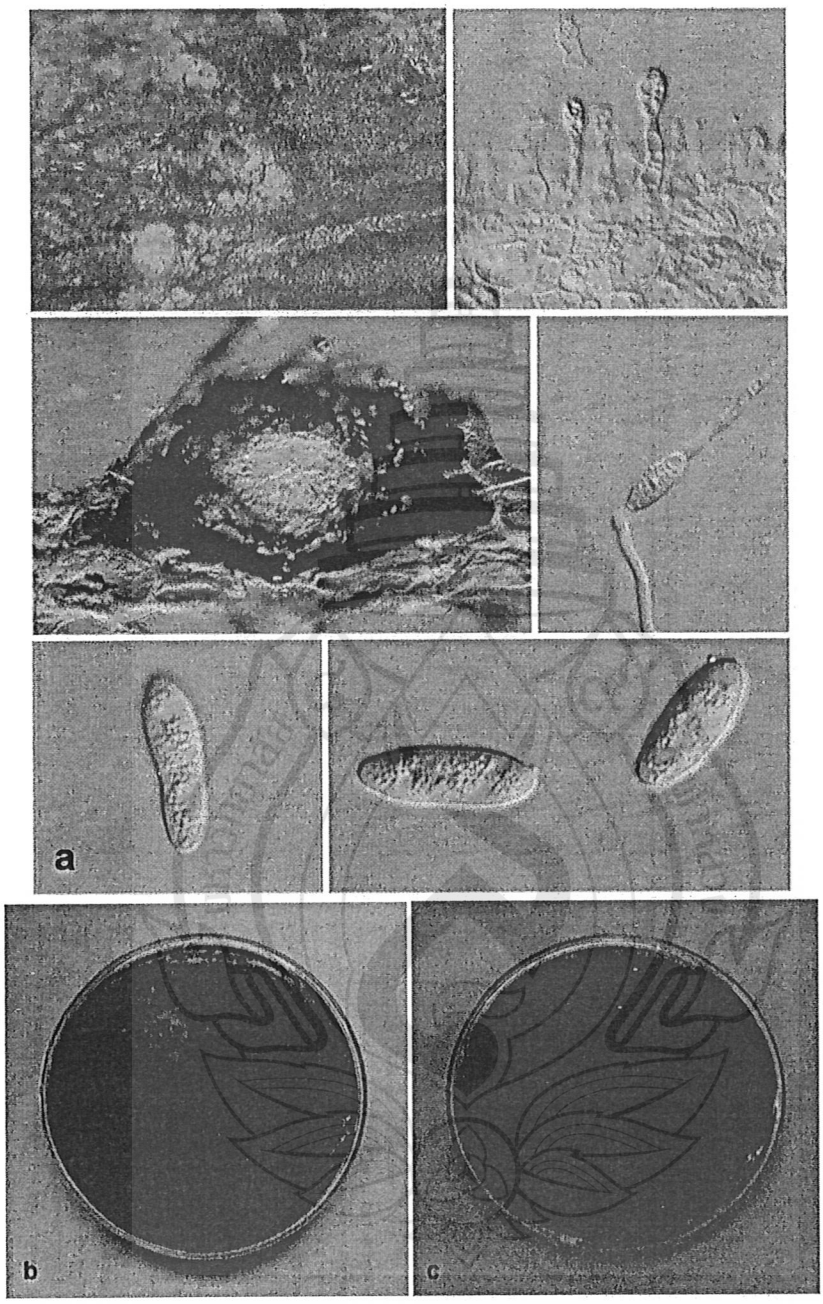
Note. (a) Morphological character on *M. liliifera* leaf. (b) Colony characteristic on SDA. at 28 °C, 30 days.

4. *Ophioceras* cf. *leptosporum* (JM18)



Note. (a) Morphological character on *M. liliifera* leaf. (b) Colony characteristic on SDA. at 28 °C, 30 days

5. *Fusicoccum aesculi* (JM19-1, JM19-2)



Note. (a) Morphological characters of strains JM19-1 and JM19-2 on *Magnolia liliifera* leaf. (b) Colony characteristic of strain JM19-1 on SDA. at 28 °C, 30 days. (c) Colony characteristic of strain JM19-2 on SDA. at 28 °C, 30 days.

BIOGRAPHY

Name Assistant Professor Dr. Ekachai Chukeatirote
Adress School of Science, Mae Fah Luang University, Chiang Rai 57100
Tel. 053-916778
Fax 053-916776
e-mail ekachai@mfu.ac.th

Educational Background

PhD in Biochemistry, University of Kent at Canterbury, UK
BSc (First Class Hons.) in Biology, Chiang Mai Univeristy, Thailand

Publications

- Dajanta K, **Chukeatirote E**, Apichartsrangkoon A. 2012. Improvement of *thua nao* production using protein-rich soybean and *Bacillus subtilis* TN51 starter culture. Annals of Microbiology 62: 785-795.
- Karunarathna SC, Yang ZL, Raspe O, Ko Ko TW, Vellinga EC, Zhao RL, Bahkali AH, **Chukeatirote E**, Degreef J, Callac P and Hyde KD. 2011. *Lentinus giganteus* revisited: new collections from Sri Lanka and Thailand. Mycotaxon 118: 57-71.
- Wikee S, Udayanga D, Crous PW, **Chukeatirote E**, McKenzie EHC, Bahkali AH, Dai DQ and Hyde KD. Phyllosticta—an overview of current status of species recognition. Fungal Diversity 51: 43-61.
- Liu JK, Jones EBG, **Chukeatirote E**, Bahkali AH, Hyde KD. 2011. *Lignincola conchicola* from palms with a key to the species of *Lignincola*. Mycotaxon 117: 343–349.
- Ko Ko TW, McKenzie EHC, Bahkali AH, To-anun C, Chukeatirote E, Promputtha I, Abd-Elsalam KA, Soytong K, Wulandari NF, Sanoamuang N, Jonglaekha N, Kodsueb R, Cheewangkoon R, Wikee S, Chamyuang S, Hyde KD. 2011. The need for re-inventory of Thai phytopathogens. Chiang Mai Journal of Science 38: 625-637.
- Manamgoda DS, Cai L, Bahkali AH, **Chukeatirote E** and Hyde KD. 2011. *Cochliobolus*: an overview and current status of species. Fungal Diversity 51: 3-42.
- Liu JK, Phookamsak R, Jones EBG, Zhang Y, Ko Ko TW, Hu HL, Boonmee S, Doilom M, **Chukeatirote E**, Bahkali AH, Wang Y and Hyde KD. 2011. *Astrosphaeriella* is polyphyletic, with species in *Fissuroma* gen. nov., and *Neoastrosphaeriella* gen. nov. Fungal Diversity 51: 135-154.
- Chomnunti P, Schoch CL, Aguirre-Hudson B, Ko-Ko TW, Hongsanan S, Jones EBG, Kodsueb R, Phookamsak R, **Chukeatirote E**, Bahkali AH and Hyde KD. 2011. *Capnodiaceae*. Fungal Diversity 51: 103-134.
- Boonmee S, Zhang Y, Chomnunti P, **Chukeatirote E**, Tsui CKM, Bahkali AH and Hyde KD. 2011. Revision of lignicolous *Tubeufiaceae* based on morphological reexamination and phylogenetic analysis. Fungal Diversity 51: 63-102.
- Dajanta K, Apichartsrangkoon A and **Chukeatirote E**. 2011. Free-amino acid profiles of thua nao, a Thai fermented soybean. Food Chemistry 125: 342–347.
- Dajanta K, Apichartsrangkoon A and **Chukeatirote E**. 2011. Volatile profiles of thua nao, a Thai fermented soy product. Food Chemistry 125: 464-470.
- Dajanta K, **Chukeatirote E** and Apichartsrangkoon A. 2011. Analysis and characterisation of amino acid contents of thua nao, a traditionally fermented soybean food of Northern Thailand. International Food Research Journal 18: 588-592.
- Wikee S, Cai L, Noireung P, McKenzie EHC, Su YY, **Chukeatirote E**, Thi HN, Bahkali AH, Moslem MA, Abdelsalam K and Hyde KD. 2011. *Colletotrichum* species from Jasmine (*Jasminum sambac*). Fungal Diversity 46: 171–182.

Name Associate Professor Dr. Kevin Hyde
Adress School of Science, Mae Fah Luang University, Chiang Rai 57100
Tel. 053-916248
Fax 053-916776
e-mail kdhyde3@gmail.com

Educational Background

D.Sc. in Fungal Diversity, University of Wales, UK
B.Sc. in Zoology, University of Wales, UK

Publications

- Aung OM, Soytong K, Hyde KD, 2008. Diversity of entomopathogenic fungi in rainforests of Chiang Mai Province, Thailand. Fungal Diversity, 30, 15-22.
- Cai L, Guo XY, Hyde KD, 2008. Morphological and molecular characterisation of a new anamorphic genus Cheirosporium, from freshwater in China. Persoonia, 20, 53-58.
- Duong LM, McKenzie EHC, Lumyong S, Hyde KD, 2008. Fungal succession on senescent leaves of Castanopsis diversifolia in Doi Suthep-Pui National Park, Thailand. Fungal Diversity, 30, 23-36.
- Hyde KD, Zhang Y, 2008. Epitypification: should we epitypify? Journal of Zhejiang University-Science B, 9, 842-846.
- Kodsueb R, McKenzie EHC, Lumyong S, Hyde KD, 2008a. Diversity of saprobic fungi on Magnoliaceae. Fungal Diversity, 30, 37-53.
- Kodsueb R, McKenzie EHC, Lumyong S, Hyde KD, 2008b. Fungal succession on woody litter of Magnolia liliifera (Magnoliaceae). Fungal Diversity, 30, 55-72.
- Pinruan U, Sakayaroj J, Hyde KD, Jones EBG, 2008. Thailandiomyces bisetulosus gen. et sp nov (Diaporthales, Sordariomycetidae, Sordariomycetes) and its anamorph Craspedodidymum, is described based on nuclear SSU and LSU rDNA sequences. Fungal Diversity, 29, 89-98.
- Swe A, Jeewon R, Pointing SB, Hyde KD, 2008. Taxonomy and molecular phylogeny of Arthrobotrys mangrovispora, a new marine nematode-trapping fungal species. Botanica Marina, 51, 331-338.
- Than PP, Jeewon R, Hyde KD, Pongsupasamit S, Mongkolporn O, Taylor PWJ, 2008a. Characterization and pathogenicity of Colletotrichum species associated with anthracnose on chilli (Capsicum spp.) in Thailand. Plant Pathology, 57, 562-572.