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รายงานวิจัยฉบับสมบูรณ์

การปรับปรุงวิธีการที่เหมาะสมต่อการจัดจำแนกวงศ์วานวิวัฒนาการของเชื้อราในกลุ่ม *Colletotrichum* ที่เป็นสาเหตุที่สำคัญของโรคพืช

A practical phylogeny-based approach for revision of the important pathogenic genus *Colletotrichum*.

โดย

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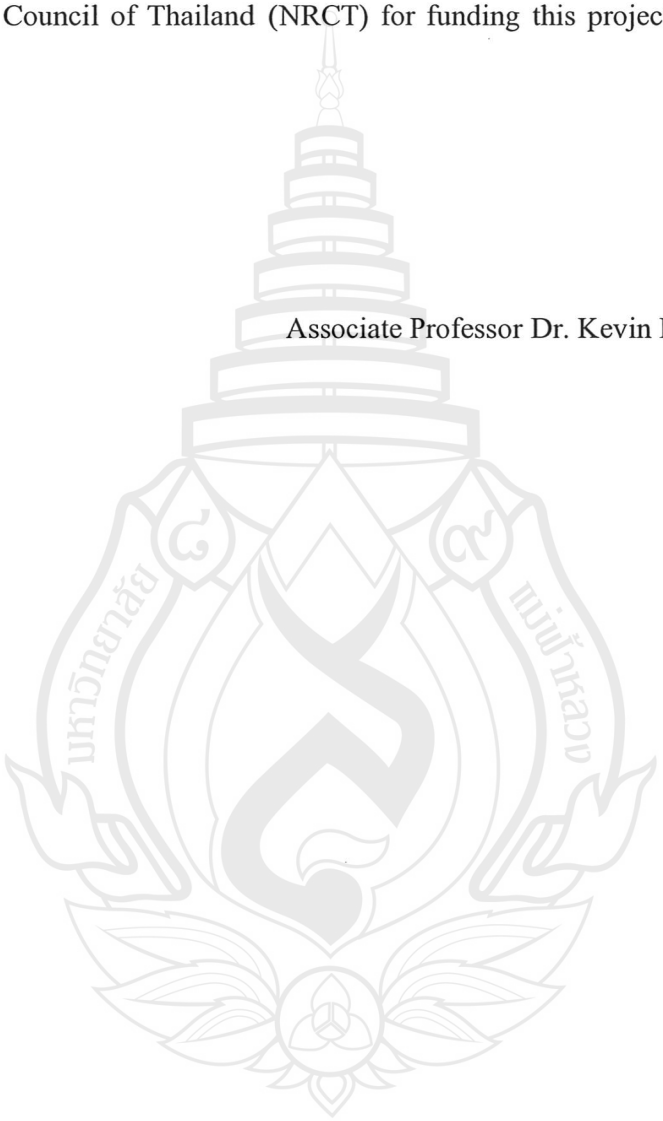
Associate Professor Dr. Kevin D. Hyde



บทสรุปผู้บริหาร (EXECUTIVE SUMMARY)

1. ความสำคัญและที่มาของปัญหาในการการวิจัย (Rationale and review)

Species concepts in the important pathogenic genus *Colletotrichum* are currently based largely on morphology in culture. Host-specificity is now considered to operate only at the microspecies level. Assumptions in the past that *Colletotrichum* species are host-specific has led to more than 650 species having been named (http://www.ukncc.co.uk/cabipages/Names/NAMES.ASP). This has severely constrained accurate exchange of information on *Colletotrichum* species.

Morphology and culture-based systems are generally appropriate for diagnosis of species aggregates, but are of little use to distinguish the evolutionary units of relevance to plant pathologists. Hence the outcome of the project will be a significant refinement of current *Colletotrichum* taxonomy based on phylogenetic evidence potentially leading to molecular identification systems and diagnostic tools. This in turn will lead to a better understanding of the requirements for plant breeders who require knowledge of species they need to breed plant resistance against.

2. วัตถุประสงค์ของโครงการวิจัย (Objectives of the research)

i. To create a preliminary multigene-based phylogeny of the genus *Colletotrichum* using nuclear and mitochondrial genomes, ribosomal DNA, β-tubulin, TEF1α, and other appropriate genes.

ii. To determine the utility for phylogenetic inferences of single gene sequence data sets as compared to combined data sets at the generic level using *Colletotrichum* species.

iii. To indicated species concepts within the genus by linking molecular result and morphology.

3. ขอบเขตของโครงการวิจัย (Scope of the research)

The genus *Colletotrichum* contains about 50 species, which cause plant diseases often known as anthracnose. We will collect more than 200 isolates in Thailand (mostly northern part) and some specimens from elsewhere in Thailand. In this study we will develop a practical phylogeny-based approach for the identification of *Colletotrichum*, focusing on Thai species and the *C. gloeosporioides* complex. Variations in the mitochondrial genome, ribosomal DNA, β-tubulin, TEF1α and other appropriate genes will be investigated; all are in wide use in other fungal genera to resolve problems in identification. The successful outcome of this project will have important practical implications to the plant pathology, plant breeding and quarantine communities and result in important publications.

4. ระเบียบวิธีวิจัยและผลผลิตจากการวิจัย (Methodology and the research output)

Research Plan from October 2010 to September 2013
Year 1 (October 2010 to September 2011): (12 months).

Anthracnose diseased samples will be collected in Thailand and specimens will be procured from elsewhere as needed for this study. Diseased samples will be observed and any the *Colletotrichum* species associated with the disease tissues will be isolated by single spore isolation techniques. Cultures will be grown in PDA at room temperature for one week and the morphology of selected species will be observed and documented by stereomicroscope and compound microscope. More than 100 isolates will be obtained. Molecular sequence data, initially ITS, will be used to characterize species and decide which strains need further study. All the cultures will be deposited at MFLU culture collection (MFLUCC, Mae Fah Luang University, Thailand). We will also work towards epitypification of other species.

5. ประโยชน์ที่ได้รับ (Benefit)

Colletotrichum species are important pathogens causing disease of crops and ornamental plants and it is essential we can accurately identify species for plant disease control, plant breeding programs, quarantine regulations and important publications. We will survey of *Colletotrichum* species infecting crops, fruits and other plants in Thailand and will isolate endophytes from healthy grasses. By identifying the disease causal agents we will establish which species infect which plants in Thailand, we expect to discover several new species and also work towards epitypification of other species. Phylogenetic analyses of ITS and morphology will be used to characterize species that need further work and we will look towards establishing a single gene(s) that can readily identify species. Ultimately we will develop a practical phylogeny for the identification of *Colletotrichum* species, by focusing on Thai species. Our research will involve international collaborations so that the techniques used to classify *Colletotrichum* species will be globally accepted.

6. แผนการถ่ายทอดเทคโนโลยีหรือผลการวิจัยสู่กลุ่มเป้าหมาย

Systematics, plant pathologists, plant health practitioners, plant breeders and quarantine officers, since they cannot name organisms confidently

บทคัดย่อ

เชื้อราในสกุล *Colletotrichum* ซึ่งมีจำนวนสปีชีส์ประมาณ 50 สปีชีส์ ซึ่งจากรายงานการค้นพบ *Colletotrichum* สปีชีส์ พบว่า โดยส่วนใหญ่เป็นเชื้อสาเหตุของโรคแอนแทรคโนส ซึ่งเชื้อราในสกุล *Colletotrichum* มีความสามารถในการแพร่ กระจาย และ ระบาดไปยังพื้นที่ต่างๆ ได้อย่างกว้างขวาง โดยเฉพาะอย่างยิ่ง เป็นสาเหตุหลักในการสร้างความเสียหายทางพืชเศรษ ฐกิจ อาทิเช่น ธัญพืช พืชผัก พืชตระกูลถั่ว ไม้ดอกไม้ประดับ และ ไม้ผล ในงานวิจัยฉบับนี้ ผู้วิจัยได้นำเสนอ การพัฒนาการศึกษาวิวัฒนาการ ของเชื้อราในสกุล *Colletotrichum* เพื่อใช้ในการจัดจำแนกในระดับสปีชีส์อย่างถูกต้องและแม่นยำ เพื่อประโยชน์ในการแก้ไขปัญหา หรือการป้องกันโรคที่เกิดจากเชื้อราในสกุล *Colletotrichum* ยิ่งไปกว่านั้นยังนำไปถึงประโยชน์ด้าน การกักกันโรค และ การตกแต่งพันธุกรรม ได้ในอนาคต โดยงานวิจัยฉบับนี้ได้ได้มีการเก็บรวบรวมตัวอย่างเพื่อใช้ในการศึกษา จากต่างสถานที่ภายในประเทศไทย ต่าง พืชอาศัย รวมไปถึงส่วนต่างๆของพืชอาศัย เพื่อนำไปสู่การศึกษาในระดับโมเลกุล ผู้วิจัยได้ใช้วิธี Polymerase chain reaction และ Sequencing analyses โดยใช้ ITS, Actin, CAL, GPDH, GS และ TUB ดังนั้นในรายงานฉบับนี้ได้จึงนำเสนอ 83 sequence ที่เป็นผลจากการวิเคราะห์รหัสทางพันธุกรรม ITS และ 26 sequence ใน Actin นอกจากนี้ยังมี CAL, GPDH, GS และ TUB sequence อีกด้วย

Keywords: *Colletotrichum*, แอนแทรคโนส, วงศ์วานวิวัฒนาการ, เชื้อราโรคพืช, ลักษณะสัณฐานวิทยา

ABSTRACT

The genus *Colletotrichum* (sexual state "Glomerella") are important pathogens causing serious disease of plants and infected crops are subjected of import control (quarantine) and plant breeding programs. *Colletotrichum* species have a worldwide distribution and are associated with leaf spots, fruit anthracnose and when serious infections occur they are responsible for reducing economic plant yields (e.g. of cereals, vegetables, legumes, ornamental plants and fruits). They are also obligate symbionts and occur in a symptomless parts of plants as endophytes and the relationships between life modes (i.e. can the fungi switch modes) is poorly establish. Previous identification and classification was based on host association and morphological characteristics. Molecular sequence data analysis has become commonplace in classifying plant pathogenic genera like *Colletotrichum*, which have been found to comprise several species complexes. Initially ITS and morphology was used to characterize species, however, they could not resolve species well. Recent multigene phylogenetic analysis have involved actin (*act*), calmodulin (*cal*), chitin synthase (*chs1*), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and ITS gene regions as well as morphology and pathogenicity testing so at present there are about 100 described species and this is increasing monthly. There are also four accepted species complexes and *C. gloeosporioides* is the most important. Recently multigene phylogenetic analysis confirmed that *C. gloeosporioides* is a species complex that comprises 22 morphologically similar, phylogenetically distinct species. However, ITS, beta tubulin (*tub2*), DNA lyase and the intergenic region of *apn2* and *MAT1-2-1* genes (*ApMat*)) have also been used to identify new lineages and new species within this species-complex and presently, there are 28 accepted species names within the species complex. There is however, no consensus among mycologists as to which gene markers should be used to define and delimit a species within the species complex.

At the beginning of this study (October 2011) there were more 50 confirmed "molecular" species in the genus causing plant diseases often known as anthracnose with 15 species known from Thailand. In the first year we collected more than 100 fresh specimens of various disease plants and fruits from difference places in

Thailand. More than 100 strains were isolated from the fresh specimens and we initiated a survey of *Colletotrichum* species infecting fruits in Thailand and also those which are endophytes of healthy grasses. We also started to sequence these isolates and carry out morphological as well as pathogenicity studies. We identified several new species and also worked towards epitypification of other species and the first publications will appear in year two of the grant. We are also involved in developing a practical phylogeny and morphology based approach for the identification of *Colletotrichum* species, focusing on Thai species. However, since this is a global problem we have also chosen to collaborate with Brazilian, Chinese and Indian colleagues in order to bring greater depth to the research and international agreement to the findings. The latter was achieved with the formation of the International Subcommission on *Colletotrichum* taxonomy (http://www.fungaltaxonomy.org/subcommissions) of which our group is a founder member.

Keywords: *Colletotrichum*, Phylogeny, Plant pathogen, Taxonomy

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ABBREVIATION AND SYMBOLS

ACT	=	Actin
CTAB	=	Hexadecyltrimethylammonium bromide
DNA	=	Deoxyribonucleic acid
ITS	=	Internal transcribed spacer
LSU	=	Large subunit (28S rDNA)
PDA	=	Potato dextrose agar
RFLP	=	restriction fragment length polymorphism
Sp.	=	Species
TEF 1-α	=	Translation elongation factor 1-alpha
β-tubulin	=	Beta-tubulin
μm	=	micrometer

CHAPTER 1
INTRODUCTION

1.1 Background

Species concepts in the important pathogenic genus *Colletotrichum* are currently based largely on morphology in culture. Host-specificity is now considered to operate only at the micro-species level. An assumption in the past that *Colletotrichum* species are host-specific has led to numerous problems in identification, with more than 650 species having been named (http://www.ukncc.co.uk/cabipages/Names/NAMES.ASP). This has severely constrained accurate exchange of information on *Colletotrichum* species.

Morphology and culture-based systems are generally appropriate for diagnosis of species aggregates, but are of little use to distinguish the evolutionary units of relevance to plant pathologists. Hence the outcome of the project will be a significant refinement of current *Colletotrichum* taxonomy based on phylogenetic evidence potentially leading to molecular identification systems and diagnostic tools. This in turn will lead to a better understanding of the requirements for plant breeders who require knowledge of species they need to breed plant resistance against.

1.2 Objectives

1.2.1 To create a preliminary multigene-based phylogeny of the genus *Colletotrichum* using nuclear and mitochondrial genomes, ribosomal DNA, β-tubulin, TEF1α, and other appropriate genes.

1.2.2 To determine the utility for phylogenetic inferences of single gene sequence data sets as compared to combined data sets at the generic level using *Colletotrichum* species.

1.2.3 To elucidate species concepts within the genus by linking molecular and morphological approaches.

1.3 Scope of research

The genus *Colletotrichum* contains about 50 species, which cause plant diseases often known as anthracnose. *Colletotrichum* species are worldwide in distribution and cause major damage to cereals, vegetables, legumes, ornamental plants and fruit trees. The current naming of *Colletotrichum* species is largely based on a combination of morphological and cultural characteristics. Due to limited numbers of morphological character-suites available in culture coupled with inherent phenotypic plasticity, precise identification of the species has always been difficult. Physiological specialization within species and overlapping host ranges mean that our current classification system is impracticable for users. This causes problems to systematics, plant pathologists, plant health practitioners, plant breeders and quarantine officers, since they cannot name organisms confidently. In our proposal we will develop a practical phylogeny-based approach for identification of *Colletotrichum*, focusing on Thai species and the *C. gloeosporioides* complex. Variations in the mitochondrial genome, ribosomal DNA, β-tubulin, TEF1α and other appropriate genes will be investigated; all are in wide use in other fungal genera to resolve problems in identification and taxonomy. The successful outcome of this project will have important practical implications to the plant pathology, plant breeding and quarantine communities and important publications.

CHAPTER 2

LITERATURE REVIEW

2.1 The need for species recognition

The genus *Colletotrichum* causes various plant diseases often known as anthracnose and is worldwide in distribution (Sutton, 1992). *Colletotrichum* species cause major damage to crops in tropical, subtropical and temperate regions (Than *et al.*, 2008 and Hyde *et al.*, 2009a, 2010). Cereal, vegetables, legumes, ornamentals and fruit trees may be seriously affected by this pathogen (Freeman, 2000). Also, ***Colletotrichum*** sp. is cosmopolitan and has been shown that multiple species can infect single host or single species can infect multiple hosts (Cai *et al.*, 2009 and Hyde *et al.* 2009). *Colletotrichum* species are also commonly isolated as endophytes, and latent and quiescent infections by these species on several hosts have been reported (Bills, 1996; Brown *et al.*, 1998 and Photita *et al.*, 2004, 2005). Their ability to cause latent infection i.e. infection without visible symptom makes them one of the most successful pathogens causing post-harvest disease in a wide range of crop species (Sutton, 1992).

At least nine different *Colletotrichum* species, *C. capsici*, *C. coccodes*, *C. crassipes*, *C. dematium*, *C. destructivum*, *C. gloeosporioides*, *C. lindemuthianum*, *C. trifolii* and *C. truncatum* for example, have been reported on legumes in tropical and temperate regions (Lenne, 1992). These legume hosts include many important human food sources including grain legumes, root crops and fruits, and pasture plants, medicinal plants, timber trees and ornamentals (Anonymous, 1979). All of these *Colletotrichum* species are reported to infect at least two hosts, and *C. capsici*, *C. gloeosporioides* and *C. lindemuthianum* are reported to have the widest host ranges among these nine. These reports however, are not backed up by voucher specimens and their occurrence cannot be verified. If we want to breed legumes that are resistant to anthracnose caused by *Colletotrichum* species we must know which species infect which hosts.

Colletotrichum gloeosporioides is a particularly large complex comprising taxa which cause diseases of a wide range of crops. The taxa have been isolated as pathogens, endophytes and saprobes and it is not clear whether these different lifestyles are associated with specific lineages or have evolved many times. It is therefore particularly important that we gain an understanding of the diversity of organisms within this complex.

Colletotrichum sp. is the anamorphic stage of several species of *Glomerella* sp. and has a taxonomic history of about 200 years (Corda, 1837). There are 17 acknowledged generic synonyms for *Colletotrichum* sp. and two further names are dubiously included, and there are about 900 species names assigned to this genus (Sutton, 1980, 1992). The identification and characterization of *Colletotrichum* species are mainly based on morphological and cultural criteria or a combination of both. It has become apparent that the classification system presently used has limited scope since some species names assigned to collection and isolates lack the precision required by users. The numbers of morphological characters derived from growth in culture are limited, and growth conditions have been rarely standardized. Moreover the inherent phenotypic plasticity of individual isolates creates confusion in identification. There are group species or species complexes such as *C. gloeosporioides*, *C. dematium*, *C. lindemuthianum* which are known to be represented by at least nine distinct subtaxa (Sutton, 1992).

Under these circumstances the species name has limited practical significance to the plant pathologist involved in disease management and quarantine, and the breeder involved in resistant breeding. The evolution of different systems for identification of species over time has largely been the result of subtle changes in the concept concerning the importance of different aspects of morphology combined with ideas about host range and host-pathogen relationships for particular taxa. Despite these amendments the current concept of *Colletotrichum* sp. systematics is still very broad, unreliable and unpredictable being based on the combination of classical criteria such as conidial shape and size, presence, absence and morphology of setae, presence of sclerotia and appressoria and symptom expression on host. Moreover, the current classification system for *Colletotrichum* in general is unsatisfactory because

the constituent species are inadequately defined (Cannon *et al.*, 2000; Than *et al.*, 2008a).

Molecular approaches are being used to resolve problems in fungal taxonomy and fungal identification by many workers (Ma *et al.*, 1997; Ranghoo *et al.*, 1999; Rollo *et al.*, 1995; Zhang *et* al., 1997; Than *et al*, 2008a.b). Because of the shortcomings of *Colletotrichum* sp. systematic based on cultural characteristics and morphology there is need for a combined approach including the use of molecular data. Various DNA-based systems have been used to study phylogeny, systematic, genetic diversity and population structure of *Colletotrichum* species. These markers include restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), amplified restriction fragment length polymorphisms (AFLP), rDNA internal transcriber spacer (ITS1 to ITS2) and small subunit ribosomal RNA (18S rDNA). RFLP was found extremely useful to determine genetic relationships within *C. gloeosporioides* from *Stylosanthes* spp (Braithwaite *et al.*, 1990). The same marker was used to study diversity in *C. gloeosporioides* from *Stylosanthes guianensis*, and avocado and almond (Freeman *et al.*, 1996; Kelemu *et al.*, 1999). RAPD was used to study genetic diversity and variability in *C. lindemuthianum*, *C. acuminatum* and *C. gloeosporioides* (Balardin *et al.*, 1997; Gonzalez *et al.*, 1998; Kelemu *et al.*, 1999; Lander *et al.*, 1999; Sicard *et al.*, 1997). Similarly AFLP was used to characterize *C. lindemuthianum* isolates (Gonzalez *et al.*, 1998), however these days these techniques are considered crude.

Cannon *et al.* (2000) stated that data derived from nucleic acid analyses should provide the most reliable framework to build a classification of *Colletotrichum* sp., as DNA characters were not directly influenced by environmental factors. Most fungal phylogenetic studies utilized sequences from the ribosomal gene cluster, since they were present in large numbers as tandem repeats and evolved as a single unit (Mitchell *et al.*, 1995). In particular, sequence analysis of the internal transcribed spacer (ITS) regions which lie between the 18S and 5.8S genes and the 5.8S and 28S genes, has proved useful in studying phylogenetic relationships of *Colletotrichum* species because of their comparative variability (Sreenivasaprasad *et al.*, 1994; 1996; Moriwaki *et al.*, 2002; Photita *et al.*, 2005; Shenoy *et al.*, 2007; Than *et al.*, 2008a,b).

The ITS region was used to analyze *Colletotrichum* species from various fruits, to study intraspecific diversity in *C. acuminatum* and *C. lindemuthianum* and to study phylogeny and systematic of several *Colletotrichum* spp. (Freeman *et al.*, 2000, 2001; Sicard *et al.*, 1997; Sreenivasaprasad *et al.*, 1996). Small subunit rRNA was used to infer phylogenetic placement of *Athelia bambacina*, *Aureobasidium pullunans* and *C. gloeosporioides* (Illingworth *et al.*, 1991). Besides these A+T rich DNA analysis, ß-tubulin genes have been used to study *Colletotrichum* species (Freeman *et al.*, 2000, 2001; Thon and Royse, 1999; Than *et al.*, 2008a,b). Sequence analysis of protein coding genes such as partial β-tubulin gene, has also been applied to resolve phylogenetic relationships among *C. acutatum* species complexes (Sreenivasaprasad and Talhinhas, 2005). Sequences of introns from two genes (glutamine synthase and glyceraldehyde-3-phosphate dehydrogenase) were also used to evaluate a diverse collection of isolates of *C. acutatum* (Guerber *et al.*, 2003; MacKenzie *et al.*, 2008). *C. acutatum* isolates clustered into groups (Guerber *et al.*, 2003; MacKenzie *et al.*, 2008; Peres *et al.*, 2008). These groups might represent phylogenetically distinct species of *C. acutatum sensu lato* (Guerber *et al.*, 2003). Yun *et al.* (1999) stated that, because of the high intra-species variability and the low inter-species variability, MAT1-2 mating type sequences gave strong support for branches, allowing differentiation of closely related *Cochliobolus* spp. whose relationships were not resolved by ITS sequences alone. Consequently, Du *et al.*(2005) confirmed that MAT1-2 mating type was useful in differentiating the groups of isolates from the species complexes (*C. graminicola*, *C. gloeosporioides* and *C. acutatum*).

A combined application of molecular diagnostic tools along with traditional morphological characterization is an appropriate and reliable approach for studying *Colletotrichum* species complexes (Cannon *et al.*, 2000). Than *et al.* (2008a) differentiated isolates of chilli anthracnose from Thailand into three species: *C. acutatum*, *C. capsici* and *C. gloeosporioides*, based on morphological characterization, sequencing based on rDNA-ITS region and partial ß tubulin gene and pathogenicity testing. Hong and Kim (2007) reported that Korea isolates of *C. acutatum* were phylogenetically separated from the global groups of *C. acutatum* A1 to A8 based on the sequences in partial beta-tubulin 2 (exons 3-6). Restriction

fragment length polymorphisms (RFLP) of ITS region resulting from *Alu*I, *Rsa*I and *Bam*HI digestions have also been employed to differentiate *Colletotrichum* species from chilli anthracnose in Taiwan region (Sheu *et al*., 2007).

The current classification of *Colletotrichum* species is broad and has a limited practical significance. It is well accepted that the systematic of the genus *Colletotrichum* awaits a detailed investigation and refinement. In this proposal we will initiate the work to refine *Colletotrichum* taxonomy focusing on Thailand taxa and in particular the *C. gloeosporioides* complex. Various molecular techniques such as PCR amplification of rDNA; β-tubulin, TEF1α and other appropriate gene sequences will be used in this study. We will also determine the utility for phylogenetic inferences of single gene sequence data sets as compared to combined data sets at the generic level using *Colletotrichum* species.

CHAPTER 3

MATERIAL AND METHODS

3.1 Material

3.1.1 Petri dish

3.1.2 Loop

3.1.3 Needle

3.1.4 Blade

3.1.5 Slide

3.1.6 Cover slide

3.1.7 Forceps

3.1.8 Mortar and pestle

3.1.9 Tubes

3.1.10 Tips

3.1.11 Pipette/Autopipette

3.1.12 Gel Electrophoresis

3.1.13 Autoclave

3.1.14 Hot air oven

3.1.15 Lamina air flow

3.1.16 Microscopes (Optical and Stereo microscope)

3.1.17 Microtome

3.1.18 Media (Potato dextrose agar, Difgo and Agar)

3.1.19 Chemical

3.1.19.1 Dye (Lactophenol cotton blue stain)

3.1.19.2 Ethanol

3.1.19.3 DNA extract kit

3.1.19.4 Agarose gel

3.1.19.5 Distilled water

3.2 Method

3.2.1 Fungal isolates and cultures

Strains of *Colletotrichum* spp. were isolated by single spore isolation method on the water agar (WA) and Potato dextrose agar (PDA, Difgo). After that cultures were grown on PDA at room temperature (25-30˚C) for one week. And then they were deposited at MFLU culture collection (MFLUCC, Mae Fah Luang University, Thailand).

3.2.2 Molecular analysis

3.2.2.1 DNA extraction

DNA extracted from the fungal mycelium that were grown on PDA petridis (one week). Re-suspended fungal mycelium in 1-2 ml of sterile water. Suspensions vortexed briefly and left in the dark at 25˚C for 24 hrs to encourage spore germination and/or mycelium were softened. Total genomic DNA extracted by the adapted CTAB method used to extract fungal genomic DNA from fungi (Ford *et al.*, 2000).

3.2.2.2 Polymorphisms in nuclear DNAs

A nuclear DNA probe, GcpR1, from the bean anthracnose pathogen *C. lindemuthianum* had been found very useful to differentiate between species of *Colletotrichum* (Rodriguez and Yoder, 1991). This probe was also used to differentiate between *C. gloeosporioides* from Almond and Avocado (Freeman *et al.*, 1996). The same probe used to study inter-specific differences between these *Colletotrichum* species.

3.2.2.3 Analysis of A + T-rich DNA associated with mt DNA

Polymorphisms in A+T rich DNA revealed by restriction enzyme H*ae*III used to characterize and differentiate various *Colletotrichum* isolates (Freeman and Shabi, 1996; Freeman *et al.*, 1996; Hodson *et al.*, 1993; Sreenevasaprasad *et al.*, 1992). The restriction enzyme H*ae*III recognizes and cleaves the DNA sequence GGCC. Hence, most of the nuclear DNA digested to the fragment of less than 2 kb whereas A+T rich DNA is cleaved infrequently by this enzyme (Freeman *et al.*, 1996).

3.2.2.4 DNA fragment amplification (for ITS, β-tubulin and TEF 1-α genes)

Universal PCR primers available for amplification of ITS 1 and ITS 2 regions between the small and large nuclear rDNA including the 5.8S rDNA used as previously described by (White *et al.*, 1990). Similarly conserved regions of β-tubulin and TEF 1-α genes amplified using specific primers. Amplification reactions carried out in an automated thermal cycler using the basic amplification protocol described by Guo *et al.* (2001).

3.2.2.5 DNA sequencing

PCR products purified by Promega Wizard DNA purification kits. The purified DNA sequenced on an automated sequencer using fluorescent dye-labeled sequencing primers (ALFExpress, Pharmacia). The amplification product with multiple bands were appearing by electrophoresis in 1% low-melting temperature agarose gel, visualized with ethidium bromide, excised separately, purified and sequenced. When the two products were too closed for gel elution they cloned into pGEM-T Easy vector with an overnight ligation. Recombinants vector could identified and plasmid DNA did extracted, purified and sequenced. All of the data made available to the scientific community via submission to GenBank.

3.2.2.6 Data analysis

RFLP auto-radiograms of nDNA RFLP and gel photographs of mtDNA were scored for the presence (1) or absence (0) of band separately. A multi-locus genotype based on these two set of data were constructed for each study isolate. Genetic similarity between and within species were computed to construct UPGMA tree using the NTSYS-pc version 2.1 (Rolf, 2000). nDNA RFLP data for *C. lindemuthianum* populations from Hong Kong and Southern China were subjected to similar analysis, and the genetic diversity within and between the study populations were estimated by Gleason and Shannon indices.

PCR sequenced data of each gene was aligned for all isolates and with published sequences from GenBank and EMBL databases with computer program (eg. SeqApp, Clustal X) and then aligned manually. Non-informative sites was omitted or re-coded in the analysis. All characters were treated as unordered. Alignment data was subjected to three methods of phylogenetic analysis; maximum Parsimony (MP), Weighted parsimony (WP) and Maximum-likelihood (ML) using

PAUP* 4.0 (Swofford, 1998). Out group rooting was used to determine polarity. Bootstrapping and decay indices were used to measure branch robustness.

The techniques involved in the approach described are familiar with the investigators. Dr. Hyde has extensive knowledge in collection, isolation, identification and growth of fungal cultures. Drs Crous and Rampai had hand-on experience in the specific molecular and physiological evaluation techniques. Dr Hyde had many years experience in identification of *Colletotrichum* species and in relating molecular and morphological species concepts.

CHAPTER 4

RESULTS

4.1 Fungal isolates and cultures

In year one, we obtained 109 strains of *Colletotrichum* species (Table 4.1) from difference hosts and host parts and difference geographical regions in Thailand. We have preserved these in MFLU herbaria and MFLUCC culture collection and have carried out DNA sequencing for both.

Table 4.1 List of *Colletotrichum* spp. collection in Thailand.

MFLUCC	Host	Collection Site
10-0610	Jujube	Chiang Rai
10-0611	Strawberry	Chiang Rai
10-0612	Strawberry	Chiang Rai
10-0613	Strawberry	Chiang Rai
10-0614	Mango	Chiang Rai
10-0615	Strawberry	Chiang Rai
10-0616	Jujube	Chiang Rai
10-0617	*Mangifera* sp. small	Chiang Rai
10-0618	Mango	Chiang Rai
10-0619	Roseapple	Chiang Rai
10-0620	Jujube	Chiang Rai
10-0621	Citrus	Chiang Rai
10-0622	Strawberry	Chiang Rai
10-0623	Jujube	Chiang Rai
10-0624	Green jambu	Chiang Rai
10-0625	Roseapple	Chiang Rai
10-0626	Caryota urens	Chiang Rai

Table 4.1 List of *Colletotrichum* spp. collection in Thailand. (Continued)

MFLUCC	Host	Collection Site
10-0627	Dragon fruit	Chiang Rai
10-0628	Banana	Chiang Rai
10-0629	Citrus	Chiang Rai
10-0630	Rose apple	Chiang Rai
10-0631	Banana	Chiang Rai
10-0632	Banana	Chiang Rai
10-0633	Pine apple	Chiang Rai
10-0634	Strawberry	Chiang Rai
10-0635	Cassawarry plum	Chiang Rai
10-0636	Cassawarry plum	Chiang Rai
10-0637	Banana	Chiang Rai
10-0638	Banana	Chiang Rai
10-0639	Banana	Chiang Rai
10-0640	Banana	Chiang Rai
10-0641	Strawberry	Chiang Rai
10-0642	Strawberry	Chiang Rai
10-0643	Banana	Chiang Rai
10-0644	Unknown	Chiang Rai
10-0645	Strawberry	Chiang Rai
10-0646	Banana	Chiang Rai
10-0647	Banana	Chiang Rai
10-0648	Banana	Chiang Rai
10-0649	Banana	Chiang Rai
10-0650	Neem	Chiang Rai
10-0651	Neem	Chiang Rai
10-0652	Rose apple	Chiang Rai
10-0653	*Citrus* sp.	Chiang Rai
10-0654	*Citrus* sp.	Chiang Rai

Table 4.1 List of *Colletotrichum* spp. collection in Thailand. (Continued)

MFLUCC	Host	Collection Site
10-0655	Papaya	Chiang Rai
10-0656	Dragon fruit	Chiang Rai
10-0657	Papaya	Chiang Rai
10-0658	Dragon fruit	Chiang Rai
10-0659	*Amaranthus* sp.	Chiang Rai
10-0660	*Annona* sp.	Chiang Mai
10-0661	*Annona* sp.	Chiang Mai
10-0662	Wild Pepper	Mae Kathong
10-0663	Wild Pepper	Mae Kathong
10-0664	*Hibiscus* sp.	Chiang Rai
10-0665	*Schlegera* sp.	Chiang Rai
10-0666	Wild Legume	Chiang Rai
10-0667	*Mirabilis* sp.	Chiang Rai
10-0668	Banana	Chiang Rai
10-0669	Aeschynanthus radicans	Southern Thailand
10-0670	Long kong Banan	Southern Thailand
10-0671	Boa mango	Southern Thailand
10-0672	Mango	Southern Thailand
10-0673	Mango	Southern Thailand
10-0674	Banana	Southern Thailand
10-0675	Nongyang Banana	Southern Thailand
10-0676	*Ficus* sp.	Chiang Mai
10-0677	Cassawarry plum	Chiang Rai
10-0678	Cofee berries	Chiang Mai
10-0679	Cofee berries	Chiang Mai
10-0680	Cofee berries	Chiang Mai
10-0681	Cofee berries	Chiang Mai
10-0682	Cofee berries	Chiang Mai

Table 4.1 List of *Colletotrichum* spp. collection in Thailand. (Continued)

MFLUCC	Host	Collection Site
10-0684	*Ficus* sp.	Chiang Rai
11-0456	-	France
11-0460	Oak, Quercus	France
11-0534	*M. indica*	Chiang Rai
11-0535	Monocot plant	Chiang Rai
11-0536		Chiang Rai
11-0538		Chiang Rai
11-0539		
11-0541		
11-0542	*M. indica*	Chiang Rai
-	*Panicum* sp.	Chiang Rai
-	*Panicum* sp.	Chiang Rai
-	*Pennisetum* sp.	Chiang Rai
-	*Panicum* sp.	Chiang Rai
-	*Panicum* sp.	Chiang Rai
-	*Pennisetum* sp.	Chiang Rai
-	*Panicum* sp.	Chiang Rai
-	*Panicum* sp.	Chiang Rai
-	*Panicum* sp.	Chiang Rai
-	*Pennisetum* sp.	Chiang Rai
-	*Pennisetum* sp.	Chiang Rai
-	*Panicum* sp.	Chiang Rai
-	*Pennisetum* sp.	Chiang Rai
-	Lemmon grass	Chiang Rai
-	*Panicum* sp.	Chiang Rai
-	*Pennisetum* sp.	Chiang Rai
-	*Pennisetum* sp.	Chiang Rai
-	Lemmon grass	Chiang Rai

Table 4.1 List of *Colletotrichum* spp. collection in Thailand. (Continued)

MFLUCC	Host	Collection Site
	Pennisetum sp.	Chiang Rai
	Pennisetum sp.	Chiang Rai
-	*Panicum* sp.	Chiang Rai
-	*Panicum* sp.	Chiang Rai
-	*Panicum* sp.	Chiang Rai
-	*Panicum* sp.	Chiang Rai
-	*Panicum* sp.	Chiang Rai
-	*Panicum* sp.	Chiang Rai

4.2 Molecular analysis

For 1 year, we did PCR and Sequecing analysis on ITS, Actin, CAL, GPDH, GS and TUB region. We obtained 83 sequences of ITS and 26 sequences of Actin, CAL, GPDH, GS and TUB region (Table 4.2). We will continue this work.

Table 4.2 Result of PCR for *Colletotrichum* strains collected.

MFLUCC	Region							
	ITS	SSU	LSU	Actin	CAL	GAPDH	GS	TUB
10-0610	/	x	x	x	x	x	x	x
10-0611	/	x	x	x	x	x	x	x
10-0612	/	x	x	x	x	x	x	x
10-0613	/	x	x	x	x	x	x	x
10-0614	/	x	x	x	x	x	x	x
10-0615	/	x	x	x	x	x	x	x
10-0616	/	x	x	/	/	/	/	/
10-0617	/	x	x	x	x	x	x	x
10-0618	/	x	x	x	x	x	x	x
10-0619	/	x	x	x	x	x	x	x
10-0620	/	x	x	x	x	x	x	x
10-0621	/	x	x	/	/	/	/	/
10-0622	/	x	x	x	x	x	x	x
10-0623	/	x	x	x	x	x	x	x
10-0624	/	x	x	/	/	/	/	/
10-0625	/	x	x	/	/	/	/	/
10-0626	/	x	x	x	x	x	x	x
10-0627	/	x	x	x	x	x	x	x
10-0628	/	x	x	x	x	x	x	x
10-0629	/	x	x	x	x	x	x	x
10-0630	/	x	x	/	/	/	/	/
10-0631	/	x	x	x	x	x	x	x

Table 4.2 Result of PCR for *Colletotrichum* strains collected (Continued)

MFLUCC	Region							
	ITS	SSU	LSU	Actin	CAL	GAPDH	GS	TUB
10-0632	/	x	x	x	x	x	x	x
10-0633	/	x	x	x	x	x	x	x
10-0634	/	x	x	x	x	x	x	x
10-0635	/	x	x	/	/	/	/	/
10-0636	/	x	x	/	/	/	/	/
10-0637	/	x	x	x	x	x	x	x
10-0638	/	x	x	x	x	x	x	x
10-0639	/	x	x	x	x	x	x	x
10-0640	/	x	x	/	/	/	/	/
10-0641	/	x	x	x	x	x	x	x
10-0642	/	x	x	x	x	x	x	x
10-0643	/	x	x	x	x	x	x	x
10-0644	/	x	x	x	x	x	x	x
10-0645	/	x	x	x	x	x	x	x
10-0646	/	x	x	x	x	x	x	x
10-0647	/	x	x	x	x	x	x	x
10-0648	/	x	x	x	x	x	x	x
10-0649	/	x	x	x	x	x	x	x
10-0650	/	x	x	/	/	/	/	/
10-0651	/	x	x	/	/	/	/	/
10-0652	/	x	x	/	/	/	/	/
10-0653	/	x	x	/	/	/	/	/
10-0654	/	x	x	/	/	/	/	/
10-0655	/	x	x	/	/	/	/	/
10-0656	/	x	x	/	/	/	/	/
10-0657	/	x	x	/	/	/	/	/
10-0658	/	x	x	x	x	x	x	x

Table 4.2 Result of PCR for *Colletotrichum* strains collected (Continued)

MFLUCC	Region							
	ITS	SSU	LSU	Actin	CAL	GAPDH	GS	TUB
10-0659	/	x	x	x	x	x	x	x
10-0660	/	x	x	/	/	/	/	/
10-0661	/	x	x	x	x	x	x	x
10-0662	/	x	x	x	x	x	x	x
10-0663	/	x	x	x	x	x	x	x
10-0664	/	x	x	x	x	x	x	x
10-0665	/	x	x	x	x	x	x	x
10-0666	/	x	x	x	x	x	x	x
10-0667	/	x	x	x	x	x	x	x
10-0668	/	x	x	/	/	/	/	/
10-0669	/	x	x	/	/	/	/	/
10-0670	/	x	x	x	x	x	x	x
10-0671	/	x	x	x	x	x	x	x
10-0672	/	x	x	x	x	x	x	x
10-0673	/	x	x	/	/	/	/	/
10-0674	/	x	x	/	/	/	/	/
10-0675	/	x	x	x	x	x	x	x
10-0676	/	x	x	/	/	/	/	/
10-0677	/	x	x	/	/	/	/	/
10-0678	/	x	x	/	/	/	/	/
10-0679	/	x	x	x	x	x	x	x
10-0680	/	x	x	x	x	x	x	x
10-0681	/	x	x	/	/	/	/	/
10-0682	/	x	x	/	/	/	/	/
10-0684	/	x	x	x	x	x	x	x
11-0456	/	x	x	x	x	x	x	x
11-0460	/	x	x	x	x	x	x	x

Table 4.2 Result of PCR for *Colletotrichum* strains collected

MFLUCC	Region							
	ITS	**SSU**	**LSU**	**Actin**	**CAL**	**GAPDH**	**GS**	**TUB**
11-0534	/	x	x	x	x	x	x	x
11-0535	/	x	x	x	x	x	x	x
11-0536	/	x	x	x	x	x	x	x
11-0538	/	x	x	x	x	x	x	x
11-0539	/	x	x	x	x	x	x	x
11-0541	/	x	x	x	x	x	x	x
11-0542	/	x	x	x	x	x	x	x

* /: yes and x: No.

CHAPTER 5
CONCLUSION

Colletotrichum species have a worldwide distribution and are important pathogens subjected to import control and plant breeding programs. Previous identification and classification was based on host-association and morphological characteristics. Molecular sequence data, initially ITS and morphology was used to characterize species, however, they could not resolve species well. Recent multigene phylogenetic analysis as well as morphology and pathogenicity testing have resolved about 100 *Colletotrichum* species. There are four important species complexes. *C. gloeosporioides* species complex is the most important and multigene phylogenetic analysis have confirmed that *C. gloeosporioides* comprises 22 morphologically similar, phylogenetically distinct species. However, ITS, beta tubulin (*tub2*), DNA lyase and the intergenic region of *apn2* and *MAT1-2-1* genes (*ApMat*)) have also used shown new lineages within this species-complex and presently, there are 28 accepted species names within the complex. No consensus among mycologists as to which gene markers should be used to define and delimit a species within the species complex exists. In October 2011 there were more 50 confirmed "molecular" species of *Collettorichum* with 15 species known from Thailand. In year one we collected and isolated more than 100 strains of various disease plants and fruits from difference places in Thailand. We sequenced these isolates and study their morphology as well as pathogenicity studies. We identified several new species and also worked towards epitypification of other species; the first publications will appear in year two. We are involved in developing a practical phylogeny and morphology based approach for identification of *Colletotrichum* species, focusing on Thai species. Because this is a global problem we also collaborate with Brazilian, Chinese and Indian colleagues in order to bring greater depth to the research and international agreement to the findings. The latter was achieved with the formation of the International Subcommission on *Colletotrichum* taxonomy (http://www.fungaltaxonomy.org/ subcommissions) in August 2012 in Beijing; our group is a founder member.

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Field of Specialization Skill

Mycology, Plant Pathology

- Project Title: Diversity, molecular taxonomy of nematophagous fungi and characterisation of microbial enzymes associated with nematode infection (awarded ****)

Principal Investigator: Dr. KD Hyde, Principal investigator.

Investigator: Dr. R Jeewon, Co-Investigator

Project Status: Completed

Project Objective: The main objectives of this project are:

- To survey nematophagous fungi in Hong Kong.

- To investigate the genetic and evolutionary links between virulent and avirulent NF based on rDNA and protein sequences.

- To extract and purify extracellular enzymes from virulent microbes involved innematode infection.

- To test the ability of the expressed proteins to break down the cuticle of nematodes.

- Evaluate the expressed proteins in nematode biocontrol. Resulted in 3 SCI publications

- Project Title: Evolutionary relationships of loculoascomycetes (fungi) based on a phylogenetic Approach (awarded *****)

Principal Investigator: Dr. KD Hyde.

Co-Investigator: Dr. R Jeewon., Dr. A. Aptroot., Dr. J.M. Moncalvo.

Project Status: Completed

Project Objective:

- To verify whether Luttrell's hypothesis that loculoascomycetes are phylogentically distinct from unitunicate ascomycetes based on several genes.

- To assess the usefulness of different genes used in fungal molecular phylogenetics.

- To test the hypothesis that certain named anamorphic fungi are part of the life cycle of their associated telemorphs.

- To validate (or refute) the various current terms for morphological structures of fruiting bodies and interascal filaments.

- Resulted in 5 SCI publications

- Project Title: Molecular evolution of genes for phylogenetic analysis of the class Sordariomycetes (Mycota) – (awarded ****)

Principal Investigator: Dr. KD Hyde.

Co-Investigator: Dr. ECY Liew., Dr. J.M. Moncalvo ,Dr. D.S. Hibbett.,

Project Status: Completed.

Project Objective:

This resulted in 3 SCI publications

Selected publications:

1. **Hyde, K.D.** (2003). Mycology and its future in the Asia region. Fungal Diversity 13: 59-68. Impact Factor = 3.59 (Journal Citation Reports 2007).

2. Whitton, S.R., McKenzie, E.H.C. and **Hyde, K.D.** (2003). Microfungi on the Pandanaceae: Zygosporium, a review of the genus and two new species. Fungal Diversity 12: 207-222. Impact Factor = 3.59 (Journal Citation Reports 2007).

3. Tsui, C.K.M., Goh, T.K., and **Hyde, K.D.** (2003). Reflections on the genus Vanakripa, with a description of V. ellipsoidea sp. nov. Mycologia 95: 124-127. Impact Factor = 1.808 (Journal Citation Reports 2007).

4. Tang, A., **Hyde, K.D.** and Corlett, R.T.C. (2003). Diversity of fungi on wild fruits in Hong Kong. Fungal Diversity 14: 165-185. Impact Factor = 3.59 (Journal Citation Reports 2007).

5. Paulus, B., Gadek, P. and **Hyde, K.D.** (2003). Estimation of microfungal diversity in tropical rain forest leaf litter using particle filtration: the effects of leaf storage and surface treatment. Mycological Research 107: 748-756. Impact Factor = 1.86 (Journal Citation Reports 2007).

6. Pointing, S.B., Parungao, M.M. and **Hyde K.D.** (2003). Production of wood-decay enzymes, mass loss and lignin solubilization in wood by tropical Xylariaceae. Mycological Research 107: 231-235. Impact Factor = 1.86 (Journal Citation Reports 2007).

7. Guo, L.D., Huang, G.R., Wang, Y., He, W.H., Zheng, W.H. and **Hyde, K.D.** (2003). Molecular identification of white morphotype strains of endophytic fungi from Pinus tabulaeformis. Mycological Research 107: 680-688. Impact Factor = 1.86 (Journal Citation Reports 2007).

8. Yanna, Ho, W.H. and **Hyde, K.D.** (2003). Can ascospore ultrastructure differentiate between the genera Linocarpon and Neolinocarpon and species therein? Mycological Research 107: 1305-1313. Impact Factor = 1.86 (Journal Citation Reports 2007).

9. Jeewon, R., Liew, E.C.Y. and **Hyde, K.D.** (2003). Molecular systematics of the Amphisphaeriaceae based on cladistic analyses of partial LSU rDNA gene sequences. Mycological Research 107: 1392-1402. Impact Factor = 1.86 (Journal Citation Reports 2007).

10. Lu, B.S. and **Hyde, K.D.** (2003). Gigantispora gen. nov. (Xylariaceae, Ascomycotina) from decorticated twigs in the USA, a new combination for Anthostoma gigantispora. Nova Hedwigia 76: 201-206. Impact Factor = 0.77 (Journal Citation Reports 2007).

11. Pinnoi, A., McKenzie, E.H.C., Jones, E.B.G. and **Hyde, K.D.** (2003). Palm fungi from Thailand. Custingophora undulatistipes sp. nov. and Vanakripa minutellipsoidea sp. nov. Nova Hedwigia 77: 213-219. Impact Factor = 0.77 (Journal Citation Reports 2007).

12. Tsui, C.K.M., Hodgkiss, I.J. and **Hyde, K.D.** (2003). Three new species of Aquaticola (Ascomycetes) from tropical freshwater habitats. Nova Hedwigia 77: 161-168. Impact Factor = 0.77 (Journal Citation Reports 2007).

13. Lam, D.M., Lumyong, S., **Hyde, K.D.** and Jeewon, J. (2004). Emarcea castanopsicola gen. et sp. nov. from Thailand, a new xylariaceous taxon based on morphology and DNA sequences. Studies in Mycology 50: 253-260. Impact Factor = 5.9 (Journal Citation Reports 2007).

14. Bucher, V.V.C., **Hyde, K.D.**, Pointing, S.B. and Reddy, C.A. (2004). Production of wood decay enzymes, mass loss and lignin solubilization in wood by marine ascomycetes and their anamorphs. Fungal Diversity 15: 1-14. Impact Factor = 3.59 (Journal Citation Reports 2007).

15. Tsui, C.K.M. and **Hyde, K.D.** (2004). Biodiversity of fungi on submerged wood in a stream and estuaries in the Tai Ho Bay, Hong Kong. Fungal Diversity 15: 205-220. Impact Factor = 3.59 (Journal Citation Reports 2007).

16. Ho, W.H., Yanna, and **Hyde, K.D.** (2004). A new type of conidial septal pore in fungi. Fungal Diversity 15: 171-186. Impact Factor = 3.59 (Journal Citation Reports 2007).

17. Luo, J., Yin, J.F., Cai, L., Zhang, K. and **Hyde, K.D.** (2004). Freshwater fungi in Lake Dianchi, a heavily polluted lake in Yunnan, China. Fungal Diversity 16: 93-112. Impact Factor = 3.59 (Journal Citation Reports 2007).

18. Lee, S.W., Ho, W.H. and **Hyde, K.D.** (2004). Ultrastructure of the asci and ascospores of Torrentispora fibrosa. Fungal Diversity 16: 87-91. Impact Factor = 3.59 (Journal Citation Reports 2007).

19. Guo, L.D., Xu, L., Zheng, W.H. and **Hyde, K.D.** (2004). Genetic variation of Alternaria alternata, an endophytic fungus isolated from Pinus tabulaeformis as determined by random amplified microsatelites (RAMS). Fungal Diversity 16: 53-65. Impact Factor = 3.59 (Journal Citation Reports 2007).

20. Photita, W., Lumyong. S., Lumyong, P., McKenzie, E.H.C. and **Hyde, K.D.** (2004). Are some endophytes of Musa acuminata latent pathogens? Fungal Diversity 16: 131-140. Impact Factor = 3.59 (Journal Citation Reports 2007).

21. Fryar, S.C., Booth, W., Davies, J., Hodgkiss, I.J. and **Hyde, K.D.** (2004). Distribution of fungi on wood in the Tutong River, Brunei. Fungal Diversity 17: 17-38. Impact Factor = 3.59 (Journal Citation Reports 2007).

22. Kumar, D.S.S. and **Hyde. K.D.** (2004). Biodiversity and tissue-recurrence of endophytic fungi from Tripterygium wilfordii. Fungal Diversity 17: 69-90. Impact Factor = 3.59 (Journal Citation Reports 2007).

23. Pinruan, U., McKenzie, E.H.C., Jones, E.B.G. and **Hyde, K.D.** (2004). Two new species of Stachybotrys, and a key to the genus. Fungal Diversity 17: 145-157. Impact Factor = 3.59 (Journal Citation Reports 2007).

24. Jeewon, R., Liew, E.C.Y. and **Hyde, K.D.** (2004). Phylogenetic evaluation of species nomenclature of Pestalotiopsis in relation to host association. Fungal Diversity 17: 39-55. Impact Factor = 3.59 (Journal Citation Reports 2007).

25. **Hyde, K.D.** (2004). Fungal Conservation: Issues and Solutions. The Quarterly Review of Biology 79: 80-81. Impact Factor = 3.8 (Journal Citation Reports 2007).

26. Bucher, V.V.C., **Hyde, K.D.**, Pointing, S.B. and Reddy, C.A. (2004). Production of wood decay enzymes, mass loss and lignin solubilization in wood by diverse freshwater fungi. Microbial Ecology 48: 331-337. Impact Factor = 2.56 (Journal Citation Reports 2007).

27. Fryar, S.C., Davies, J., Booth, W., Hodgkiss, I.J. and **Hyde, K.D.** (2004). Succession of fungi on dead and live wood in brackish water. Mycologia 96: 219-225. Impact Factor = 1.808 (Journal Citation Reports 2007).

28. Ho, W.H. **Hyde, K.D.,** Hodgkiss, I.J. and Yanna (2004). Cataractispora receptaculorum, a new freshwater ascomycete from Hong Kong. Mycologia 96: 411-417. Impact Factor = 1.808 (Journal Citation Reports 2007).

29. Kodsueb, R., Lumyong, S., Lumyong, P., McKenzie, E.H.C., Ho, W.H. and **Hyde, K.D.** (2004). Acanthostigma and Tubeufia species, including T. claspisphaeria sp. nov. from submerged wood in Hong Kong sp. nov. Mycologia 96: 667-674. Impact Factor = 1.808 (Journal Citation Reports 2007).

30. Paulus, B., Gadek, P. and **Hyde, K.D.** (2004). Phylogenetic and morphological assessment of five new species of Thozetella from an Australian rainforest. Mycologia 96: 1074-1087. Impact Factor = 1.808 (Journal Citation Reports 2007).

31. Pinruan, U., Sakayaroj, J., Jones, E.B.G. and **Hyde, K.D.** (2004). Aquatic fungi from peat swamp palms: Phruensis brunneispora gen. et sp. nov. and its hyphomycete anamorph. Mycologia 96: 1163- 1170. Impact Factor = 1.808(Journal Citation Reports 2007).

32. Kumar, D.S.S., Cheung, H.Y., Lau, C.S., Chen, F. and **Hyde K.D.** (2004). In vitro studies of endophytic fungi from Tripterygium wilfordii with anti-proliferaive acivity on human peripheral blood mononuclear cells. Journal of Ethnopharmacology 94: 295-300. Impact Factor = 2.05 (Journal Citation Reports 2007).

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34. Cai, L., Zhang, K.Q., McKenzie, E.H.C. and **Hyde, K.D.** (2004). Linocarpon bambusicola sp. nov. and Dictyochaeta curvispora from bamboo

submerged in freshwater. Nova Hedwigia 78: 439-445. Impact Factor = 0.77 (Journal Citation Reports 2007).

35. Pinnoi, A., Pinruan, U., **Hyde, K.D.** and Lumyong, S. (2004). Submersisphaeria palmae sp. nov. and key to genus and notes on Helicoubisia. Sydowia 56: 72-78. Impact Factor = 0.556 (Journal Citation Reports 2007).

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37. Kumar, D.S.S., Lau, C.S., Wan, J.M.F., Yang, D. and **Hyde K.D.** (2005). Immunomodulatory compounds from Pestalotiopsis leucothës (HKUCC 10197), an endophytic fungus of Tripterygium wilfordii. Life Sciences 78: 147-156. Impact Factor = 2.257 (Journal Citation Reports 2007).

38. Pointing, S.B., Pelling, A.L., Smith, G.J.D., **Hyde, K.D**. and Reddy, C.A. (2005). Screening of basidiomycetes and xylariaceous fungi for lignin peroxidase and laccase gene-specific sequences. Mycological Research 109: 115-124.

39. Tang, A.M.C., Jeewon, R. and **Hyde, K.D.** (2005). Successional patterns of microfungi in fallen leaves of Castanopsis fissa (Fagaceae) in Hong Kong forest. Canadian Journal of Microbiology 51: 967-974. Impact Factor = 1.286 (Journal Citation Reports 2007).

40. Fryar, S.C., Booth, W., Davies, J., Hodgkiss, I.J. and **Hyde, K.D.** (2005). Evidence of in situ competition between fungi in freshwater. Fungal Diversity 18: 59-71.

41. Cai, L., Jeewon, R. and **Hyde, K.D.** (2005). Phylogenetic evaluation and taxonomic revision of Schizothecium based on ribosomal DNA and protein coding genes. Fungal Diversity 19: 1-21.

42. Promputtha, I., Jeewon, R., Lumyong, S., McKenzie, E.H.C. and **Hyde, K.D.** (2005). Ribosomal DNA fingerprinting in the identification of non sporulating endophytes from Magnolia liliifera (Magnoliaceae). Fungal Diversity 20: 167-186.

43. Wang, Y., Guo, L.D. and **Hyde, K.D.** (2005). Taxonomic placement of sterile morphotypes of endophytic fungi from Pinus tabulaeformis (Pinaceae) in northeast China based on rDNA sequences. Fungal Diversity 20: 235-260.

44. Ho, W.H., Yanna and **Hyde, K.D. (**2005). Endosporoideus gen. nov., a mitosporic fungus on Phoenix hanceana. Mycologia 97: 238-245 .

45. Bussaban, B., Lumyong, S., Lumyong, P., Seelanan, T., Park, D.C., McKenzie, E.H.C. and **Hyde, K.D.** (2005). Molecular and morphological characterization of Pyricularia and allied genera. Mycologia 97: 1002-1011.

46. Li, Y., **Hyde, K.D.**, Jeewon, R., Cai, L., Vijaykrishna D. and Zhang, K.Q. (2005). Phylogenetics and evolution of nematode trapping fungi (Orbiliales) estimated from nuclear and protein coding genes. Mycologia 97: 1034-1046.

47. Crous, P.W., Groenewald, J.Z., Risède, J.M., Simoneau, P. and **Hyde, K.D.** (2006). Calonectria speces and their Cylindrocladium anamorphs: species with clavate vesicles. Studies in Mycology 55: 213-226.

48. Paulus, B., Gadek, P. and **Hyde, K.D.** (2006). Successional patterns of microfungi in fallen leaves of Ficus pleurocarpa (Moraceae) in an Australian tropical rainforest. Biotropica 38: 42-51. Impact Factor = 1.7 (Journal Citation Reports 2007).

49. Tsui, C.K.M., Berbee, M.L., Jeewon, R. and **Hyde, K.D.** (2006). Molecular phylogeny of Dictyosporium and allied genera inferred from ribosomal DNA. Fungal Diversity 21: 157-166.

50. Vijaykrishna, D. and **Hyde, K.D.** (2006). Inter and intra stream variation of lignicolous freshwater fungi in tropical Australia. Fungal Diversity 21: 203-224.

51. Pinnoi, A., Lumyong, S., **Hyde, K.D.** and Jones, E.B.G. (2006). Biodiversity of fungi on the palm Eleiodoxa conferta in Sirindhorn peat swamp forest, Narathiwat, Thailand. Fungal Diversity 22: 205-218.

52. Kodsueb, R., Jeewon, R., Dhanasekaran, V., McKenzie, E.H.C., Lumyong, P., Lumyong, S. and **Hyde, K.D.**, (2006). Systematic revision of Tubeufiaceae based on morphological and molecular data. Fungal Diversity 21: 105-130.

53. Tran, T.M., Stephenson, S.L., **Hyde, K.D**. and Mongkolporn, O. (2006). Distribution and occurrence of myxomycetes in tropical forests in northern Thailand. Fungal Diversity 22: 227-242.

54. Duong, L.M., Jeewon, R., Lumyong, S. and **Hyde, K.D** (2006). DGGE coupled with ribosomal DNA phylogenies reveal uncharacterized fungal phylotypes on living leaves of Magnolia liliifera. Fungal Diversity 23: 121-138.

55. Vijaykrishna, D., Jeewon, R. and **Hyde, K.D.** (2006). Molecular taxonomy, origins and evolution of freshwater ascomycetes. Fungal Diversity 23: 367-406.

56. El-Morsy, E.M., El-Dohlob, S.M. and **Hyde, K.D.** (2006). Diversity of Alternaria alternata a common destructive pathogen of Eichhornia crassipes in Egypt and its potential use in biological control. Fungal Diversity 23: 139-158.

57. Hidayat, I., Jeewon, R., To-anun, C. and **Hyde, K.D.** (2006). The genus Oxydothis: New palmicolous taxa and phylogenetic relationships within Xylariales. Fungal Diversity 23: 159-179.

58. Kodsueb, R., Jeewon, R., Vijaykrishna, D., Lumyong, S., McKenzie, E.H.C. and **Hyde, K.D.** (2006). The family Pleosporaceae: intergeneric relationships and phylogenetic perspectives based on sequence analyses of partial 28S rDNA. Mycologia 98: 571-583.

59. Li, Y., Jeewon, R., **Hyde, K.D.,** Mo, M., and Zhang, K. (2006). Two new species of nematode trapping fungi relationships inferred from morphology, rDNA and protein gene sequence analyses. Mycological Research 110: 790-800.

60. Paulus, B., Kanowski, J., Gadek, P. and **Hyde, K.D.** (2006). Diversity and distribution of saprobic microfungi in leaf litter of an Australian tropical rainforest. Mycological Research 110: 1441-1454.

61. Cai, L., Jeewon, R. and **Hyde, K.D.** (2006). Phylogenetic investigations of Sordariaceae based on multiple gene sequences and morphology. Mycological Research 110: 137-150.

62. Shenoy, B.D., Jeewon, R., Wu, W.P.,Bhat, D.J. and **Hyde, K.D.** (2006). Ribosomal and RPB2 DNA sequence analyses suggest that Sporidesmium and morphologically similar genera are polyphyletic. Mycological Research 110: 916-928.

63. Cai, L., Jeewon, R. and **Hyde, K.D.** (2006). Molecular systematics of Zopfiella and allied genera: evidence from multiple gene sequence analysis. Mycological Research 110: 359-368.

64. Paulus, B., Gadek, P. and **Hyde, K.D.** (2005). Discostroma ficicola sp. nov. (Amphisphaeriaceae) and a key to species of Discostroma. Sydowia 58: 76-90.

65. **Hyde, K.D.** and Sarma, V.V. (2006). Biodiversity and ecological observations on filamentous fungi of mangrove palm Nypa fruticans Wurumb

(Liliopsida-Arecales) along the Tutong River, Brunei. Indian Journal of Marine Sciences 35: 297-307. 0.3

66. Aung, O.M., Kang, J.C., Liang, Z.Q., Soytong, K. and **Hyde, K.D.** (2006). Cordyceps mrciensis sp. nov. from a spider in Thailand. Mycotaxon 97: 253-240.

67. Aung, O.M., Kang, J.C., Liang, Z.Q., Soytong, K. and **Hyde, K.D.** (2006). Hymenostilbe sp. nov., a new species from Thailand. Mycotaxon 97: 240-245.

68. Zhao, R.L., Desjardin, D., Soytong, K. and **Hyde, K.D.** (2006). Proposed synonyms in Cyathus. Mycotaxon 97: 327-335.

69. Zhu, H., Cai, L., Zhang, K.Q. and **Hyde, K.D.** (2006). A new species of Acrogenospora from submerged bamboo in Yunnan, China Mycotaxon 95: 348-358.

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71. Kodsueb, R., Lumyong, S., **Hyde, K.D.**, Lumyong, P. and McKenzie, E.H.C. (2006). Acrodictys micheliae and Dictyosporium manglietiae, two new anamorphic fungi from woody litter of Magnoliaceae in northern Thailand. Cryptogamie Mycologie 27: 111-119.

72. Cabanela MV, Jeewon R, **Hyde KD**. (2007) Morphotaxonomy and phylogeny of Paoayensis lignicola gen. et sp nov (ascomycetes) from submerged wood in Paoay Lake, Ilocos Norte, the Philippines. Cryptogamie Mycologie 28, 301-310.

73. Cai L, **Hyde KD** (2007) Ascorhombispora aquatica gen. et sp nov from a freshwater habitat in China, and its phylogenetic placement based on molecular data. Cryptogamie Mycologie 28, 291-300.

74. Hu DM, Zhu H, Cai L, **Hyde KD**, Zhang KQ (2007) Sirothecium triseriale, a new chirosporous anamorphic species from China. Cryptogamie Mycologie 28, 311-314.

75. **Hyde, K.D.** and Soytong, K. (2007) Understanding microfungal diversity - a critique. Cryptogamie Mycologie 28, 281-289.

76. Kodsueb R, McKenzie EHC, Ho WH, **Hyde KD**, Lumyong P, Lumyong S (2007) New anamorphic fungi from decaying woody litter of Michelia baillonii (Magnoliaceae) in northern Thailand. Cryptogamie Mycologie 28, 237-245.

77. Huang, W.Y., Cai, Y.Z., **Hyde, K.D.,** Corke, H. and Sun, M. (2007). Endophytic fungi from Nerium oleander L (Apocynaceae): main constituents and antioxidant activity. World Journal of Microbiology & Biotechnology 23: 1253-1263.0.745

78. Cai, L. and **Hyde, K.D.** (2007). New species of Clohesia and Paraniesslia collected from China freshwater habitats. Mycoscience (In press).

79. Cai, L. and **Hyde, K.D.** (2007). Anamorphic fungi from China freshwater habitats: Dictysporium tetrasporum sp. nov., Exserticlava yunnanensis sp. nov., and new records Pseudofuscophialis lignicola and Pseudobotrytis terrestris. Mycoscience (In press).

80. Jeewon, R. and **Hyde, K.D.** (2007). Detection and diversity of fungi from environmental samples: traditional versus molecular approaches. In: Soil Biology, Volume 11 (eds. A. Varma and R. Oelmuller). Springer-Verlag, Heidelberg: 1-15.

81. Than, P.P., Shivas, R.G., Jeewon, R., Pongsupasamit, S., Marney, T.S., Taylor, P.W.J. and **Hyde, K.D.** (2008). Epitypification and phylogeny of Colletotrichum acutatum J.H. Simmonds. Fungal Diversity 28: 97-108.

82. Pinruan, U., Sakayaroj, J., **Hyde, K.D.,** Jones, E.B.G. (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.

83. Than PP, Jeewon R, **Hyde K.D.,** Pongsupasamit S, Mongkolporn O, Taylor PWJ (2008) Characterization and pathogenicity of Colletotrichum species associated with anthracnose on chilli (Capsicum spp.) in Thailand. Plant Pathology 57: 562-572. 2.01

84. Cai, L., Guo, X.Y. and **Hyde, K.D.** (2008). Morphological and molecular characterization of a new anamorphic genus Cheirosporium, from freshwater in China. Persoonia **: xx-xx.

85. Aung, O.M., Soytong, K. and **Hyde, K.D.** (2008). Diversity of entomopathogenic fungi in rainforests of Chiang Mai Province, Thailand. Fungal Diversity 30: 15-22.

86. Duong, L.M., McKenzie, E.H.C., Lumyong, S. and **Hyde, K.D.** (2008). Fungal succession on senescent leaves of Castanopsis diversifolia in Doi Suthep-Pui National Park, Thailand. Fungal Diversity 30: 23-36.

87. Kodsueb, R., McKenzie, E.H.C., Lumyong, S. and **Hyde, K.D.** (2008). Diversity of saprobic fungi on Magnoliaceae. Fungal Diversity 30: 37-53.

88. Kodsueb, R., McKenzie, E.H.C., Lumyong, S. and **Hyde, K.D.** (2008). Fungal succession on woody litter of Magnolia liliifera (Magnoliaceae). Fungal Diversity 30: 55-72.

Co-investigator

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Current position: Lecturer

Academic qualifications:

1996-1999 PhD in Biochemistry, Research School of Biosciences, University of Kent at Canterbury, UK; Project title "Evolution of CUG codon reassignment in *Candida* species" with Prof. Mick Tuite

1995-1996 MSc in Biotechnology, University of Kent, UK; Project title "Cloning of Ser-tRNA^{CAG} genes from various *Candida* species and expression in *Saccharomyces cerevisiae*" with Prof. Mick Tuite

1990-1994 BSc (First Class Hons.) in Biology, Department of Biology, Faculty of Science, Chiang Mai Univeristy, Thailand; Project title "Lactic acid

production by starch-utilising lactic acid bacteria" with Assoc. Prof. Dr. Saisamorn Lumyong.

Awards and Scholarships:

1995-1999 Postgraduate studentship sponsored by the DPST project to pursue MSc/PhD study aboard

1998 Travel grant from the Genetics Society of America (GSA), Bethesda, MD, USA (for Yeast Genetics and Molecular Biology Meeting); from the organising committee, University of Crete, Heraklion, Greece (for Evolutionary Biology Meeting); from Department of Biosciences, University of Kent, UK (for Translation UK)

1994 Prof. Dr. Dhab Nelanithi Foundation Award

1990-1994 Studentship under the DPST project, Chiang Mai University

Work experiences:

2000-present Lecturer, Mae Fah Luang University

Academic committee for Undergraduate and Postgraduate Programme in Biotechnology, Mae Fah Luang University

2001-2002 Postdoctoral Fellow, Department of Applied Chemistry, Faculty of Engineering, Oita Univeristy, Japan.

1995-1999 Teaching Assistance in the following undergraduate practical:

Nucleic Acids and Proteins, Enzyme Kinetics, Gene Cloning, Yeast Mutagenesis, Immunology, Microbiology, UKC, UK

Membership of Learning Societies:

- Editorial Board, Research Journal of Microbiology (2005 – present)
- Member of Thai Society of Biotechnology (2004 – present)
- Member of the Science Advisory Board (2004 – present)
- Member of CRN Microbiology (2004 – present)
- Member of Society of General Microbiology (1995 – 1999)

- Member of Researcher Panel of the IRPUS Project, Thailand (2005 – present)
- Member of the Thai-UK Alumni and Professional Network (2004 – present)

Selected publications:

1. Dajanta K, **Chukeatirote E** and Apichartsrangkoon A. 2008. Effect of lactoperoxidase system on keeping quality of raw cow's milk in Thailand. Int J Dairy Sci 3: 112-116.

2. Onto S, Laosat N, Suksawat W, Popluechai S, Eungwanichayapant PD and **Chukeatirote E**. 2008. Phylogenetic analysis of *Cucumis sativus* using RAPD molecular markers. *J Plant Sci* 3: 105-110.

3. Sakai K, Fujii N and **Chukeatirote E** (2007) Racemisation of L-lactic acid in pH-swing open fermentation of kitchen refuse by selective proliferation of *Lactobacillus plantarum*. *J Biosci Bioeng* 102: 227-232.

4. **Chukeatirote E**, Hanpattanakit P, Kaprom A and Tovaranonte J (2007) Antimicrobial Activity of *Senna spectabilis* and *S. tora*. *J Plant Sci* 2: 123-126.

5. Hanmoungjai W, **Chukeatirote E**, Pathom-aree W, Yamada Y and Lumyong S (2007) Identification of Acidotolerant Acetic Acid Bacteria Isolated from Thailand Sources. *Res J Microbiol* 2: 194-197.

6. **Chukeatirote E** and Thakang P (2006) Chemical composition of *thua nao*—a fermented soybean food of Northern Thailand. *Chiang Mai J Sci*. 33: 243-245.

7. **Chukeatirote E**, Chainun C, Siengsubchart A, Moukamnerd C, Chantawannakul P, Lumyong S, Boontim N and Thakang P. (2006) Microbiological and biochemical changes in *thua nao* fermentation. *Res J Microbiol* 1: 38-44.

8. Wisitrasamewong K and **Chukeatirote E** (2005) *in silico* PCR-RFLP. Thailand Innovation Awards 2005, pp. 53-54.

9. Sakai K, Mori M, Fujii A, Iwami Y, **Chukeatirote E** and Shirai Y (2004) Fluorescent *in situ* hybridization analysis of open lactic acid fermentation of kitchen refuse using rRNA-targeted oligonucleotide probes. *J Biosci Bioeng* 98: 48-56.

10. **Chukeatirote E**, Bankluay K, Kaprom A, Sampanvejsobha S and Winyayong P (2004) Microbiological quality of some tea products in Chiang Rai Province, Thailand. *Chiang Mai J Sci* 31: 185-189.

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Education Background

- B.Sc. in Agriculture (Plant Pathology) Hons. Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand. March, 2001

- Ph.D. in Biodiversity and Ethnobiology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. October, 2007

Field of Specialization Skill

Mycology, Plant Pathology

Scholarships

- Centre for Research in Fungal Diversity (2002–2005)

- The Commission on Higher Education (2005–2007)

Awards

- Aventis CropScience Award 2000- The 3rd Place Winning Team of "Innovative Rice Production Technology" Essay Contest.

- The Excellence Dissertation Award Academic Year 2007 on Thesis Entitled "Biodiversity of Saprobic Fungi on Woody Litter" from Chiang Mai University Graduate School.

Selected publications:

1. **Kodsueb, R.**, McKenzie, E.H.C., Lumyong, S. and Hyde, K.D. (2008). Diversity of saprobic fungi on *Magnoliaceae*. *Fungal Diversity* 30: 37-53.

2. **Kodsueb, R.**, McKenzie, E.H.C., Lumyong, S. and Hyde, K.D. (2008). Fungal succession on woody litter of *Magnolia liliifera* (*Magnoliaceae*). Fungal Diversity 30: 55-72.

3. **Kodsueb R.,** Jeewon R., Hyde K.D., McKenzie E.H.C., Ho W.H. and Lumyong S. (2007). Molecular phylogeny of new synnematous hyphomycete taxon from Thailand and its teleomorphic affinities to Massarinaceae (Pleosporales). *Botanical Journal of the Linnean Society* 155: 283–296.

4. **Kodsueb R.**, McKenzie, E.H.C., Ho, W.H., Hyde K.D., Lumyong P. and Lumyong S. (2007). New anamorphic fungi from decaying woody litter of. *Michelia baillonii* (Magnoliaceae) in northern Thailand. *Cryptogamie Mycologie* 28: 237–245.

5. **Kodsueb, R.**, Jeewon, R., Vijaykrishna, D., McKenzie, E.H.C., Lumyong, P., Lumyong, S. and Hyde, K.D. (2006). Systematic revision of *Tubeufiaceae* based on morphological and molecular data. *Fungal Diversity* 21: 105–130.

6. **Kodsueb R.,** Lumyong S., Hyde K.D., Lumyong P. and McKenzie E.H.C. (2006). *Acrodictys micheliae* and *Dictyosporium manglietiae*, two new anamorphic fungi from woody litter of Magnoliaceae in northern Thailand. *Cryptogamie Mycologie* 27: 111–119.

7. **Kodsueb R.,** Jeewon R., Lumyong S., Vijaykrishna D., Aptroot A., McKenzie E.H.C. and Hyde K.D. (2006). The family Pleosporaceae: intergeneric relationships and phylogenetic perspectives based on sequence analyses of partial 28S rDNA. *Mycologia* 98: 571–583.

8. **Kodsueb, R.**, Lumyong S. and Hyde K.D. (2004). Terrestrial Lignicolous Microfungi. In *Thai Fungal Diversity* (eds. E.B.G. Jones, M. Tanticharoen and K.D. Hyde). BIOTEC, Thailand: 155–161.

9. **Kodsueb R.,** Lumyong S., Lumyong S., McKenzie E.H.C., Ho W.H. and Hyde K.D. (2004). *Acanthostigma* and *Tubeufia* species, including *T. claspisphaeria* sp. nov., from submerged wood in Hong Kong. *Mycologia* 96: 667–674

10. Hyde K.D., Jeewon R, Bahl J., Bhilabut B., Bussaban B., Cai L., Damodar S.B., **Kodsueb R.**, Lam C.W.H., Lam D.M., Photita W., Promputtha I.,

Tang A.M.C., Thongkantha S., Vijaykrishna D., Yeung S.Y. (2004). Fungal studies at the University of Hong Kong. Annual Meeting of Mycological Society of America, Asheville, North Carolina, USA, 2004.

3. **Name: Dr Matchima Naradisorn**

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Education:

- 2003 – 2007 PhD (Postharvest Pathology), The University of Adelaide (Australia)
- 1996 – 1997 M.Agr. (Plant Pathology), The University of Sydney (Australia)
- 1991 – 1994 B.Sc. (Agriculture), Chiangmai University (Thailand)

Publications and proceedings:

1. **Naradisorn,** M., Able, A. J., Scott, E., Klieber, A. and Sedgley, M. 2005. Effect of preharvest calcium application on grey mould development and postharvest quality in strawberries. Acta Hort. 2005; 708:147-150.
2. **Naradisorn**, M., Sedgley, M., Scott, E. and Able, A.J. Effect of calcium lactate on grey mould development in strawberry. Poster presentation at Australasian Postharvest Horticulture Conference, Rotorua, New Zealand (27-30 September 2005) and received attendee spot prize.
3. Raksaboon, N, Ruenkum, A. and **Naradisorn**, M. Control of anthracnose in mango using n-propyl dihydrojasmonate. Proceedings of Industrial and Research Projects for Undergraduate Student Symposium (IRPUS-2009). March 26-29, 2009. Siam Paragon, Bangkok.

4. **Naradisorn**, M. and Ruenkum, A. Preliminary study on antimicrobial activity of crude extracts of pomelo albedo against Colletotrichum gloeosporioides. Proceedings of International Symposium "Go…Organic 2009". August 19-21, 2009. Bangkok, Thailand.

Current projects:

- Antimicrobial activity of pomelo albedo tissues against postharvest fungal pathogens

Postharvest handling of cut anthuriums