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ที่แยกจากต้นมะม่วง *Mangifera indica* L.

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ANTIFUNGAL PHYTOPATHOGENS AND INDOLE ACETIC ACID PRODUCTION BY ENDOPHYTIC FUNGI ISOLATED FROM MANGO *Mangifera indica* L. PLANT

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ราเอนโดไฟต์จากส่วนต่างๆของต้นมะม่วง (*Mangifera indica* L.) ได้แก่ราก กิ่ง ใบ และผล ได้ถูกคัดแยกได้ ทั้งหมด 239 ไอโซเลต จากต้นมะม่วง 3 สายพันธุ์ (อกร่อง เขียวเสวย และตลับนาก) ทั้งจากฤดูการมะม่วงดิบและฤดูมะม่วงสุก ยีสต์เอนโดไฟต์ 13 ไอโซเลตถูกแยกได้จากเฉพาะมะม่วงสุกเท่านั้น ส่วนราเส้นใยเอนโดไฟต์ 79 ไอโซเลตถูกแยกได้จากฤดูการมะม่วงดิบและ 147 ไอโซเลตถูกแยกได้จากฤดูการมะม่วงสุก ในการศึกษาครั้งนี้ได้ทดสอบการเป็นปรปักษ์ในการต้านการเจริญของราที่ก่อโรคในพืช 2 ชนิด คือ *Colletotrichum gleosporioides* และ *Lasiodiplodia theobromae* โดยวิธี dual culture และวิธี double dishes นอกจากนี้ยังได้ทดสอบการสร้างกรดอินโดลแอซีติกซึ่งเป็นสารกระตุ้นการเจริญของพืชอีกด้วย เฉพาะไอโซเลตที่มีแอคทิวิตีได้ถูกนำไปจำแนกชนิดต่อไป โดยใช้วิธี PCR-RFLP เพื่อการแบ่งราเอนโดไฟต์ออกเป็นกลุ่มก่อนที่จะเลือกตัวแทนของแต่ละกลุ่มไปทำการวิเคราะห์ลำดับเบสบริเวณ ITS ต่อไป พบว่ายีสต์เอนโดไฟต์ที่แยกได้ประกอบด้วย *Aureobasidium* sp., *Rhodotorula* sp., *Hanseniaspora* sp., *Candida* sp. และ *Cryptococcus laurentii* ส่วนราเส้นใยเอนโดไฟต์ที่แยกได้ประกอบด้วย *Aspergillus* sp., *Colletotrichum* sp., *Lasiodiplodia* sp., *Penicillium* sp., *Phyllosticta* sp., และ *Rhytidhysteron* sp. และยังพบว่า ยีสต์เอนโดไฟต์ *Candida* sp. CY.OS.09 เป็นปรปักษ์กับ *C. gleosporioides* สูงที่สุด แต่ยีสต์เอนโดไฟต์ทั้ง 13 ไอโซเลตไม่เป็นปรปักษ์กับ *L. theobromae* ส่วนราเส้นใยเอนโดไฟต์ *Colletotrichum* sp. CY.OS.162 และ *Lasiodiplodia* sp. CY.OS.132 เป็นปรปักษ์ต่อ *C. gleosporioides* และ *L. theobromae* ตามลำดับ *Colletotrichum* sp. CY.OS 161 และ*Colletotrichum* sp. CY.OS 33 สามารถสร้างสารระเหยยับยั้งการเจริญของราก่อโรคพืชได้ดีที่สุด นอกจากนี้ยังพบการสร้างกรดอินโดลแอซีติกในระดับสูงสุดจากยีสต์เอนโดไฟต์ *Aureobasidium* sp. CY.OS 13 และ ราเส้นใยเอนโดไฟต์ *Colletotrichum* sp. CY.OS.22 ผลจากการศึกษานี้อาจนำไปสู่การใช้ราเอนโดไฟท์ที่คัดแยกได้นี้ในการต้านราก่อโรคพืช อีกทั้งอาจใช้เพื่อกระตุ้นการเจริญของพืชได้ต่อไป

ภาควิชา จุลชีววิทยา ลายมือชื่อนิสิต .....

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ORLAVANH SONESOUPHAP: ANTIFUNGAL PHYTOPATHOGENS AND INDOLE ACETIC ACID PRODUCTION BY ENDOPHYTIC FUNGI ISOLATED FROM MANGO *Mangifera indica* L. PLANT. ADVISOR: ASST. PROF. CHULEE YOMPAKDEE, Ph.D., CO-ADVISOR: ASSOC. PROF. PRAKITSIN SIHANONTH, Ph.D., 140 pp.

Total endophytic fungi of 239 isolates were obtained from 3 varieties of *Mangifera indica* L. including Okrong, Talapnak and Khiaosawoey from different parts (root, twig, leaf and fruit) of raw and ripen fruit seasons. Thirteen endophytic yeast isolates were from ripen fruit season, and endophyte filamentous fungi of 79 isolates were from raw fruit season while 147 were from ripen fruit season. A study was conducted for screening of the antifungal activity against 2 phytopathogens: *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* by dual culture and double dishes methods. In addition, indole acetic acid (IAA) production of the isolates was also analyzed. Only the isolates containing activity were further identified. PCR-RFLP was used to group the isolates prior to ITS region DNA sequencing of the representatives from each group. Endophytic yeasts comprised *Aureobasidium* sp., *Rhodotorula* sp., *Hanseniaspora* sp., *Candida* sp. and *Cryptococcus laurentii*. Endophytic filamentous fungi were consisted of *Aspergillus* sp., *Colletotrichum* sp., *Lasiodiplodia* sp., *Penicillium* sp., *Phyllosticta* sp., and *Rhytidhysteron* sp. Endophytic yeast *Candida* sp. CY.OS 09 showed the highest growth inhibition activity against *C. gleosporioides* while no anti-*L. theobromae* activity could be detected from all 13 endophytic yeast isolates. For endophytic filamentous fungus *Colletotrichum* sp. CY.OS 162, and *Lasidiplodia* sp. CY.OS 132, showed the highest activities against *C. gleosporioides and L. theobromae*, respectively. The *Colletotrichum* sp. CY.OS 161 and *Colletotrichum* sp. CY.OS 33 produced the strongest antifungal volatile compound. IAA production was highest observed in endophytic yeast *Aureobasidium* sp. CY.OS 13, and endophytic filamentous fungus *Colletotrichum* sp. CY.OS 22. This study suggested a future use of isolated endophytic fungi as biocontrol agents, along with plant growth promotion capability.

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ABBREVIATIONS

µl	Microlitre
µg/l	Microgram/ litre
bp	Base pair
CTAB	Cetyl-trimethylammoim bromide
ºC	Degree in Celsius
d	Day
DCW	Dry Cell Weight
DDI	Distilled De-Ionized
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ft	Foot
g	Grams
GAE	Gallic acid equivalents
h	Hour
IAA	Indole acetic acid
kg	Kilogram
mg	Milligram
mg/g	Milligram/gram
ml	Millilitre
mm	Millimetre
min	Minutes
µM	Micromolar
mM	Millimolar
PDA	Potato Dextrose Agar
pH	Potentials of Hydrogen Ion
TE	Tris-EDTA Buffer
V	Volt
YM	Yeast extract Malt extract medium
YPD	Yeast Peptone Dextrose medium

CHAPTER I
INTRODUCTION

1.1. State of problem

Mango (*Mangifera indica* L.) in Family Anacardiaceae is an economically important fruit crop in the tropical and subtropical areas. It is delicious and widely popular summer fruit in many countries all around the world, which considered as "The King of Fruits" in India. There is also a substantial import to areas where the production is limited by the climate. Mangoes are nutritional rich as source of energy, carbohydrates, fat, protein, vitamins and minerals. It was reported that mango can contribute to fighting cancer, skin damage and prevent miscarriage (Amir 2015). Moreover, the majority of mango production is freshly consumed and about 1-2% of mango produced is processed to make products such as juices, nectars, concentrates, jams, jelly powders, fruit bars, flakes and dried fruits (Berardini et al. 2005). Mango varieties, which are either too fibrous or too soft for fresh consumption could be used for making mango juice (Heuzé et al. 2015). In addition, mango tree has been reconized as an important herb in the Ayurvedic and indigenous medicine systems for over 4000 years (Shah et al. 2010). Notably, mango leaf extracts have been reported for their various medicinal effects such as antioxidant, antimicrobial, antihelminthic, antidiabetic and antiallergic activities (Hannan et al. 2013). Studies suggested that mango possesses antidiabetic, antioxidant, antiviral, cardiotonic, hypotensive, antiinflammatory properties (Kusari et al. 2012). Various effects including antibacterial, antifungal, antihelminthic, antiparasitic, antitumor, antiHIV, antibone resorption, antispasmodic, antipyretic, antidiarrhoeal, antiallergic, immunomodulation, hypolipidemic, antimicrobial, hepatoprotective and gastroprotective effects have been suggested (Hannan et al. 2013). In many countries are great global mangoes producers such as India, China, and Thailand which produced 16,337,400 tons, 4,351,593 tons, and 2,550,600 tons respectively in the year 2014, and at present, there is an increasing demand for mangoes in the world market (CBI 2009).

However, there are number of diseases that cause infections and destroy mango fruits and mango trees. Different parts of *M. indica* are known to suffer from a number of diseases caused by fungi, bacteria and insects (Chaudhary 2012) thus, decreasing the yield and quality of mangoes. Diseases could affect all stages of its development, i.e. from nursery to the consumption of fruits. Especially at post-harvest stage, the diseases manifest themselves as several kinds of rots, die back, mildew, necrosis, scab, blotch, stem bleeding, wilt, spot, canker, sooty mold and malformation. Mango fruits both ripe and unripe are also vulnerable to a variety of diseases (Chaudhary 2012). Diseases of fruit occurring during transit and storage results in a great spoilage. Among several mango spoilage causing organisms, fungi are the most destructive, causing extensive damages during storage and transport of mango fruits (Chaudhary 2012). There are diseases which widely affect agriculture as to the economy of many countries such as: anthracnose, powdery mildew, algal leaf spot, and sooty mold.

Colletotrichum gloeosporioides is a fungal plant pathogen causing the anthracnose disease. It can infect and destroy the mango at all stages of growth such as seedlings, shoots, flowers, and fruits (Dinh et al. 2003). *Lasiodiplodia theobromae* cause canker, dieback, and fruit and root rot in over 500 different hosts, including perennial fruits and nut trees, vegetable crops, and ornamental plants. It is a common post-harvest fungal disease of commercial crops such as citrus, mango, and banana (Punithalingam 1980). Biocontrol is one of the best way for control of plant pathogens, and one of biocontrol strategy is the use of microorganisms in the agricultural field to minimize the use of chemicals and pesticides.

Endophytes are microorganisms such as bacteria, fungi and actinomycetes that live in plant tissues and live there for all or part of their life stages without causing harm to the hosts (Petrini 1991). Endophytic microorganisms are virtually found in every plant on earth and could be isolated from roots, twigs, leaves, stem and fruits. These endophytic organisms reside in the living tissues of the host plant and participate in a variety of relationships ranging from symbiotic to pathogenic conditions (Clarke et al. 2013). The potential role of the endophyte and its biologically active metabolites in its association with its host has been investigated. The fungal endophytes possess the exoenzymes necessary to invade and colonize their hosts. Furthermore, they grow well in the apoplastic washing fluid of the host, thus tend to live with the plant over the entire

lifetime (Pirttil and Frank 2011). Endophytic fungi are one of promising sources for screening of new products for pharmaceutical and agrochemical industries. Endophytic fungi grow within their plant hosts without causing apparent disease symptoms and their growth in this habitat involves continuous metabolic interaction between fungus and host (Schulz et al. 2002). Endophytic fungi can produce secondary metabolites including terpenoids, alkaloids, phenylpropanoids, aliphatic compounds, polyketides, and peptides, and volatile organic compounds such as alcohols, aldehydes, terpenes, aromatics and thiols which dominate the antimicrobial activity towards plant pests (Morath et al. 2012, Mousa and Raizada 2013). Special ones can produce bioactive compound possessing anticancer and antitumor activities (Schulz et al. 2002). Besides, some endophytes can produce plant hormones such as indole acetic acid (IAA), gibberellins and cytokinins which effect as plant growth promoters (Nutaratat et al. 2014).

This study aims to isolate endophytic fungi from various parts of mango trees. Their antifungal and indole acetic acid production activities will be evaluated. Then identification of endophytic fungi with outstanding activities will be carried out.

1.2. Objective

To isolate endophytic fungi from *Mangifera indica* L. and evaluate their antifungal activities against two phytopathogens, *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* as well as their activities on indole acetic acid production. Some endophytes with high activity will be further identification.

1.3. Possible benefits of the study

Endophytic fungi isolates which have antifungal phytopathogens and /or indole acetic acid production activities are isolated and identified.

May obtain endophytic fungi with good anti-phytopathogens for use in biocontrol and/or some endophytes with high IAA production for use as plant growth promoter.

1.4. Research Methodologies

1.4.1. Sample collection

1.4.2. Isolation of endophytic fungi

1.4.3. Antifungal phytopathogens activity assay

1.4.4. Evaluation on indole acetic acid production activity

1.4.5. Taxonomic identification of endophytic fungi

1.4.6. Data analysis

CHAPTER II
LITERATURE REVIEW

2.1. Endophytic microorganisms

2.1.1. What are endophytes?

The word “endophyte” is derived from Greek, ‘endo’ or ‘endon’ which means within, and ‘phyte’ or ‘phyton’ which means plant (Jalgaonwala et al. 2011), thus by meaning endophytes are microorganisms that live in plant tissues and can live there for all or part of their life stages without causing harm to the host (Petrini 1991). Nearly one million endophytic species are ubiquitously present in various plants (Pirttil and Frank 2011). Since the discovery of endophytes in Darnel, Germany in 1904, endophytes (bacteria, fungi and actinomycetes) have been isolated from plant tissues such as seeds, roots, stems and leaves of a wide variety of plants (Hallmann et al. 1997, Sturz et al. 2003). Many endophytes have potential to produce various metabolites which may directly or indirectly be used for agricultural, pharmaceutical, and biotransformation processes (Mousa and Raizada 2013).

2.1.2. Host-endophyte relationship

Many studies reported that endophytes are not host specific. Single endophytes can colonize a wide host range and that some strains of the same endophytic fungi isolated from different parts taken from the same host which differ in their ability to utilize different substances reviewed by Jalgaonwala et al. (2011). Moreover endophytes can be isolated from different plants which grow under different ecological and geographical conditions such as tropic, temperate, xerophilic and aquatic (Petrini 1986). Host-endophyte relationship may be varied from host to host and host to endophyte. Suitable host plant and endophyte relationship have ability to balance pathogen-host antagonism, not truly all symbiotic (Schulz et al. 1999). This includes microorganisms with different life history strategies in that it can grow saprophytically on dead or senescing tissue following an endophytic growth phase (Stone et al. 2000).

2.1.3. Plant interaction of endophytic microorganisms

The interaction of an endophyte and a plant is controlled by the genes of both organisms and modulated by the environment (Morica et al. 2012), followed by several physical and chemical barriers which is overcoming to successfully establish an association (Kusari et al. 2012). In plant communities, multiple mutualistic potential of these fungi, establishing hyphae links or inoculum reservoirs, may favour inter-plant interactions. The mutualistic interactions of endophytic microorganisms which colonize in plant roots benefit the microbial partner with reliable supply of nutrients and protect them from environmental stresses, as suggested in a review (Schulz and Boyle 2006). The plant host responds to at least some infections with mechanical defense reactions (Narisawa et al. 2004). Many literatures reported that endophyte can be detected at a particular moment within the tissues of apparently healthy plant hosts (Schulz and Boyle 2005), which described the "balanced antagonism" hypothesis (Schulz et al. 1999); (Schulz and Boyle 2005) (Figure 2.1) that was proposed on how an endophyte had activated the host defenses, ensuring self-resistance before being incapacitated by the toxic metabolites of the host, thus can grow within its host without causing visible manifestations of infection or disease (Schulz and Boyle 2006) (Figure 2.1. A). The hypothesis stated that asymptomatic colonization between the host and the endophyte is a balance of antagonisms. The plant defense mechanisms are counter acting to many virulence factors of endophytes and pathogens. Should the virulence of fungal and plant defense are balanced, the association is apparently asymptomatic and avirulent, and that environmental factors has a major role to destabilize the delicate balance of antagonisms. In case that plant defense mechanisms completely counteract the fungal virulence factors, the fungus will perish. Conversely, if the plant does not maintain a defense mechanism, a plant-pathogen relationship would have lead to plant disease (Figure 2.1. B). The environmental factors that affected to many endophytes often made them becoming pathogenic (Morica et al. 2012) (Figure 2.1. C). Recently, it was revealed that the plant-endophyte interaction might not be just equilibrium between virulence and defense, but a much more complex and precisely controlled interaction (Figure 2.1. D) reviewed by Kusari et al. (2012).

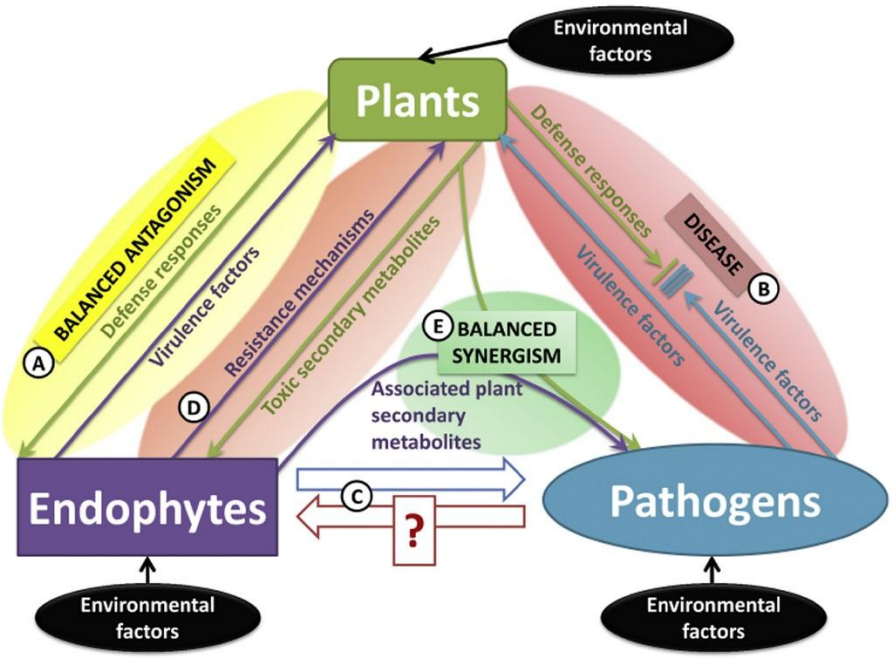


Figure 2.1 Chemical-ecological schematic interpretation of plant-fungus cost-benefit interactions with emphasis on endophytic fungi (Kusari et al. 2012).
(A) Balanced antagonism hypothesis is shown.

(B) Plant disease caused by pathogenic fungi is presented.

(C) Endophyte-pathogen reciprocity is demonstrated. The question mark (?) indicates that this phenomenon might not be universal, and further research is necessary for verification.

(D) Endophyte survival strategy is illustrated.

(E) Balanced synergism is shown.

Thus are either both commensal microorganisms that have no direct effect on plants, or beneficial microorganisms that could be used in biological control of plant pathogens or plant growth promotion (Hallmann et al. 1997, Stone et al. 2000, Sturz et al. 2000)

2.2. Endophytic fungi

An endophytic fungus lives in mycelial (except yeast cells) form in biological association with the living plant, at least for some part of life cycle. Therefore, a minimal requirement before a fungus is termed an 'endophyte' should be the demonstration of its hyphae in living tissue. Endophytic fungi are widespread in all major taxonomic groups of plants living under various environments (Schulz and Boyle 2005). Some of endophytic filamentous fungi and yeasts which isolated from varities of plants are shown in Table 2.1 and Table 2.2.

Table 2.1 Examples of endophytic filamentous fungi from varieties of plants

Plant species	Endophytic filamentous fungi	Tissue	References
Brassica napus	*Acremonium kiliense*	Leaf	Zhang et al. (2013)
	Alternaria mali	Roots, Leaf	
	Alternaria alternate	Leaves, Stems,	
	Arthrinium sp.	Roots	
	Arthrinium arundinis	Leaf	
	Aspergillus sp.	Stem	
	Aspergillus flavipes	Root	
	Botrytis cinerea	Stem	
	Chaetomium bostrychodes	Stem	
	Chaetomium globosum	Root, Stem	
	Clonostachys rosea	Roots, Stems	
	Dothidea sp.	Stems, Root	
	Epicoccum nigrum	Stem	
	Fusarium oxysporum	Stems, Leaf	
	Fusarium proliferatum	Roots	
	Fusarium solani	Roots, Leaf	
	Fusarium tricinctum	Roots	
	Guignardia vaccinii	Roots	

Table 2.1 Examples of endophytic filamentous fungi from varieties of plants (cont.)

Plant species	Endophytic filamentous fungi	Tissue	References
Brassica napus	*Hypoxylon* sp.	Stem	Zhang et al.
	Macrophomina sp	Stem, Roots	(2013)
	Nigrospora sp.	Root	
	Penicillium pinophilum	Roots, Stem	
	Periconia sp.	Root	
	Rhizoctonia solani	Stems	
	Simplicillium lamellicola	Stems	
	Sporidiobolus sp.	Stems	
Cucumis sativus	*Phoma glomerata*	Plants	Waqas et
	Penicillium sp.		al. (2012)
Eugenia jambolana	*Alternaria alternata*	Leaf, petiole	Yadav et
	Aspergillus sp.	and stem	al. (2014)
	Aspergillus aff. Fumigatus	tissue	
	Aspergillus japonicas		
	A. niger		
	Aspergillus flavus		
	Aspergillus peyronelii		
	Aspergillus terreus		
	Aspergillus tubingensis		
	Chaetomium sp.		
	Choanephora sp.		
	Coprinopsis cinerea		
	Curvularia lunata		

Table 2.1 Examples of endophytic filamentous fungi from varieties of plants (cont.)

Plant species	Endophytic filamentous fungi	Tissue	References
Eugenia jambolana	*Penicillium spinulosum* *Isaria tenuipes* *Syncephalastrum racemosum* *Trichoderma longibrachiatum* *Paecilomyces formosus*	Leaf, petiole and stem tissue	Yadav et al. (2014)
Humboldtia brunonis	*Alternaria alternate* *Aspergillus* sp. *Cunnighamella echinulata* *Curvularia clavata Jain* *Curvularia pallescens* *Debaryomyces hansenii* *Fusarium fusaroides* *Fusarium oxysporum* *Guignardia* sp. *Hypoxylon anthochroum* *Lasodiplodia theobromae* *Meyerozyma guilliermondii* *Paecilomyces lilacinus* *Pestalotiopsis* sp. *Peacilomyces lilacinus* *Phanerochaete* sp.	Leave and stems	Sheik et al. (2015)

Table 2.1 Examples of endophytic filamentous fungi from varieties of plants (cont.)

Plant species	Endophytic filamentous fungi	Tissue	References
Mangifera indica L.	*Acremonium p*ersicinum *Alternaria alternate* *Aspergillus awamori* *A. flavus* *A. fumigatus* *A. niger* *A. tamari* *Chaetomium globosum* *Cladosporium cladosporoides* *Curvularia lunata* *Drechslera hawaiense* *Epicoccum purpurascens* *F. pallidoroseum* *F. roseum* *Penicillium citrinum* *Phomopsis mangiferae* *Robillarda sessilis* *Trichoderma viride* *Verticillium albo-atrum*	leaves, stem and bark	Chaudhary (2012)
Paeonia suffruticosa	*Chaetomium* sp.	Stem	Li et al. (2003)
Psychotria flavida	*Bipolaris papendorfii* *Cylindrocladium* sp. *Curvularia lunata* *Guignardiamangiferae* *Lasiodiplodia theobromae* *Meyerozyma caribbica* *Meyerozyma guilliermondii*		Sheik et al. (2015)

Table 2.1 Examples of endophytic filamentous fungi from varieties of plants (cont.)

Plant species	Endophytic filamentous fungi	Tissue	References
Psychotria flavida	*Meyerozyma guilliermondii* *Phanerochaete chrysosporium* *Phyllosticta capitalensis* *Pestalotiopsis clavispora* *Phialemonium dimorphosporum* *Talaromyces flavus*	Leave and stems	Sheik et al. (2015)
Theobroma cacao	*Acremonium* sp. *Botryosphaeria ribis* *Colletotrichum* sp. *Colletotrichum gloeosporioides* *Fusarium decemcellulare* *Fusarium solani* *Xylaria* sp.	Tissue	Mejía et al. (2008)

Table 2.2 Examples of endophytic yeasts from varieties of plants

Plant species	Endophytic yeasts	Tissue	References
Brassica napus	*Aureobasidium pullulans* *Cryptococcus sp.* *Dioszegia zsoltii* *Dioszegia zsoltii* *Phoma crystallifera*	Stems	Zhang et al. (2013)
Citrus sinensis	*Aureobasidium pullulans* *Candida parapsilosis* *Cryptococcus flavescens* *Cryptococcus laurentii* *Pichia guilliermondii* *Rhodotorula dairenensis* *Rhodotorula mucilaginosa*	Leaves	Reviewed by Doty (2013)
Daucus sativus	*Pichia*	Roots	Reviewed by Doty (2013)
Malus domestica	*Rhodotorula* *Candida* *Pichia*	Leaves	Reviewed by Doty (2013)
Orchidanceae(orchids)-Brazil	*Rhodotorula* *Candida* *Besingtonia*	Roots	Reviewed by Doty (2013)

Table 2.2 Examples of endophytic yeasts from varieties of plants (cont.)

Plant species	Endophytic yeasts	Tissue	References
Pinus sylvestris (Scots pine)	*Hormonema* *Rhodotorula*	Buds	Reviewed by Doty (2013)
Picea mariana (black spruce)	*Black yeasts*	Needles	Khan et al. (2012)
Pinus tabulaeformis-China	*Rhodotorula*	Twigs	Reviewed by Doty (2013)
Populus trichocarpa	*Rhodotorula graminis* *Rhodotorula mucilaginosa*	Leaves	
Quercus rober (English oak)	*Cryptococcus*	Acoms	
Quercus (oak and elm)	*Filobasidium*	Canopy	
Salix	*Ogataea*	Leaf galls	
Sequoia sempervirens (coast redwood)	*Debaryomyces*	Shoots	

Table 2.2 Examples of endophytic yeasts from varieties of plants (cont.)

Plant species	Endophytic yeasts	Tissue	References
Solanum cernuum (panaceia)	*Candida* *Cryptococcus* *Kwoniella* *Meyerozyma*	Leaves, stem	Reviewed by Doty (2013)
Zea mays L.	*Williopsis saturnus*	Roots	Nassar et al. (2005)

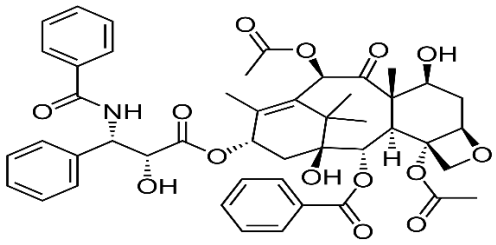
2.3. The roles of endophytic fungi

There are many bioactive compounds produced by endophytes which are new sources for discovery of novel drugs. Endophytes provide a broad variety of bioactive secondary metabolites with unique structure, including alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthones, and enzymes which are useful to pharmaceutical and agricultural industries (Tan and Zou 2001).

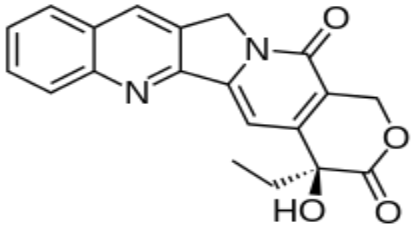
2.3.1. Anticancer compounds

Cancer is a group of disease with unregulated growth and spread of abnormal cells, so often result in death in uncontrollable (Calle and Rodriguez 2009). The anticancer drugs have some drawbacks, oftenly cause nonspecific toxicity to normal cells, meaning that it have many side effects, and are not effective against many forms of cancer (Gangadevi and Muthumary 2008, Pasut and Veronese 2009). Interestingly, bioactive compounds produced by endophytic fungi have potential anticancer drugs. For example, Taxol ($C_{47}H_{51}NO_{14}$) is a compound which interferes with the multiplication of cancer cells, reducing or interrupting their growth and spreading. Taxol had used for the treatment of breast cancer, lung cancer, and refractory ovarian cancer (Cremasco et al. 2009). Taxol is produced by *Taxomyces andreanae, Taxodium distichum, Wollemia nobilis, Phyllosticta spinarum, Bartalinia robillardoides, Pestalotiopsis terminaliae,* and *Botryodiplodia theobromae* (Pimentel et al. 2011).

Besides that, Camptothecin ($C_{20}H_{16}N_2O_4$) is the alkaloid which was obtained from endophytic fungi *Fusarium solani* isolated from *Camptotheca acuminate* (Kusari et al. 2012). Structures of anticancer compounds from endophytic fungi are shown in Figure 2.2.



Taxol



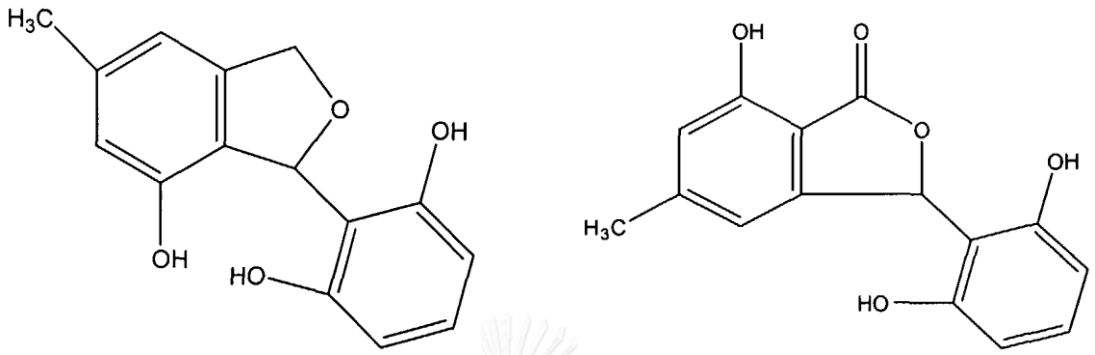
Camptothecin

Figure 2.2 Examples of structures of anticancer compounds from endophytic fungi (Pimentel et al. 2011)

2.3.2. Antioxidant compounds

The importance of antioxidant compounds are highly effective against damage caused by reactive oxygen species (ROSs) and oxygen-derived free radicals, which contribute to a variety of pathological effects, for instance, DNA damages, carcinogenesis, and cellular degeneration (Huang et al. 2007). Natural antioxidants are commonly found in medicinal plants, vegetables, and fruits. Many antioxidant compounds such as phenolics, flavonoids, pestacin and graphislactone A have potential on antioxidant activity. However, it has been reported that metabolites from endophytes can be a potential source of novel natural antioxidants (Pimentel et al. 2011). For example, phenolic and flavonoid compounds which produced by *Xylaria* sp. isolated from the medicinal plant *Ginkgo biloba*, showed that the methanol extract exhibited strong antioxidant capacity (Liu et al. 2007). Beside that Yadav et al. (2014) reported that endophytic fungi *Chaetomium* sp., *Aspergillus* sp., *A. peyronelii*, and *Aspergillus niger* strains isolated from *Eugenia jambolana* exhibited the highest antioxidant activity ranging from 50% - 80% containing 58 mg/g to 60 mg/g Gallic acid equivalents of total phenolic compounds. Pestacin ($C_{15}H_{14}O_4$) and isopestacin, 1,3-dihydroisobenzofurans were obtained from the endophytic fungus *Pestalotiopsis microspora* isolated from *Terminalia morobensis*. Other than antioxidant activity both

compounds, also possess antimycotic and antifungal activities, respectively (Harper et al. 2003, Strobel and Daisy 2003). The example structures of antioxidant compounds from endophytic fungi are shown in Figure 2.3.



Pestacin Isopestacin

Figure 2.3 Structures of antioxidant compounds from endophytic fungi (Harper et al. 2003)

2.3.3. Biocontrol potentials

The phytopathogens and plant pest including viruses, bacteria, nematodes, insects, and fungi reduced worldwide crop yield by 30-50% (Pimentel 2009). The *cassava mosaic* virus (CMV) is a plant virus which devastates the livelihoods of cassava farmers in Africa (Thresh and Cooter 2005). Amongst bacterial pathogens, *Xanthomonas* spp. Cause blights in 350 different plants including rice blight disease (*X. oryzae*) (Leyns et al. 1984). Furthermore, one of the problems in tropical and subtropical regions is nematodes that can exert their damage on plant roots (Shurtleff and Averre 2000). Nematodes in the genera *Paratrichodorus* and *Trichodorus* are also vectors of plant pathogenic viruses (Boutsika et al. 2004). Around 9,000 species of insects and mites damage crops, causing an estimated 14% loss in global crop yields (Pimentel 2009).

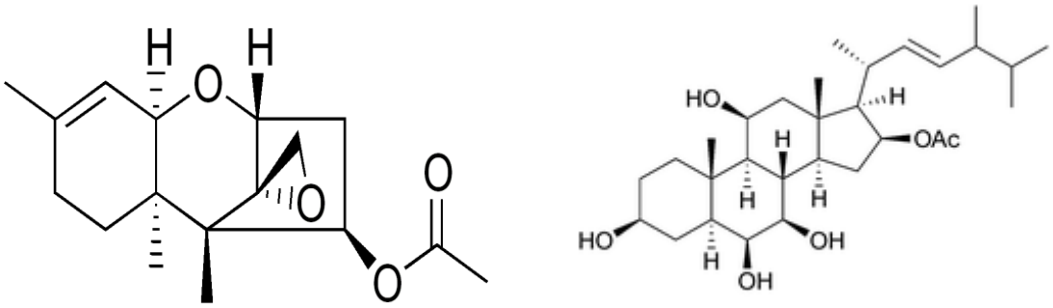
Fungi are serious phytopathogens because they can also potentially produce mycotoxins which are then consumed by humans and animals. For example, the maize and rice pathogenic fungus, *Fusarium moniliforme* produces fumonisin B1 which is associated with esophageal cancer (Gelderblom et al. 1991). *Aspergillus flavus* produces aflatoxin and causes kernel rot on pre-harvest corn and in storage (Payne and Widstrom 1992). Other serious fungal pathogens of crops include: *Magnaporthe grisea*

and *Pyricularia oryzae*, which cause rice blast diseases in Asia (Strange and Scott 2005). Besides that, *Colletotrichum* sp. is a fungal pathogen which causes Anthracnose disease that is wide damage in crop plants. To control those plant pathogens, non-chemical approach has been used for biological control (Oudejans 1991, Upadhyaya et al. 1996)

Endophytic fungi have recently been considered as an important source for screening of biocontrol agents to plant pests (insects and pathogens) and to help plant overcome abiotic stresses (drought, salt and heat) (Backman and Sikora 2008). *Rhodotorula rubra* isolated from rice tissue showed antagonistic and plant protecting activities *in vitro* against saprophytes and phytopathogens such as *Fusarium vasinfectum*, *Penicillium* spp., and *Vertillium dahlia*. (Akhtyamova and Sattarova 2013). *Nodulisporium* sp. isolated from *Mitrajyna javanica* showed strong broad spectrum antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Sacharomyces cerevisiae* and *Candida albicans (Pharamat et al. 2013)*. Zhang et al. (2014) isolated endophytic fungi from *Brassica napus*, and found that *Fusarium oxysporum* showed antagonistic effect towards pathogenic fungi (*Sclerotia sclerotium* and *Botrytis cinerea*). In addition, *F. oxysporum* also promotes growth of oilseed rape (*Brassica napus*). Three endophytic fungi viz. *Robillarda sessilis*, *Phomopsis mangiferae* and *Phomopsis guepinii* isolated from Mango leaves (*Magifera indica*), grown in India, posess antagonistic activity against pathogenic fungi which cause leaf spot diseases in leaves mango (*Gloeosporium maniferae*, *Fusarium monilifore*, *Lasiodiplodia theobromae*) (Chaudhary 2012).

Secondary metabolites, as antimicrobial compounds which produced by endophytic fungi such as terpenoids, alkaloids, phenylpropanoids, aliphatic compounds have potential to control crop pathogens (Mousa and Raizada 2013). For example: *Trichodermin* is a terpenoid compound which was isolated and characterized from *Trichoderma harzianum*, an endophytic fungus living in *Ilex cornuta*, an evergreen holly shrub from EastAsia. Trichodermin has been reported to protect the Solanaceous plant against pathogens *Alternaria solani* and *Rhizoctonia solani* (Chen et al. 2007). Penicisteroid A is a steroid which was isolated from the culture extract of *Penicillium chrysogenum* that showed antifungal activity against *Aspergillus niger* (plant black

mold) (Gao et al. 2011). The structures of Trichodermin and Penicisteroid A are shown in figure 2.4.

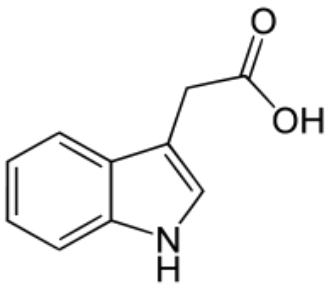


Trichodermin (12,13-epoxytrichothecene) *Penicisteroid A*

Figure 2.4 Structures of Trichodermin and Penicisteroid A that were isolated from endophytic fungal strains (Mousa and Raizada 2013).

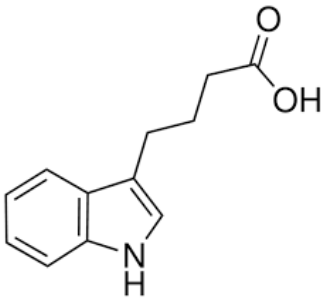
2.3.4. Plant growth promoters

Endophytic microorganisms also play role in plant growth promotion. They can produce phytohormones such as auxins, gibberellins or cytokinin, which are essential in plant growth. Auxins are a group of compounds which have an indole ring structure including indole- 3- butyric acid (Narisawa et al.), 2-phenylacetic acid (PAA) and indole-3- acetic acid (IAA) (Figure 2.5). They showed a positive effect on plant growth by stimulating seed germination, root initiation, cell elongation, and seedling growth (El-Tarabily 2008). Indole-3- acetic acid (IAA), is a plant growth hormone of the auxin family. It has been shown to stimulate cell division, cell elongation, cell differentiation, light and gravitational responses, and regulation of leaf fall and fruit ripening (Trotsenko et al. 2001, Teale et al. 2006). Clarification of IAA biosynthesis pathway was reported that L-tryptophan is a substrate required for IAA synthesis (Figure 2.6) (Mano and Nemoto 2012).



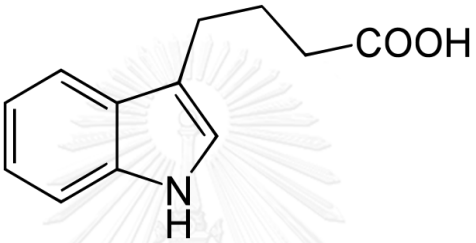
Indole- 3- acetic acid (IAA)

$C_{10}H_9NO_2$



Indole- 3- butyric acid (Narisawa et al.)

$C_{12}H_{13}NO_2$



2-phenylacetic acid (PAA)

$C_8H_8O_2$

Figure 2.5 Examples of the auxin family

The IAA production had been reported in several groups of bacteria and fungi, especially *Rhodotorula*, the first endophytic yeast were isolated from the *Populus* plant that was also shown to produce IAA (Xin et al. 2009). Not just only that, *R. grammis* and *R. mucilaginosa* from poplar (*Populus trichocarpa*) produced growth hormones as indole acetic acid and carries the nitrogenase gene required for nitrogen fixation. They also increased plant growth and fruit yields when grown in nitrogen-limited soil (Khan et al. 2012). Whereas the epiphytic yeast *Candida maltosa*, isolated from phylloplane showed relatively high concentrations of IAA (121.4-234.1 mg/l) when cultivated in YPD broth supplemented with 0.1 % L-tryptophan (Limtong and Koowadjanakul 2012). Endophytic fungi isolated from *Brassica napus*, *Fusarium tricinctum* and *Penicillium pinophilum* strongly promoted growth of oilseed rape (*Brassica napus*) (Zhang et al. 2014).

Beside that Waqas et al. (2012) reported that the endophytic fungi *Phoma glomerata* and *Penicillium* sp. isolated from cucumber plants had potential to produce gibberellins and indole acetic acid. Furthermore, they asist host- cucumber plants, helping significantly increased the plant biomass and related growth parameters under salinity and drought stress.

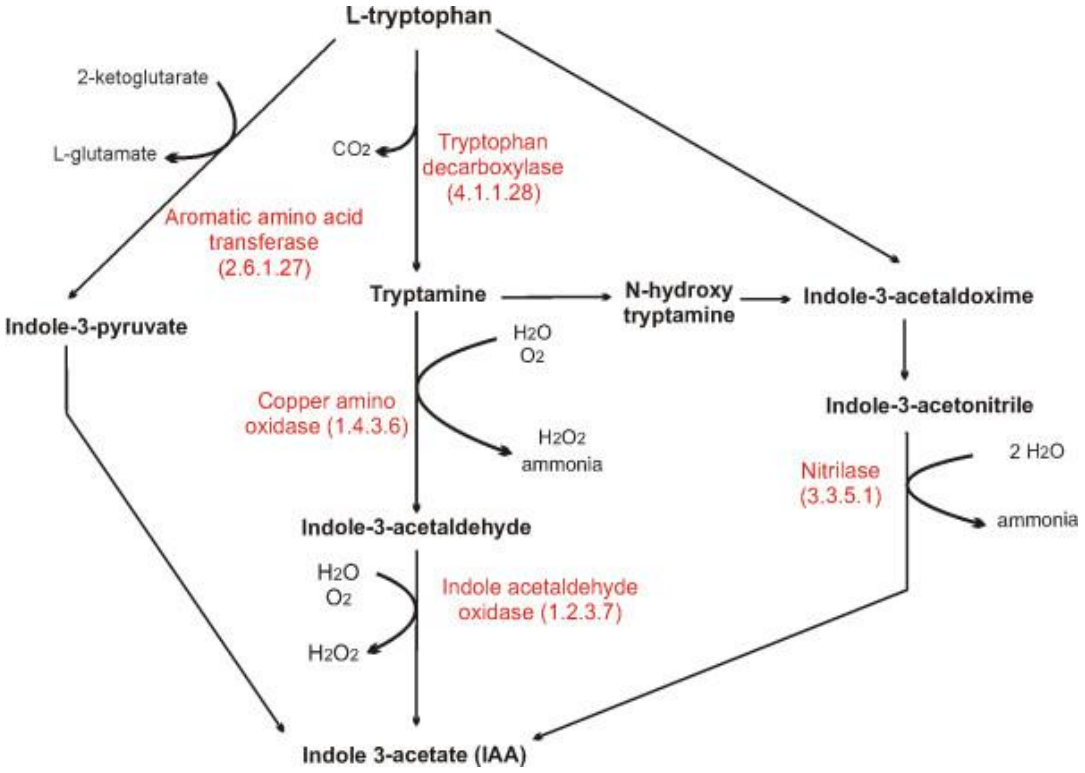


Figure 2.6 Indole-3-acetate (IAA) biosynthesis pathways (Mano and Nemoto 2012)

In addition, endophytic fungi also produce extracellular hydrolases as a resistance mechanism against pathogenic invasion and obtain nutrition from host such as: pectinase, cellulose, lipase, and laccase as identified in *Monotospora* sp. (Sunitha et al. 2013). Hydrolytic enzymes (amylase, cellulase and laccase) with various industrial applications are also of major interest, and found that *Cladosporium* sp., *Rhizoctonia* sp., *Aspergillus* sp., *Chaetomium* sp., *Biosporus* sp., *Fuzarium* sp., *Curvularia* sp., *Cladosporium* sp., *Colletotrichum* sp. were isolated from seven medicinal plants where

they were found producing extracellular enzymes such as amylase, protease, cellulase and lipase. Whereas a *Rhizoctonia* sp. isolate showed the highest production of amylase enzyme i.e. 0.26U/ml, and productivity of protease was the highest in *Biosporus* sp. (11 U/ml), *Colletotrichum* sp. showed better yield of cellulase i.e.0.013 U/ml and *Cladosporium* sp. (0.72 U/ml) showed greater production of lipase respectively (Patil et al. 2015). In another report *Alpinia calcarata*, *Bixa orellana*, *Calophyllum inophyllum* and *Catharanthus roseus* isolated from medicinal plants were screened for extracellular enzymes production such as amylase, cellulase, laccase, lipase, pectinase and protease on solid media. And found that 64% of fungi screened showed positive for lipase, 62% for amylase and pectinase, 50% showed for lipase, 32% showed for cellulase, 30% for laccase and only 28% showed positive for protease respectively (Sunitha et al. 2013).

From these literature, we can conclude that endophytic fungi are sources of bioactive metabolites which is useful for pharmaceutical, agricultural, and industries.

2.4. Mangifera indica L.

Figure 2.7 Mango trees (Rameshng 2010)

Kingdom:	Plantea
Division:	Magnoliophyta
Class:	Mangnoliopsida
Family:	Anacardiaceae
Genus:	*Mangifera*
Scientific name:	*Mangifera indica* L.
Common name:	Mango
Description:	Mango trees grow up to 35–40 m (115–131 ft) tall, with a crown radius of 10 m (33 ft). The trees are long-lived, as some specimens still fruit after 300 years.
Cultivation:	The mango is now cultivated in most frost-free tropical and warmer subtropical climates.

Mango (*Mangifera indica* L.) is an economically important fruit crop in the tropical and subtropical areas of the world. Global production of the mango has doubled in thirty years to around 35 million tons in 2009. Asia, where the mango is native, is the largest mango producer, representing 77% of global production, followed by America (13%) and Africa (10%) (UNCTAD 2012). On the Asian continent, many countries are the main global producers such as India, China and Thailand, which are top mango producers for the year 2011, about 15.19, 4.35 and 2.60 (millions tons), respectively reported by (UN 2012). At present, there is an increased demand for mango in the world market, especially the European United (Heuzé et al.) market. According to forecasts from the Food and Agricultural Organization, net imports by the EU will increase to 224 thousand tons in 2014, with an annual increase of 2.5%. The USA market is expected to increase 1% per year, and the worldwide mango imports are expected to increase 1.4% annually until 2014 (CBI 2009).

2.5. Plant diseases in Mangoes

There are number of diseases that cause infection and destroy mangoes and mango trees, thus, decreasing the yield and quality of mangoes. The diseases had affected at all stages of its development *i.e.*, from nursery to the consumption of fruits. It is estimated that the production could be increased by 28% if the crop is protected against various diseases (Rawal 1998). Lists of mango diseases are in the Table 2.3.

Table 2.3 Examples of mango diseases caused by plant pathogens and pests.

Diseases	Caused from	Characteristic of disease
Powdery mildew	*Oidium mangifera* Berthet	The white, powdery mycelial and spore growth of the fungi which forms on leaf surfaces and shoots and sometimes on flowers and fruits. Powdery mildews may infect new or old foliage.
Felt fungus	*Septobasilium bogoriense* Pat.	The fungus is brown, felty, perennial, lichen-like, and may frequently surround small tree branches, and is superficial on the plant, but parasitizes the insects which are feeding on the plant host.
Gummosis and twig blight	*Botryodiplodia theobromae*	This fungus attacks plants in different parts. Decline complex is observed in the form of twig blight, tip dieback, gummosis and bark splitting.

Table 2.3 Examples of mango diseases caused by plant pathogens and pests. (cont.)

Diseases	Caused from	Characteristic of disease
Sooty mold	*Meliola mangiferae* *Capnodium mangiferae*	The black fungus appears as a black staining or powdery coating on leaves and stems, and affects the plant indirectly by shading the leaves which interferes with photosynthesis.
Algal leaf spot	*Cephaleuros virescens*	The disease is characterized by grayish, green, brown or orange cushion-like blotches on the leaf surface. The algae produce spores, the spores infect leaf tissue causing small, greenish circular spots that may age to light brown or reddish brown.
Mango malformation	*Fusarium moniliforme*	A fungal disease which causes abnormal flower and leaf development, resulting in reduced plant growth and fruit yields.

From: Horticulture (2006)

Table 2.3 Examples of mango diseases caused by plant pathogens and pests. (cont.)

Diseases	Caused from	Characteristic of disease
Black spot	*Xanthomonas compestris pv mangiferae indicae*	Canker disease cause by a bacterium. It affects leaves, petioles, fruits and tender stems, initially producing water-soaked lesions and later turning into typical cankers.
Lethierry	*Idioscopus clypealis* *Idioscopus niveosparsus*	The most common and destructive species of hoppers which cause heavy damage to mango crop. Large number of nymphs and adult insects puncture and suck the sap of tender parts, thereby reducing the vigour of the plants.
Oriental fruit fly	*Baetrocera spp*	A very serious pest of a wide variety of fruits and vegetables throughout its range and damage levels can be anything up to 100% of unprotected fruit.

From: Horticulture (2006)

In addition, antracnose and rot diseases also cause mango diseases by fungal pathogens. They are important phytopathogens that need to be controlled they can infect and spread in wide crop plants as the following:

Colletotrichum gloeosporioides Penz (Anthracnose) causes serious disease in many host plants, and distributed in all mango planting regions of the world (Nelson 2008). It can infect and destroy the mango at all stages of growth such as seedlings, shoots, flowers, and fruits.

(1) (2)

Figure 2.8 Anthracnose disease infects on fruits (1), and leaves (2) of mango (Nelson 2008).

Lasiodiplodia theobromae is a plant pathogen with very wide host range, it is recognized as an important wood pathogen and has been reported to cause cankers, dieback, and fruit and root rots in over 500 different hosts, including perennial fruit and nut trees, vegetable crops, and ornamental plants. It is a common post-harvest fungal disease of commercial crop such as citrus, mango, and banana (Punithalingam 1980).

(1) (2)

Figure 2.9 *L. theobromae* infects on fruits (1), and twig (2) of mango.
(1) Farungsang et al. (2011)

(2) Sandesh (2015)

Lao People's Democratic Republic (Lao PDR) or Laos is a country located in the tropical zone (Figure 2.10) Laos shows high biodiversity including flora and fauna. Approximately 41.5 % of Lao PDR is covered with forest which contains an estimated 8,000–11,000 species of flowering plants. The country's fauna includes 166 reported species of reptiles and amphi-bians, at least 700 bird species, 90 known species of bats and at least 100 species of large mammals (MAF and STEA 2003). However, only 201 fungal species was identified as Ascomycota 24 species, Basidiomycota 44 species, Deuteromycota 133 species (Phengsintham et al. 2012). Still there was no report on endophytic fungi diversity.

Even though mangoes in Laos are not commercially grown fruit crop of the country, but most gardens and orchards contain mango trees, and these are organic gardens, so biocontrol is needed.

From: (Ezilon 2015)

Figure 2.10 Lao PDR's map, the square box (Vientian) is the location of organically mango trees sampling in this study.

This study aimed to isolate endophytic fungi from various parts of *Mangifera indica* L. for use as biological control against plant pathogens, especially those that causes diseases in mango trees. In addition, the plant growth promoting activity from the isolated endophytic fungi was also evaluated.

CHAPTER III
MATERIALS AND METHODOLOGY

3.1. Materials

3.1.1. Materials

1. 30°C Incubator (Thermo Scientific, USA)
2. 37°C Incubator (Thermo Scientific, USA)
3. 96 well plate (low binding) (NUNC, Sweden)
4. Agarose gel electrophoresis Mini gel electrophoresis system (Mupid-ex, Japan)
5. Alcohol burner
6. Autoclave MLS 3020 (Sanyo, Japan)
7. Balance PB3002 (Mettler-Toledo, Switzerland)
8. Beaker (Pyrex, USA)
9. Bench-top centrifuge (Eppendrof)
10. Centrifuge tubes 15 and 50 ml (Corning Incorporation, USA)
11. Cover slips (Nissho Nipro, Japan)
12. Cork borer 5 mm
13. Cryotube (Corning Incorporation, USA)
14. Deep freezer ULT 1786 (Forma Scientific, USA)
15. Deep freezer MDF-U332 (Sanyo electric, Japan)
16. Disposable syringe (Nissho Nipro, Japan)
17. DNA Thermal Cycle 2400 (Bio-Rad, USA)
18. Erlenmeyer Flasks 125, 250 and 500 ml (Corning Incorporation, USA)
19. Filter paper (Whatman, England)
20. Gel Documentation and Quantity one program Version 4.4.1 (Bio-Rad, USA)
21. Glass beads (0.45-0.52 mm) (Sigma, USA)
22. Haemacytometer (Mettler Toledo, Switzerland)
23. Heat box (Labnet international, Inc.)
24. Hot air oven UE600 (Memmert, Germany)

25. Inverted microscrope (Olympus, USA)
26. Laminar flow Clean model V4 (LAB Service, Thailand)
27. Measuring cylinder (Pyrex, USA)
28. Microcentrifuge tubes 1.50 ml and 0.2 ml (Axygen Scientific, USA)
29. Micropipette P10 P20 P100 P200 P1000 and P5000 (Gilson, France)
30. Microplate reader Elx 800 (Bio-tek instrument, USA)
31. Micro refrigerated centrifuge (Centrifuge 5424 R, eppendorf)
32. Microscope slides (Nissho Nipro, Japan)
33. Microwave oven (Samsung, Kore
34. Multinational pipet (Rainin Instrument, LLC a METTLER TOLEDO Company, USA)
35. Parafilm (Parafilm®M, USA)
36. PCR tube 200 µl (Corning Incorporation, USA)
37. Pipette aid (Drummond, USA)
38. Petri dish plates (Greiner Bio-One)
39. Power supply for electrophoresis (Atto, Japan)
40. Refrigerator Tiara (Mitsubishi Electric, Thailand)
41. Rotary vacuum evaporator N-1NW (Eyela, Japan)
42. Seropipettes 1, 5 and 10 ml (Pyrex, USA)
43. Sonicator RK 100 (Bandelin, Germany)
44. Syringe filter CA-CN 13 mm 0.22 µM (Restek, Thailand)
45. Tip p10, p200, p1000 (Axygen Scientific, USA)
46. Vortex mixer Genic II G-560E (Scientific Industries, USA)
47. Water bath shaker NST 2000 (Eyela, Japan)

3.1.2. Chemicals

1. 3-Indole acetic acid (Bio basic CANADA INC.)
2. Absolute ethanol (Lab Scan analytical science, USA)
3. Alcohol 70%, 95%
4. Agar/ Agarose (CONDA, SpA)
5. Ampicillin G (T.P.Drug laboratories (1969) Co., ltd, Thailand)
6. DNA ladder 100 bp and 1 kb (New England Biolabs inc., USA)

7. Dimethyl sulfoxide (DMSO) (Sigma, USA)
8. dNTPs mix (Fermentas, Canada)
9. Colorimetric dry
10. Chloroform (Lab Scan analytical science, USA)
11. CutSmart buffer (New England Biolabs inc., USA)
12. Ferric chloride ($FeCl_3$)
13. Glycerol (Carlo ERBA, France)
14. Glucose (Bio basic CANADA INC.)
15. *HaeIII* (New England Biolabs inc., USA)
16. *HhaI* (New England Biolabs inc., USA)
17. *HinfI* (New England Biolabs inc., USA)
18. Kanamycin sulfate (T.P.DRUG LABORATORIES (1969) CO., LTD, Thailand)
19. L-Tryptophan (Bio basic CANADA INC.)
20. Lithium acetate (Sigma, USA)
21. Malt extract (Bio basic CANADA INC.)
22. Paraffin oil (CARLO ERBA Reagents, Ronado (MI))
23. Perchloric acid ($HClO_4$)
24. Peptone (Becton, Dickinson and Company, France)
25. Phenol (Merck, Gemany)
26. Potato dextrose Broth (TM MEDIA, TITAN BIOTECH LTD. India)
27. Proteinase K (Fermentas, Canada)
28. RNase I (Fermentas, Canada)
29. Sodium hypochlorite (HAITER, Cao industrial. Thailand)
30. *Taq* DNA polymerase (New England Biolabs inc., USA)
31. ThermoPol buffer (New England Biolabs inc., USA)
32. Triton X-100 (Sigma, USA)
33. Tris buffer pH 8.8 (Preparation is described in appendix)
34. Tris buffer pH 6.8 (Preparation is described in appendix)
35. Yeast extract (Bio Springer, France)

3.2. Methodologies

3.2.1. Sample collection

Healthy, fruitful and organically grown mango trees (*Mangifera indica L.*) were sampled from the same trees in Phontan village, Saysattha district, Vientiane capital, Laos (17°57'N, 102°38'E) in two seasons (raw fruit season: April 2014 and ripen fruit season: May 2014). Three varieties of mango were collected, including Okrong (*Mangifera indica* L. cv 'Okrong'), Talapnak (*Mangifera indica* L. cv 'Talapnak'), Khiaosawoey (*Mangifera indica* L. cv 'Khiaosawoey') (kasetporpeang 2013). Six mango trees were collected from an organic farm as the same area that the location was shown in figure 3.1. The collected parts were roots, twigs, leaves, and fruits (figure 3.2). The samples were individually placed in plastic bags kept on ice and brought delivered to laboratory as soon as possible. The samples were washed with tap water to remove soil and dust particles.

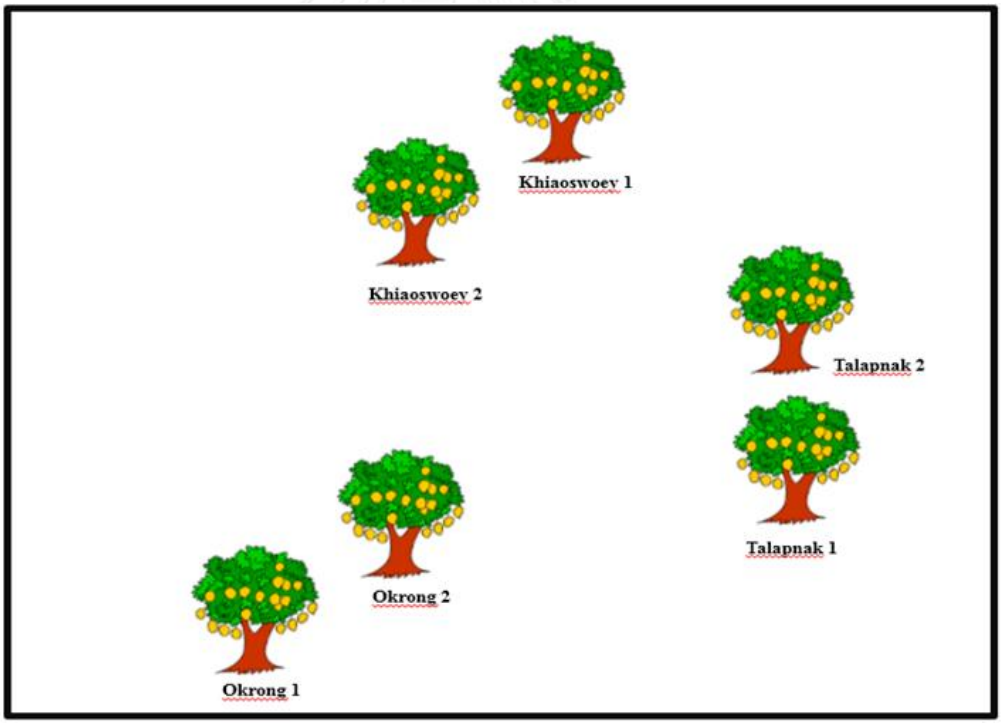


Figure 3.1 Place of mango sample collection from an organic farm at Phontan village, Saysattha district, Vientiane capital, Laos. Six mango trees (Okrong 2, Talapnak 2, and Khiaoswoey 2). Okrong 1 is far from Okrong 2 (5 m), Talapnak 1 is far from Talapnak 2 (7 m), and Khiaoswoey 1 is far from khiaoswoey 2 (4 m).

Figure 3.2 Parts of mango trees used for endophytic fungi isolation. Two season (raw and ripen sets), including leaves, twigs, fruits and roots.

3.2.2. Isolation of endophytic fungi

The collected leaves and fruits were cut into 3-cm squares. The twigs and roots were cut into 3-cm-long segments. The sample pieces were surface sterilized in 70% ethanol (v/v) for 1 min, then in 5% sodium hypochlorite (v/v) for 5 min, again in a 70% ethanol (v/v) for 1 min, and finally were rinsed in sterile water 3-5 times (3 min each) (Zhang et al. 2014). The surface-disinfected plant tissues were blotted dry on sterile tissue paper. Both terminal ends of each sample were cut off using a sterile razor blade. The remaining part were cut into small pieces and placed on potato dextrose agar (PDA) and yeast malt agar (YM agar) (Appendix A (1 and 2)) with the addition of antibiotics (Ampicillin and Kanamycin (100 µg/ml each)). Sterility checks were carried out for each sample to monitor the efficiency of the disinfection procedure. For sterility checks, 0.1 ml of some of the last wash was spread onto a PDA plate and incubated at 30°C. After seven days, if no growth of microorganism can be detected in the sterility check, the recovered fungi or bacteria grown on the screened plates were considered to be endophytes (Silva et al. 2012). After pure culture was obtained, they were preserved by storing at -80°C in 15% glycerol-containing YM broth and PDB medium in case of yeasts. For the mold isolates were stored in mineral oil and in distilled sterilized water in a cold room (4°C).

3.2.3. Antifungal phytopathogens activity assay

The mango pathogens, *Colletotrichum gloeosporioides* Penz, and *Lasiodiplodia theobromae*, were used in this study was obtained from Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Thailand.

3.2.3.1 Evaluation of the *in vitro* antagonism between endophytic fungi and phytopathogenic fungi by dual culture method.

Endophytic yeasts inoculum were prepared in YPD broth (Appendix A (3)) and incubated on a reciprocal shaker at 180 strokes/min at 30°C, for 24 h. A loop full of inoculum was transferred onto PDA by plating and the plated cultures were incubated at 30°C for 48 h. To evaluate the control of fungal growth by yeast, an assay in which yeast and fungi were grown side by side was set up (4 cm of distance), as adapted from Rosa et al. (2010). A control plate were prepared with only fungal inoculation. The experiments were performed in triplicate. Mycelial growth of pathogen was measured at 3 days for *L. theobromae* and 10 days for *C. gloeosporioides* (the time when the pathogen grew up to full plate; control plate) of incubation at 30°C.

Endophytic filamentous fungi were prepared on PDA and incubated at 30°C for 7 days and after that a mycelia agar plug done by a cork borer (5 mm in diameter) was placed on a PDA dish (test dish) and a mycelial agar plug of the pathogenic fungi from the margin of a 7 days old culture was placed on test dish side by side (4 cm of distance). Mycelial growth was measured at 3 days for *L. theobromae* and 10 days for *C. gloeosporioides* (the time when the pathogen grew up to full plate; control plate) and incubated at 30°C, as followed that described in Li et al. (2003). Width of the phytopatogen growth was then measured.

3.2.3.2 Antifungal volatile compounds (VOCs) of endophytic fungi

The endophytic fungal isolates in this study were tested for production of antifungal volatile organic compounds (VOCs) using the double-dishes method as described by Huang et al. (2011). Briefly, a mycelial agar plug of an endophyte was placed on PDA in a Petri dish and incubated at 30°C for 7 days. Another fresh PDA dish containing an agar plug with mycelia of pathogenic fungi was placed inversely over a bottom dish containing the seven-day-old culture of an endophyte to establish a double-dish set, and sealed with parafilm. In the control treatment, a bottom PDA dish

inoculated with the pathogenic fungi was placed inversely over a bottom dish containing PDA without an endophyte to make a double-dish set, which were also sealed with parafilm. The experiments were performed in triplicate for each endophyte and the control. Diameter of the pathogenic fungi colony in each double-dish set was measured after incubation at 30°C for 3 days for *L. theobromae* and 10 days for *C. gloeosporioides* (a control plates grew at full of plate) and the percentage of growth inhibition was calculated.

3.2.4. Evaluation of indole acetic acid production activity

The indole acetic acid (IAA) production by endophytic fungi were tested for their plant growth promoting activity using adapted method from Xin et al. (2009), and (Resende et al. 2014). Endophytic yeast isolates were prepared on YPD agar and incubated at 30°C for 24 h. One loop of inoculum was transferred into 5 ml of YPD broth with 1% tryptophan. The culture was incubated in a reciprocal shaker at 180 strokes/min at 30°C for 7 days. Culture supernatant was collected by centrifugation for 5 min at 8000 rpm. The supernatant was filtered through sterile Millipore membranes (pore size 0.22 µm) and collected in sterile tubes.

For endophytic filamentous fungi isolates, five pieces (5 mm diameter) were cut from the edges of growing cultures and inoculated into 100 ml PDB supplemented with L-tryptophan (1% w/v) and incubated with shaking (200 rpm) for 30 days, in the absence of light. After that the cultures were centrifuged at 8000 rpm for 10 min at 4°C to get supernatant and were filtered through sterile Millipore membranes (pore size 0.22 µm) and collected in sterile tubes. One hundred microliter culture supernatant of endophytic fungi was pipetted into wells of 96 wells plate and 100 µl of Salkowski reagent (Appendix B (1)) (Nutaratat et al. 2014)was added, mixed and left for 30 min for red color development. The intensity of the color was determined by optical density at 540 nm using a 96 wells plate spectrophotometer. IAA stock (Appendix B (2)) was used to calculate standard curve, 0.1 mg/ml (2.85 mM of IAA) to 0.0015 mg/ml (0.025 mM of IAA).

3.2.5. Endophytic fungi identification

3.2.5.1 Morphological Identification

Endophytic fungi that showed high antifungal and/or indole acetic acid production activities were identified. As described by Zhang et al. (2014), morphology of the colonies (color and mycelia) and spores (conidia, blastospores, sporangiospores or ascospore) produced by each fungal isolate were examined and used to determine the taxonomic status of the isolates. Slide culture technique was used for observing the see mycelium growth and spores morphology on microscope and on inverted microscope.

3.2.5.2 Molecular Identification

3.2.5.2.1 Genomic DNA extraction of endophytic fungi

For endophytic yeast isolates

Genomic DNA of endophytic yeasts was extracted by modified CTAB method (adapted from Arlorio et al. (1999)). Endophytic yeasts were prepared by plating on YPD agar and incubated at 30°C, 2-5 days. Cultures from 3-5 colonies were added to 5 ml YPD broth in test tubes. They were incubated on a reciprocal shaker at 180 strokes/min at 30°C, overnight, one milliliter of cell suspension was transferred to sterilized microcentifuge and then centrifuged at 13000 rpm, 5min to get cell pellets, washed cells by DDI and centrifuged at 13000 rpm, 5 min again. Supernatant was poured, and 600 µl CTAB lysis buffer was added (Appendix B (9)), vortexed for 3 min then 10 µl proteinase k (10 mg/ml) was added, gently mixed and incubated at 65ºC for 1 h (occasionally shaking, every 15 min). Next, the tubes were centrifuged at 13000 rpm, 5 min to obtain supernatant. After adding equal volume of phenol chloroform, the tube were gently mixed, centrifuged at 13000 rpm, 5min to separate aqueous layer. Then, aqueous layer was pipetted into a new tube and chloroform was added and the tube were gently mixed, centrifuged at 13000 rpm, 5min to obtain aqueous layer, and then cool isopropanol was added and placed at -20ºC for overnight. Then, the tubes were centrifuged at 13000 rpm, 5min, the supernatant was poured, and DNA pellet was washed twice with 70% ethanol. Finally, the tubes were centrifuged at 13000 rpm, 5 min, followed by pouring out the supernatant and the DNA pellets were dried at 65ºC. Extracted DNA was finally dissolved with 20µl TE.

For endophytic filamentous fungi isolates

Genomic DNA of endophytic filamentous fungi were extracted by glass bead (Modified from Koshland (2003) method). The endophytic filamentous fungi were cultivated on PDA at 30°C for 2-5 days to obtain fresh colonies. The colony was raked by hook and transferred to a sterilized microcentifuge. Two hundred µl of lysis buffer solution (Appendix B (10)), 10 µl RNase A, 10 µl proteinase K (10mg/ml) were added and then vortexed and incubated at 65ºC for 30 min. After that 0.3 g of glass beads, 100 µl TE, and 200 µl chloroform were added vortexed for 3 min. The mixtures were centrifuged at 13000 rpm, 5min, Supernatant were collected to a new tube and were added with 10 µl of 4M Ammonium Acetate, 1 ml of ethanol, and then the tube was mixed by inversion, and put at -20ºC for overnight. Next the tubes were centrifuged at 13000 rpm, 15 min. The DNA pellets were washed with 70% ethanol and finally dried and resuspend in 20 µl TE.

3.2.5.2.2. DNA amplification by Polymerase Chain Reaction (PCR)

Genomic DNA from (3.2.5.2.1.) was amplified by PCR. The internal transcribed spacer (Compant et al.) region of rDNA (ITS1-5.8S rDNA-ITS2) of each isolate was amplified using the universal primers ITS1 (3'-TCCGTAGGTGAACCTGCGG- 5') and ITS4 (3'-TCCTCCGCTTATTGATATGC-5'), using procedures as described by Zhang et al. (2010). The components of the reaction were performed at 50 µl of total volume which its content is shown in Table 3.1.

Table 3.1 The components of Polymerase Chain Reaction (PCR) for ITS amplification

Component	Final concentration	Volume (µl)
10X Thermolpol buffer	1X	5
10 mM dNTPs	0.2 mM	1
ITS1 10 µM	0.2 µM	1
ITS4 10 µM	0.2 µM	1
Taq Polymerase 5 u/µl	-	0.25
Distilled De- Ionized (DDI)	-	40.75
500 ng Template (Genomic DNA)	-	1
Total		50

The reaction of PCR were mixed and run on DNA thermal cycles set with respects program:

Initial denaturation	95ºC	30 sec	
Amplification			
Denaturation	95ºC	30 sec	
Annealing	50ºC	30 sec	35X (Cycles)
Extension	68ºC	1 min	
Final Extension	68ºC	5 min	
Hold	25ºC	∞	

3.2.5.2.3. Restriction fragment length polymorphism (RFLP)

Polymerase Chain Reaction (PCR) amplified products were cut by three enzymes such as *Hae* III, *Hha* I, and *Hinf* I for comparison on profile of restriction fragment. PCR products of 45 µl by addition of 5 µl 3M Sodium acetate and 125 µl cold ethanol then incubated at -20ºC for overnight. After that, the tubes were centrifuged at 14,000 ×g for 10 min at 4ºC and washed with 70% ice cold ethanol and centrifuged again at 14,000 rpm for 10 min, dried and dissolved in 30 µl DDI. Next, each 10 µl of the PCR product was restricted with the enzymes which were mixed with 1.5 µl CutSmart buffer, 1 µl enzyme, and 2.5 µl DDI, and then incubated at 37ºC for 1h. Finally 3 µl of 6x loading dye was added to the tube.

Restricted products were subjected to electrophorese for separation DNA fragment in 2% agar gel (Appendix B (7)) in 1X TAE buffer (Appendix B (5)), 40 ml agar gel was electrophoreses at 100 V for 45 min. DNA Ladder 100 bp was used as a standard DNA marker. Based on difference in profile of restriction fragment, the representative each group were randomly chosen for DNA sequencing.

The ITS sequences were compared with that of the most closely-related fungal species (identity values higher than 95%) in the NCBI database (ICBI) using the BLASTN program. The obtained sequencing data in combination with data on morphology of colony and spore morphology were used to identify the taxonomic status of the investigated fungal isolates.

3.2.6. Data analysis

All experimental data were analyzed:

3.2.6.1 The endophytic fungi isolates were calculated for statistical difference analysis on colonization and isolation rate by the following:

1. Season: Raw and Ripen
2. Mango variety
3. Molds and Yeasts species

3.2.6.2 Data from antifungal phytopathogens activity studies were analyzed for percentage of growth inhibition.

The percentage of growth inhibition were calculated from the following equation:

C = Control (no treatment with endophytic fungi)

T = Treated with endophytic fungi

$$\text{Inhibition } (\%) = \frac{\text{Growth diameter of C} - \text{Growth diameter of T}}{\text{Growth diameter of C}} \times 100$$

CHAPTER IV
RESULTS

4.1. Isolation of endophytic fungi

4.1.1. Endophytic yeasts in mango trees

Endophytic yeasts were isolated from different parts (root, twig, leaf, and fruit) of mango trees at different seasons (raw fruit and ripen fruit). A total 13 isolates of yeast endophyte (CY.OS 01 - CY.OS 13) were obtained from 6 mango trees (2 Okrong “OR” trees, 2 Talapnak “TN” trees and 2 Khiaosawoey “KW” trees). No endophytic yeast could be isolated from mango parts taken from raw fruit season. All 13 isolates were from ripen fruit reason: 4 isolates from OR1, 3 isolates from TN1, 3 isolates from TN2, and 3 isolates from KW2 that was shown in Appendix C (Table S1).

4.1.2. Endophytic filamentous fungi in mango trees

Endophytic filamentous fungi were isolated from different parts (root, twig, leaf, and fruit) of mango trees at different seasons (raw fruit and ripen fruit). Seventy nine isolates were obtained from raw fruit season Appendix C (Table S2), and 147 isolates were obtained from ripen fruit season Appendix C (Table S3). A total 226 isolates of filamentous fungi were obtained from 6 trees of mango (2 Okrong “OR” trees, 2 Talapnak “TN” trees and 2 Khiaosawoey “KW” trees).

Endophytic fungi isolates isolated from raw and ripen fruit seasons isolated on different media (YM and PDA) were shown in Table 4.1. Total endophytic fungi obtained was 239 isolates. Number of isolates obtained from TN1 and OR2 were higher than others while those isolated from KW2 showed the lowest number. Number of isolates from raw fruits were higher in PDA medium than in YM medium.

Table 4.1 Number of endophytic fungi isolates from raw and ripen fruits of different variety and media used for isolation.

Mango variety	Fruit seasons						
	Raw			Ripe			Total of raw and ripe
	YM	PDA	Total	YM	PDA	Total	
OR1	4	8	12	8	15	23	35
OR2	13	18	31	12	8	20	51
TN1	1	13	14	25	14	39	53
TN2	7	1	8	10	21	31	39
KW1	1	6	7	18	9	27	34
KW2	3	4	7	10	10	20	27
Total	29	50	79	83	77	160	239

YM= Yeast extract Malt extract medium, PDA= Potato Dextrose Agar
OR1= Okrong 1, OR2= Okrong 2, TN1= Talapnak 1, TN2= Talapnak 2
KW1= Khiaosawoey 1, KW2= Khiaosawoey 2

Endophytic fungi isolated from various parts of mango tree showed the number of isolates obtained from ripen fruit season were higher than those from raw season. Number of endopytic fungi isolated from twigs were the highest either from raw or ripen fruit season. While those isolated from fruits were the lowest number. Interestingly, no endophytic fungi could be isolated from raw fruits. (Table 4.2)

Table 4.2 Number of endophytic fungi were isolated from various parts of fruit seasons of mango.

Mango variety	Parts of mango tree										
	Raw fruit season					Ripen fruit season					Total
	Leaf	Twig	Fruit	Root	Total	Leaf	Twig	Fruit	Root	Total	
OR1	0	11	0	1	12	6	6	4	7	23	35
OR2	3	23	0	5	31	5	8	0	7	20	51
TN1	2	10	0	2	14	12	10	9	8	39	53
TN2	0	3	0	5	8	0	16	6	9	31	39
KW1	0	6	0	1	7	3	10	4	10	27	34
KW2	0	6	0	1	7	3	3	6	8	20	27
Total	5	59	0	15	79	29	52	29	49	147	239

YM= Yeast extract Malt extract medium, PDA= Potato Dextrose Agar
OR1= Okrong 1, OR2= Okrong 2, TN1= Talapnak 1, TN2= Talapnak 2
KW1= Khiaosawoey 1, KW2= Khiaosawoey 2

4.2. Anti-fungal phytopathogen activity assay

4.2.1. Evaluation of the *in vitro* antagonism between endophytic fungi and phytopathogenic fungi by dual culture method.

For endophytic yeasts isolated from mango trees, antagonistic effect against *C. gloeosporioides* and *L. theobromae* observed (Figure 4.1). In the dual cultures on PDA 3.15% - 15.78% growth inhibition was noted. Isolate CY.OS 09 showed 15.78% growth inhibition against *C. gloeosporioides* which was the strongest antagonistic activity among all isolates. However, no endophytic yeast exhibited antifungal activity against *L. theobromae* could be detected. Appendix D (Table S4).

For endophytic filamentous fungi isolates from mango trees. It was found that all of 226 filamentous fungi isolates showed different antagonistic activities (Figure 4.2). All filamentous fungi showed growth inhibition against *C. gloeosporioides* ranging from 0.74% - 81.48%, while isolate CY.OS 162 showed the highest of percent growth inhibition at 81.48%. For antagonistic activity against *L. theobromae*, 162 isolates showed 0.375 – 50.37% growth inhibition. The isolate CY.OS 132 also showed the strongest *L. theobromae* growth inhibition Appendix D (Table S5).

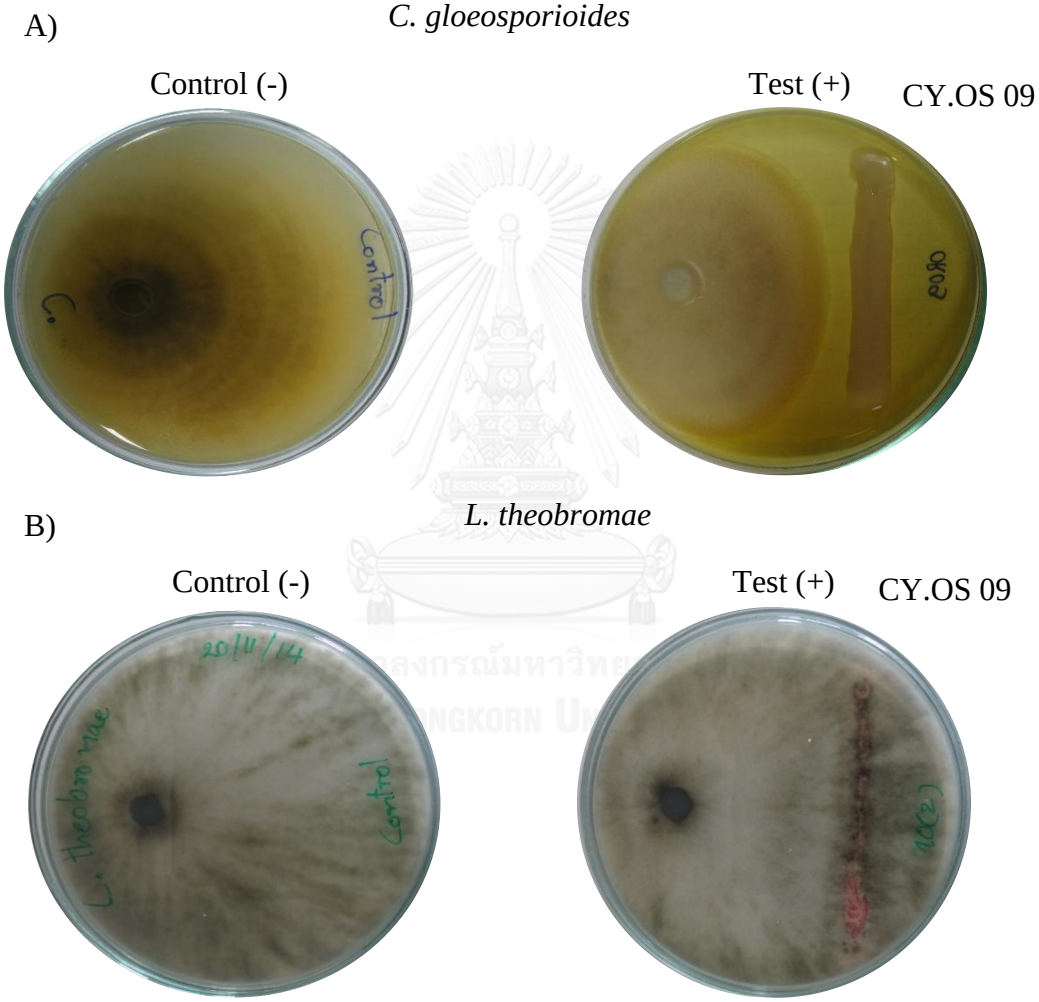


Figure 4.1 Antagonistic affect against plant pathogens *C. gloeosporioides* and *L. theobromae* by CY.OS 09. In vitro evaluation on PDA medium: *C. gloeosporioides* (A) or *L. theobromae* (B) (Control) (Left) and endophytic yeast isolate CY.OS 09 (right).The plates were incubated at 30°C for 10 d in case of *C. gloeosporioides* and 3 d in case of *L. theobromae*, respectively.

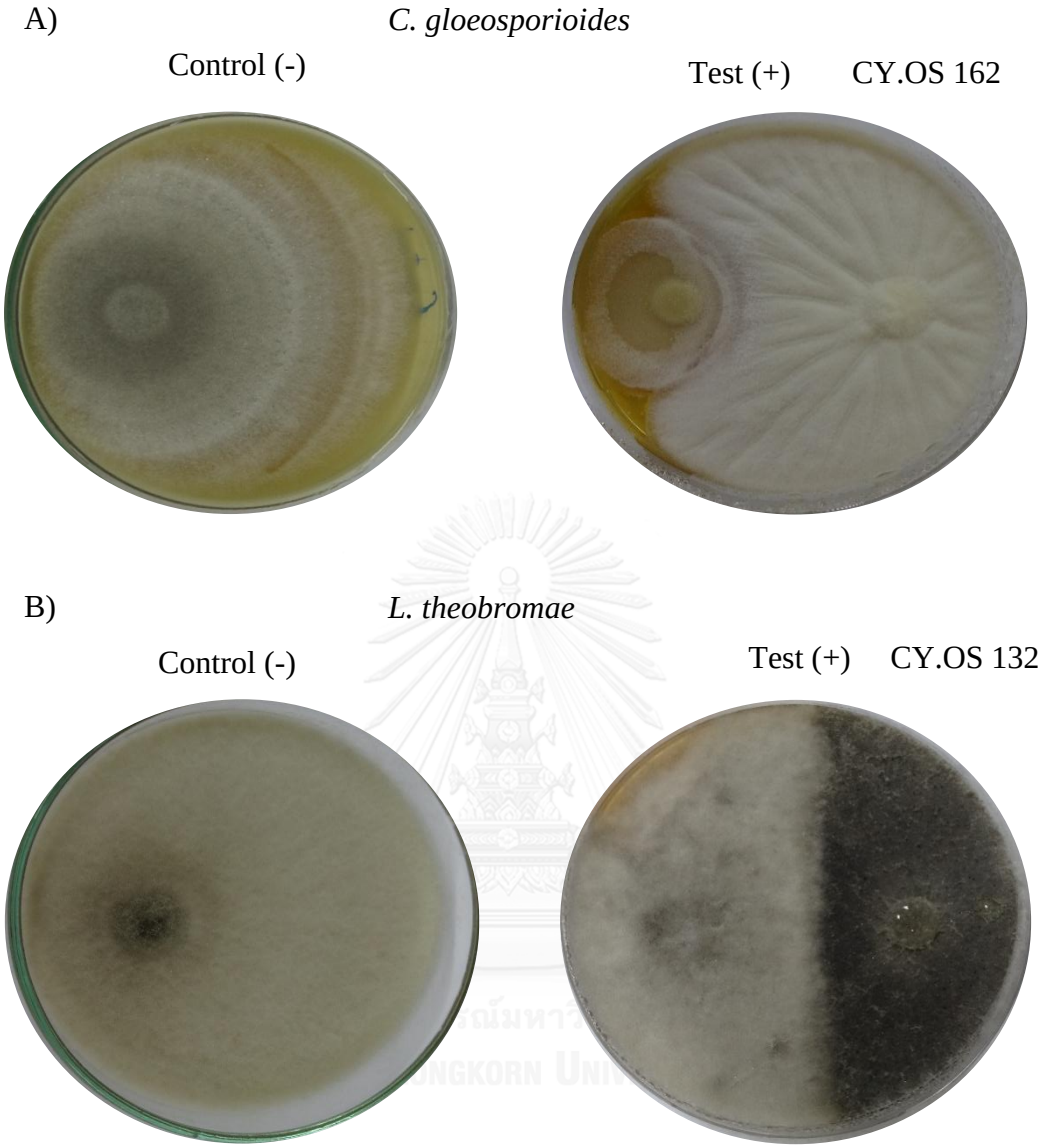


Figure 4.2 An example of antagonistic effect by endophytic filamentous fungi isolates against plant pathogens *C. gloeosporioides* and *L. theobromae.* (A). *In vitro* evaluation on antagonism between CY.OS 162 and *C. gloeosporioides* on PDA medium: *C. gloeosporioides* alone (Control) (Left) and with the endophyte CY.OS 162 (right) the plates were incubated at 30°C for 10 d. (B) *in vitro* evaluation on antagonism between CY.OS 132 and *L. theobromae* on PDA medium: *L. theobromae* alone (Control) (left) and with endophyte CY.OS 132 (right). The plates were incubated at 30°C for 3 d.

4.2.2. Evalution on antifungal volatile compounds of the endophytic fungi

Production of anti-fungal volatile compounds from the endophytic fungi isolates against *C. gloeosporioides and L. theobromae* was assayed by double dishes method. For endophytic yeast 13 isolates (Figure 4.3) against *C. gloeosporioides*, high percentage of inhibition could be observed in particular in these four isolates: CY. OS 01, CY. OS 03, CY. OS 08, and CY. OS 10 showing 42%, 38%, 40%, and 46%, respectively. For endophytic yeasts exhibiting antifungal activity against *L. theobromae*, no antifungal volatile compounds could be detected (Appendix D (Table S4)). For all 226 endophytic filamentous fungi isolates against *C. gloeosporioides and L. theobromae* (Figure 4.4), only 88 isolates showed activity against *C. gloeosporioides* ranging from 1.11% - 14.81% growth inhibition, and CY.OS 161 (CY.OS 170, and 227 are the same isolates) showed the strongest among isolates. Fifty isolates had activity of anti-fungal volatile compound against *L. theobromae* ranging from 1.85% - 34.44% growth inhibition. The isolate CY.OS 33 showed the highest activity among the isolates. All results were shown in Appendix D (Table S5).

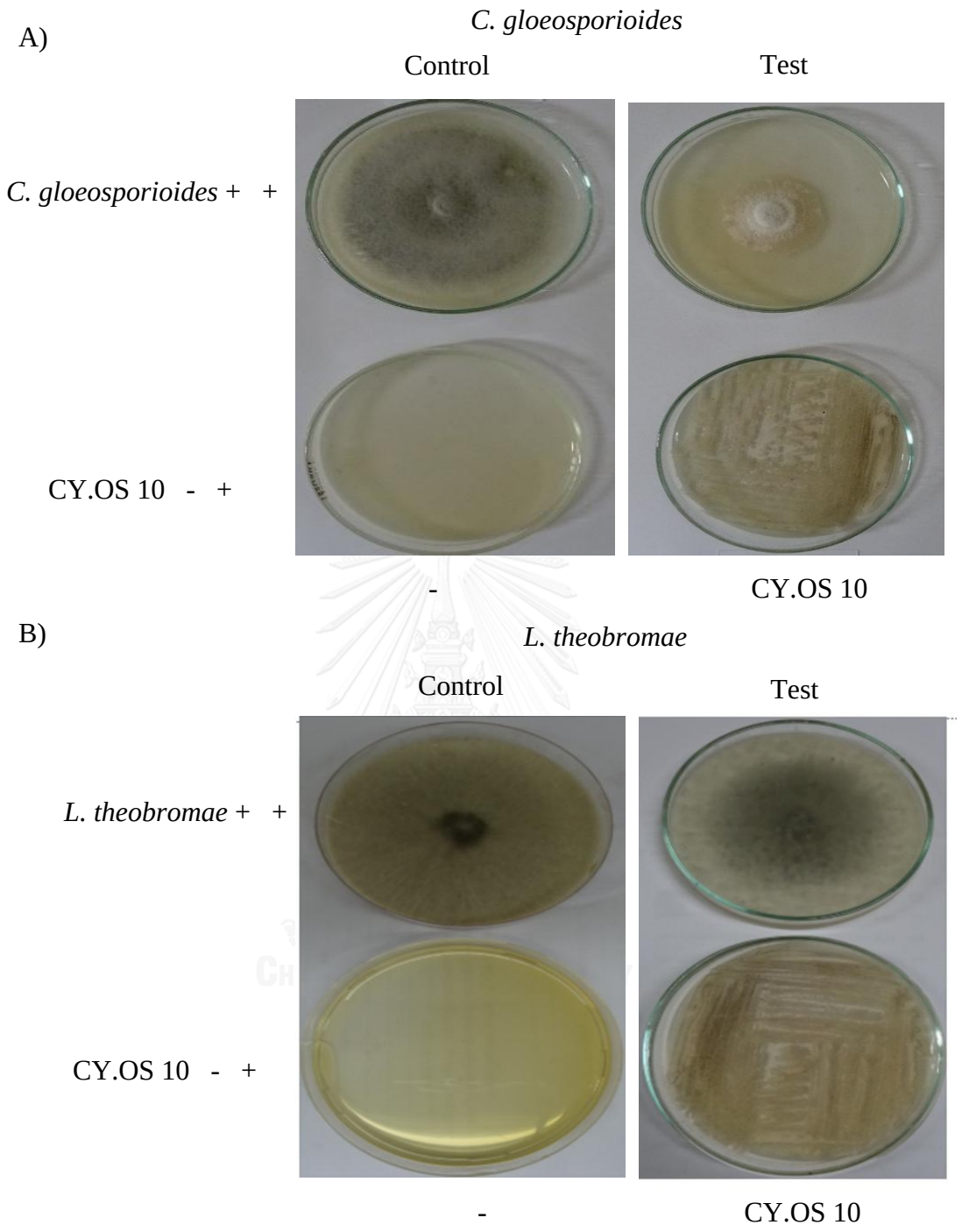


Figure 4.3 Effect of volatile organic compounds (VOCs) production against phytopathogens of endophytic yeast by double dishes method. The plate inoculated with either *C. gloeosporioides* (A), or *L. theobromae* (B) were inverted to either none (as control, left panel) or CY.OS 10 containing plate (as test, right panel). The double dishes sets were incubated at 30°C for 10 d (in case of *C. gloeosporioides*) and 3 d (in case of *L. theobromae*).

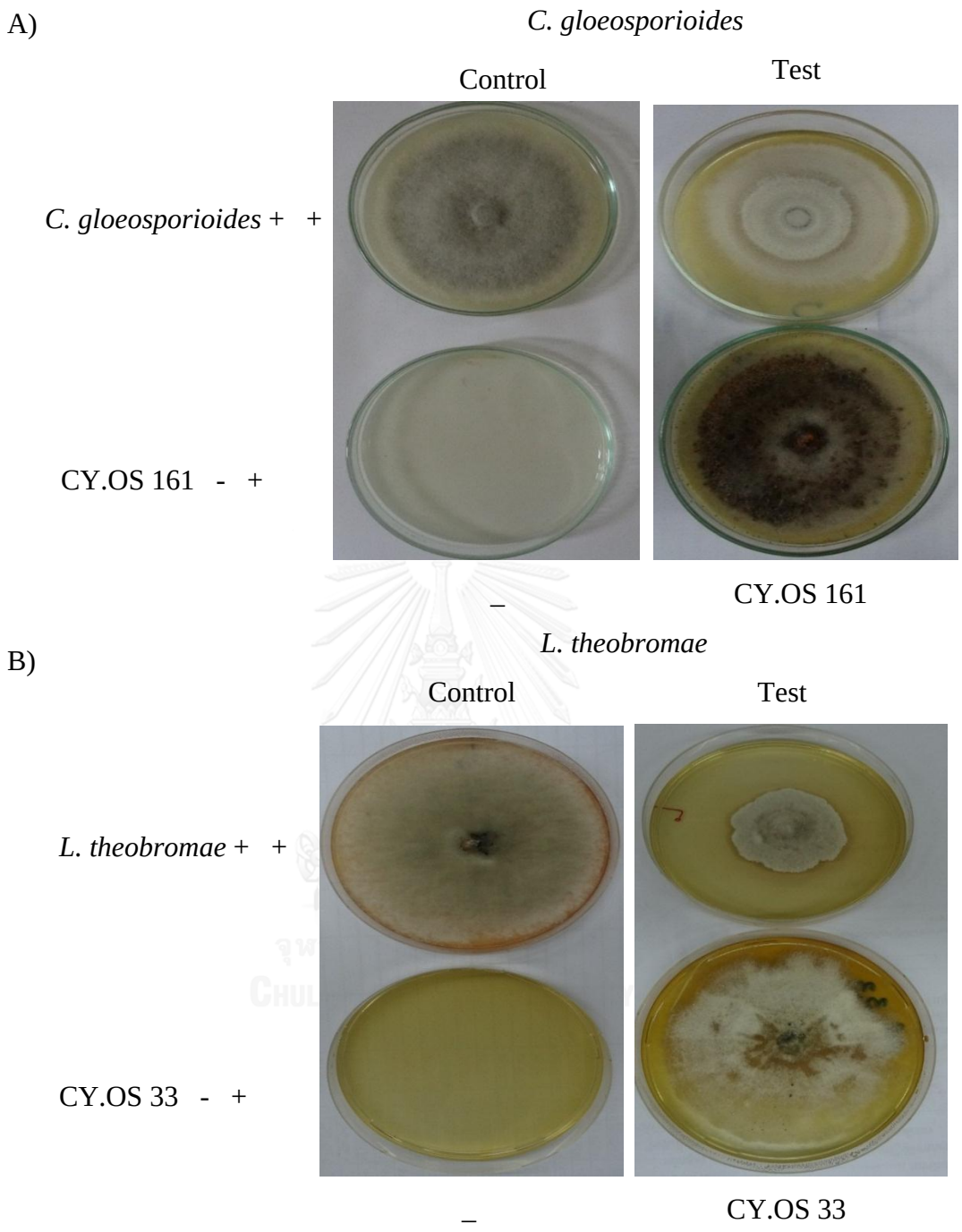


Figure 4.4 Effect of volatile organic compounds (VOCs) production of endophytic filamentous fungi by double dishes method. The plate inoculated with either *C. gloeosporioides* (A) or *L. theobromae* (B) were inverted to either none (as control, left panel) or CY.OS 161(as test, right panel) or CY.OS 33 containing each plate (as test, right panel) respectively. The double dishes sets were incubated at 30°C for 10 d (for *C. gloeosporioides*) and 3 d (in case of *L. theobromae*).

4.3. Evaluation on indole acetic acid production activites

IAA production was determined from samples of 239 endophytic fungi isolates. IAA of concentration, 0.1 mg/ml (2.85 mM of IAA) to 0.003125 mg/ml (0.043 mM of IAA) was used to calculate standard curve (Appendix E (Figure S1 and S2)). The example of colorimetric assay using Salkowski reagent (Isolates: 140-157) (Figure 4.5). Total of 239 endophytic fungi isolates revealed that 54 isolates could produce IAA ranging from 3.64 mg/g DCW – 96.22 mg/g DCW. Only 8 endophytic yeasts produced IAA ranging from 16.13 mg/g DCW – 53.64 mg/g DCW and CY.OS 13 showed the highest IAA production which were shown in Appendix E (Table S6). However, 46 endophytic filamentous fungi isolates produced IAA ranging from 3.64 mg/g DCW – 96.22 mg/g DCW. Endophytic filamentous fungi isolate CY.OS 22 showed the highest producer for IAA as 96.22 mg/g DCW as shown in Appendix E (Table S7).

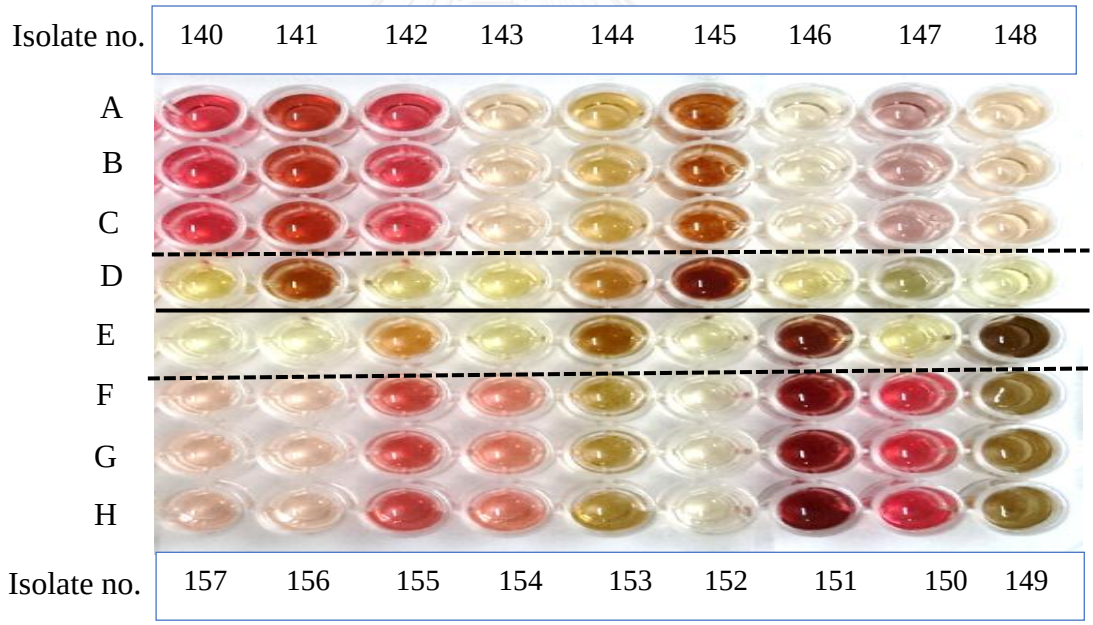


Figure 4.5 Example of colorimetric assay using Salkowski reagent of 18 endophytic filamentous fungi isolates. Four wells of each column A-D and E-H represented the IAA assay for each isolate. Row A-C or F-H were triplicate experiments of each isolate. Row D or E was supernatant only as control of supernatant color.

Results from Figure 4.5 showed that isolate no. 140, 142, and 150 displayed IAA production activity while isolate no. 141, 144, 145, 149, 151, 153, and 155 could produce color pigment and these were considered as no IAA producing activity as well as those of endophytes no. 143, 146, 148, 152, 156, and 157.

4.4. Endophytic fungi identification

4.4.1. Morphological identification of endophytic fungi

Endophytic fungi that showed high antifungal and/or indole acetic acid production activities were chosen for identification. Morphologies of colonies (color and mycelia) and spores (conidia, blastospores, sporangiospores) were examined and determined by slide culture technique (Figure 4.6). Results of slides culture of endophytic filamentous fungi showed mostly hyphae but rare in conidia or spore which made it difficult to identify by morphology. Therefore, molecular identification was paid more attention.

4.4.2. Molecular identification of endophytic fungi

The endophytic fungi isolates were grouped according to their PCR-RFLP profiles (Figure 4.7 and 4.8). Then, the PCR- amplified ITS region of representatives of each group were subjected to DNA sequencing. A representative from each group was shown on Table 4.3, and ITS sequence output was shown in Appendix F.

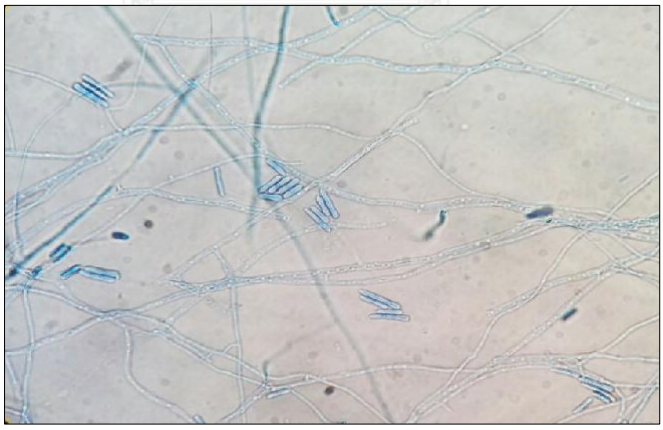
(1) CY.OS 16

Endophytic filamentous fungus CY.OS 16 had white colony, white mycelium and produced conidiophores, elongate, conidia hyaline, l-celled, and ovoid or oblong.

A) Macroscopic morplology

Top view (10 Days) Bottom view

B) Microscopic morphology



Morphological structure of endophytic filamentous fungus **CY.OS 16**, 40X (7 Days)

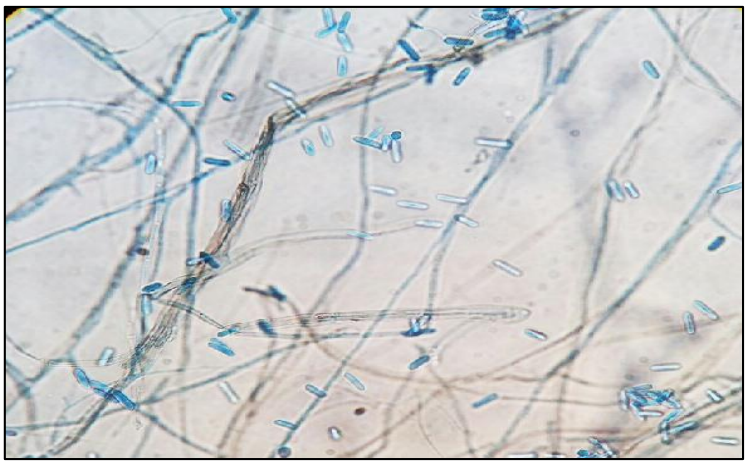
(2) CY.OS 22

Endophytic filamentous fungus CY.OS 22 had white colony, white mycelium and produced conidiophores, elongate, conidia hyaline, l-celled, and ovoid or oblong.

A) Macroscopic morplology

Top view (10 Days) Bottom view

B) Microscopic morphology



Morphological structure of endophytic filamentous fungus CY.OS 22, 40X (5 Days)

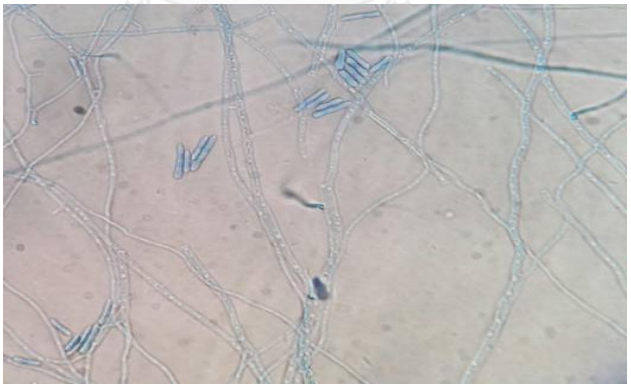
(3) CY.OS 33

Endophytic filamentous fungus CY.OS 33 had white colony, white mycelium and produced conidiophores, elongate, conidia hyaline, l-celled, and ovoid or oblong.

A) **Macroscopic morplology**

Top view (10 Days) Bottom view

B) **Microscopic morphology**



Morphological structure of endophytic filamentous fungus CY.OS 33, 40X (7 Days)

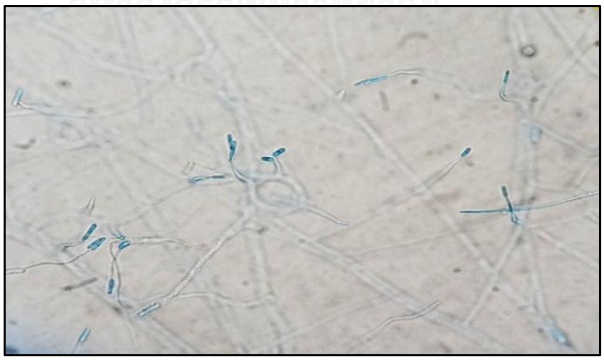
(4) CY.OS 64

Endophytic filamentous fungus CY.OS 64 had white colony, white mycelium and produced conidiophores, elongate, conidia hyaline, l-celled, and ovoid or oblong.

A) Macroscopic morplology

Top view (10 Days) Bottom view

B) Microscopic morphology



Morphological structure of endophytic filamentous fungus CY.OS 64, 40X (7 Days)

(5) CY.OS 69

Endophytic filamentous fungus CY.OS 69 had gray colony, and was slown growth, mycelium and conidia could not be observed from slide culture presentation under microscope.

A) **Macroscopic morplology**

Top view (28 Days) Bottom view

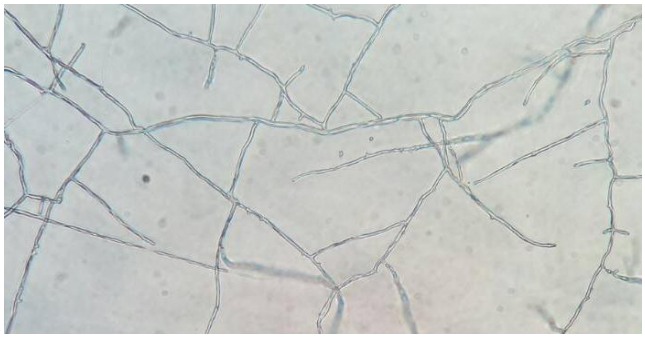
(6) CY. OS 75

Endophytic filamentous fungus CY.OS 75 had white colony, white mycelium on PDA plate. Only mycelium but not spore or conidia were observed from slide culture preparation under microscope.

A) **Macroscopic morplology**

Top view (5 Days) Bottom view

B) Microscopic morphology



Morphological structure of endophytic filamentous fungus CY. OS 75, 40X (5 Days)

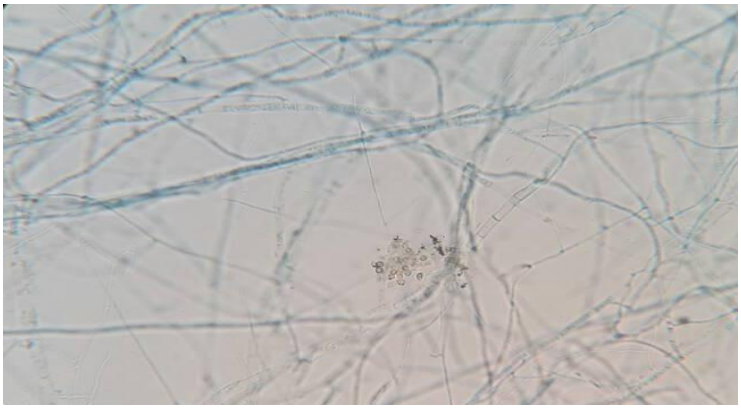
(7) CY.OS 150

Endophytic filamentous fungus CY.OS 150 had white colony, white mycelium on PDA plate. Only mycelium but not spore or conidia were observed from slide culture preparation under microscope.

A) **Macroscopic morplology**

Top view (3 Days) Bottom view

B) Microscopic morphology

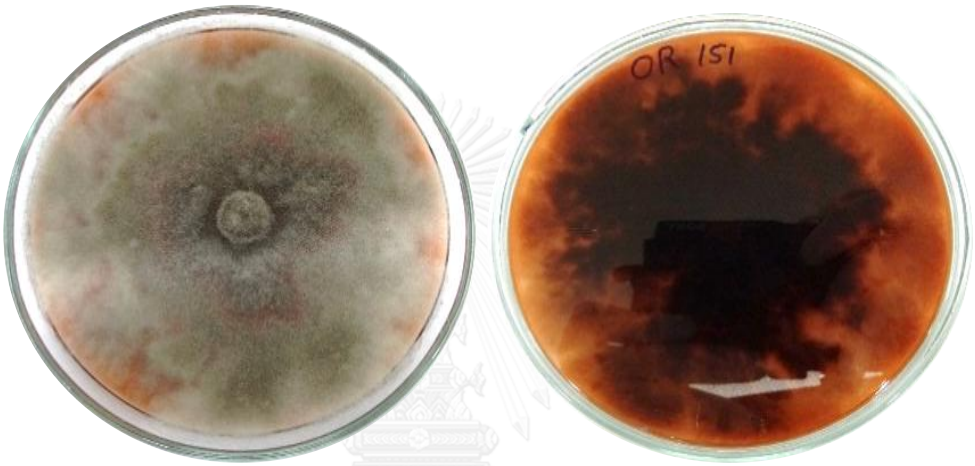


Morphological structure of endophytic filamentous fungus CY.OS 150, 40X (3 Days)

(8) CY.OS 151

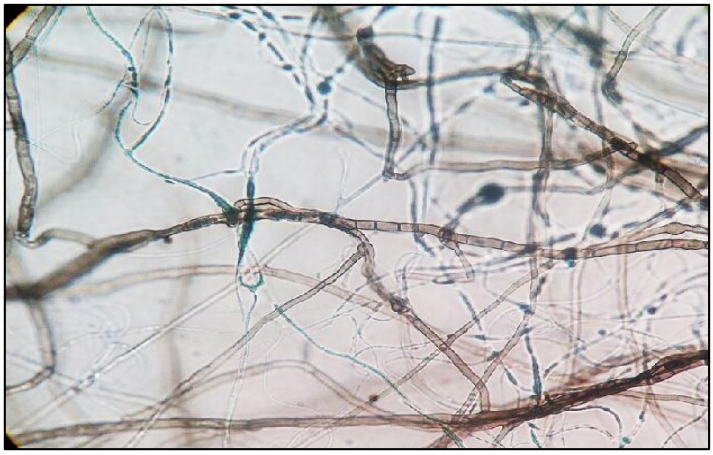
Endophytic filamentous fungus CY.OS 151 had gray/black colony, gray mycelium on PDA plate. Only mycelium but not spore or conidia were observed from slide culture preparation under microscope.

A) **Macroscopic morplology**



Top view (5 Days) Bottom view

B) **Microscopic morphology**



Morphological structure of endophytic filamentous fungus CY.OS 151, 40X (5 Days)

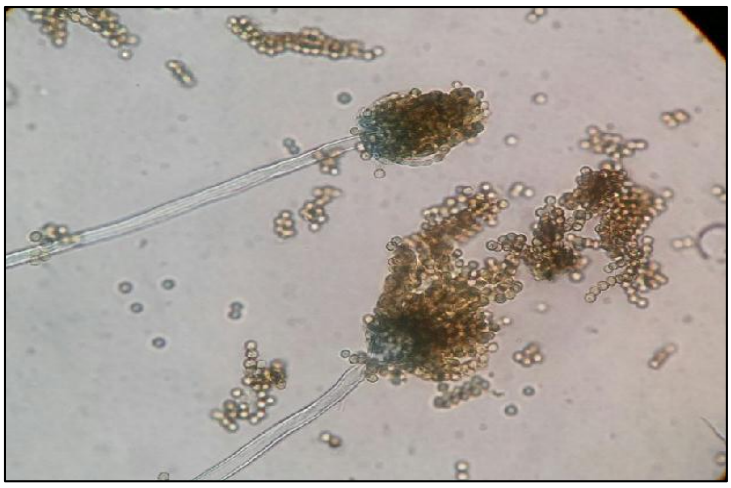
(9) CY.OS 153

Endophytic filamentous fungus CY.OS 153 had green colonies. Under microscope, a brush – like conidiosphore could be observed from slide culture preparation.

A) Macroscopic morphology

Top view (4 Days) Bottom view

Microscopic morphology

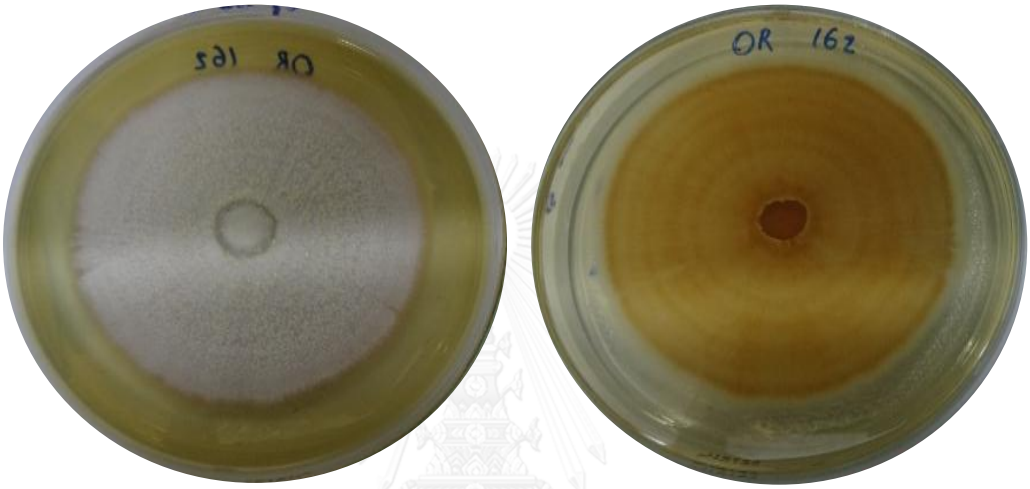


Morphological structure of endophytic filamentous fungus CY.OS 153, 40X (5 Days)

(10) CY.OS 162

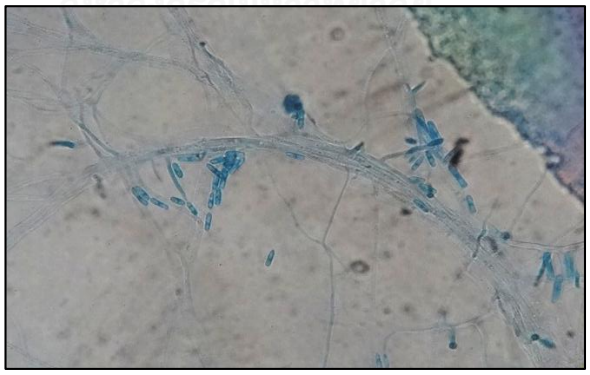
Endophytic filamentous fungus CY.OS 162 had white colony, white mycelium and produced conidiophores, elongate, conidia hyaline, l-celled, and ovoid or oblong.

A) Macroscopic morphology



Top view (7 Days) Bottom view

B) Microscopic morphology



Morphological structure of endophytic filamentous fungus CY.OS 162, 40X (5 Days)

(11) CY.OS 185

Endophytic filamentous fungus CY.OS 185 had green/black colony, and showed slow growth characteristic. Mycelium and conidia from slide culture preparation could not be found under microscope.

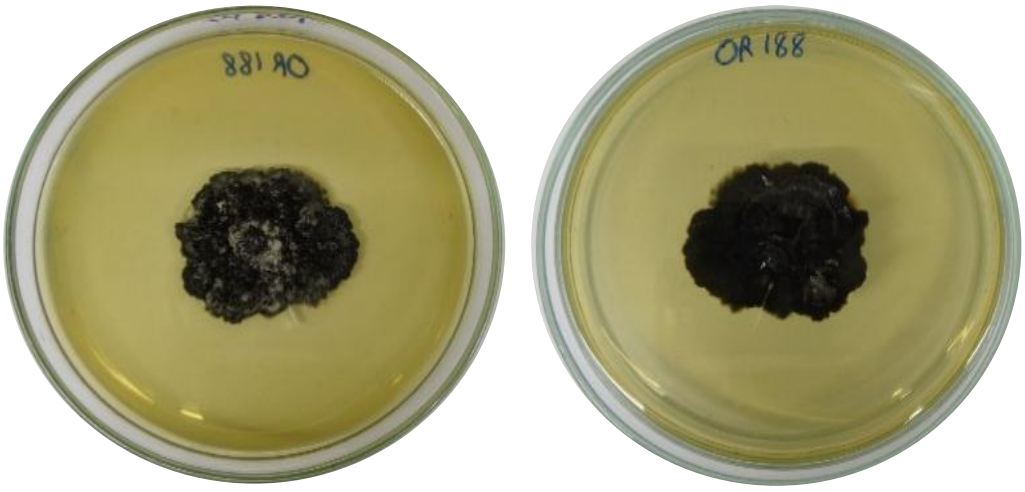
A) Macroscopic morphology

Top view (24 Days) Bottom view

(12) CY.OS 188

Endophytic filamentous fungus CY.OS 188 had black colony, and was slow growth. Mycelium and conidia from slide culture preparation could not be detected under microscope.

A) **Macroscopic morphology**



Top view (24 Days) Bottom view

(13) CY.OS 197

Endophytic filamentous fungus CY.OS 197 had white colony, white mycelium. Only mycelium but not spore or conidia were detected under microscope.

A) Macroscopic morphology

Top view (4 Days) Bottom view

B) **Microscopic morphology**

Morphological structure of endophytic filamentous fungus CY.OS 197, 40X (3 Days)

(14) CY.OS 209

Endophytic filamentous fungus CY.OS 209 had white colony, white mycelium and produced conidiophores, elongate, conidia hyaline, l-celled, and ovoid or oblong.

A) Macroscopic morphology

Top view (12 Days) Bottom view

B) **Microscopic morphology**

Morphological structure of endophytic filamentous fungus CY.OS 209, 40X (7 Days)

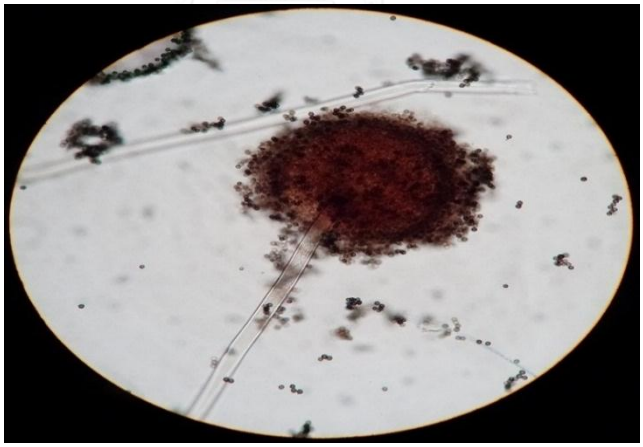
(15) CY.OS 211

Endophytic filamentous fungus CY.OS 211 had black colonies and produced a brush – like conidiosphore as observed under microscope of the slide culture preparation.

A) **Macroscopic morphology**

Top view (3 Days) Bottom view

B) **Microscopic morphology**



Morphological structure of endophytic filamentous fungus **CY.OS 211,** 40X (3 Days)

Figure 4.6 Examples of morphology identification of endophytic filamentous fungi isolates by macroscopic and microscopic examination. Endophytic filamentous fungi isolates CY.OS 16, 22, 33, 64, 69, 75, 150, 151, 153, 162, 185, 188, 197, 209, and CY.OS 211 were cultivated on PDA plate at 30ºC until indicated time. Top view (left) and Bottom view (right) were shown (A). The slide cultures of the isolates stained with methylene blue were examined using 40X magnification under microscope (B).

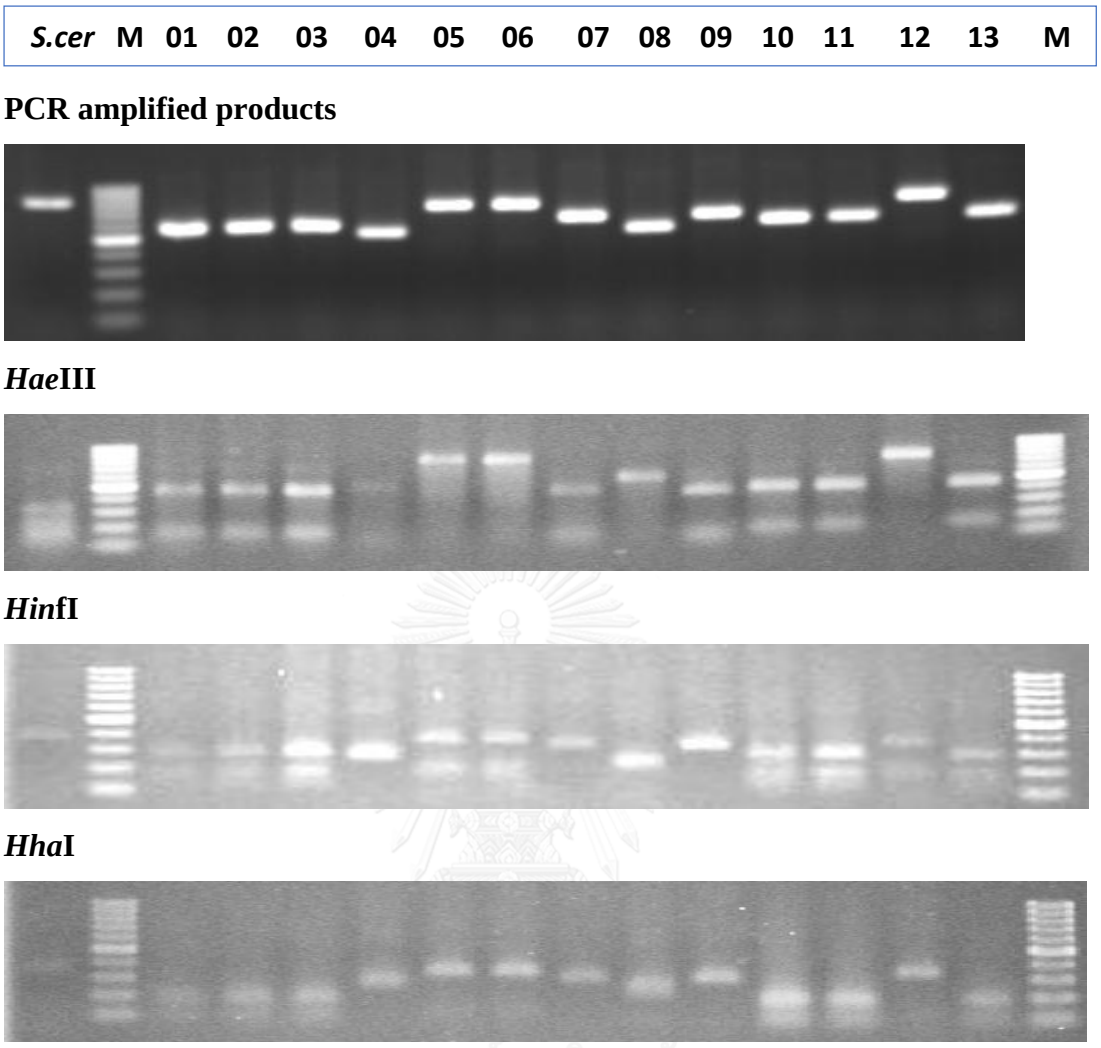
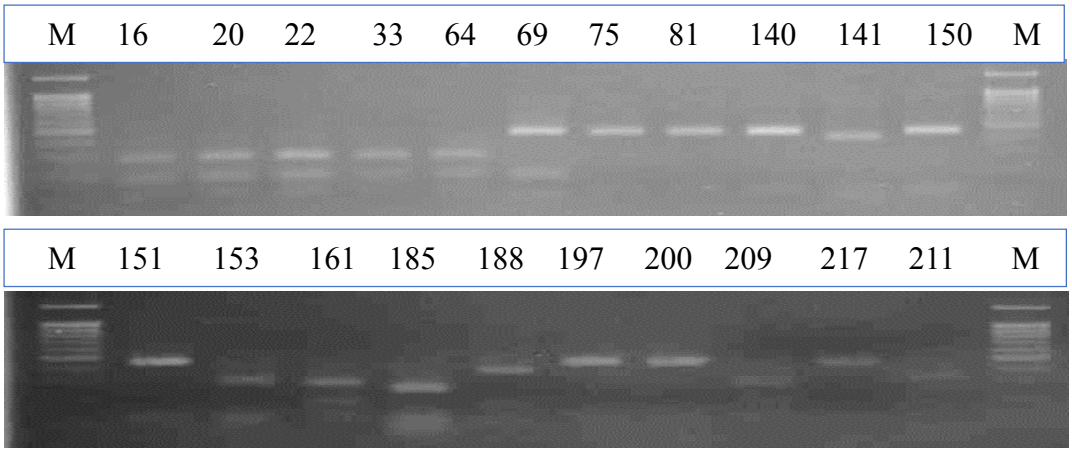
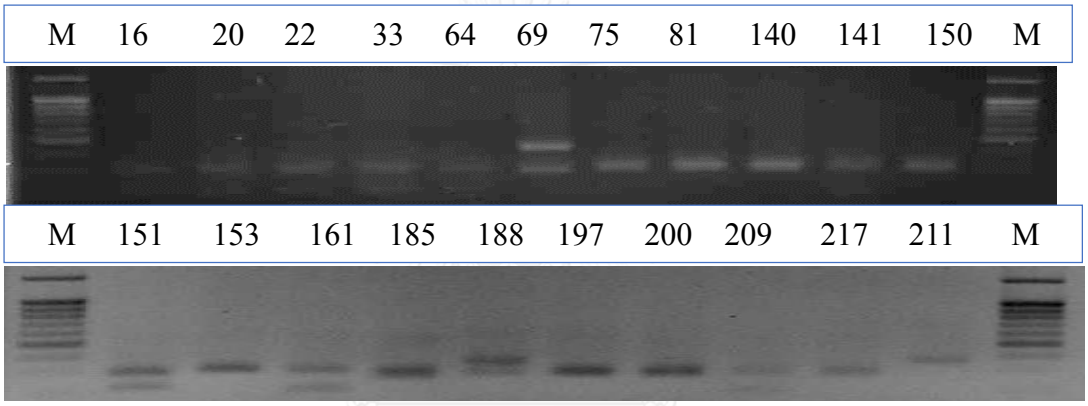


Figure 4.7 PCR- RFLP profiles of endophytic yeasts 13 isolates (CY.OS 01 – CY.OS 13). The PCR amplified fragment was individually cut with restriction enzymes *Hae*III, *Hin*fI, and *Hha*I. The reaction was incubated at 37ºC for 1 h. The restricted DNA fragments were separated on 2% agar gel by electrophoreses. *S. cer*: *Saccharomyces cerevisiae* and M: 100 bp. DNA ladder.

***Hae*III**



***Hin*fI**



***Hha*I**

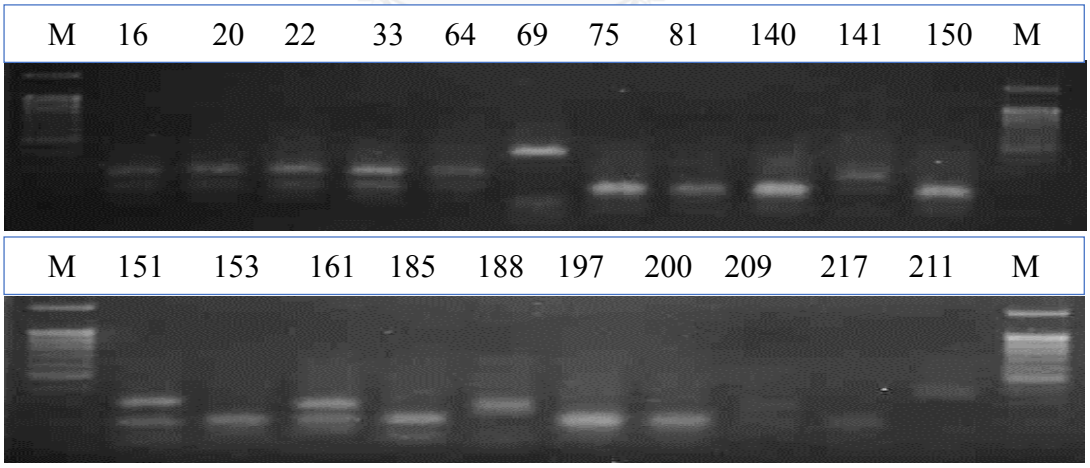


Figure 4.8 PCR- RFLP profiles of endophytic filamentous fungi 21 isolates The PCR amplified fragment was individually cut with restriction enzymes *Hae*III, *Hin*fI, and *Hha*I. The reaction was incubated at 37ºC for 1 h. The restricted DNA fragments were separated on 2% agar gel by electrophoreses. M: 100 bp. DNA ladder.

Table 4.3 Grouping of endophytic fungi isolates by PCR-RFLP and their activities

Group	Representative	Isolates	Activities
1	CY.OS 03	CY.OS 01, 02, 03, 10, 11, 13	IAA production and Antifungal
2	CY.OS 04	CY.OS 04	IAA production
3	CY.OS 05	CY.OS 05, 06, 12	Antifungal
4	CY.OS 07	CY.OS 07, 09	Antifungal
5	CY.OS 08	CY.OS 08	IAA production
6	CY.OS 20	CY.OS 16, 17, 20,161, 140	IAA production
7	CY.OS 22	CY.OS 21, 22, 25, 98, 110, 111, 122,	IAA production
8	CY.OS 33	CY.OS 33, 113, 114, 117	IAA production and Antifungal
9	CY.OS 64	CY.OS 64, 155	IAA production
10	CY.OS 69	CY.OS 69	IAA production
11	CY.OS 75	CY.OS 75, 81, 110, 133, 134, 141, 160, 212	IAA production
12	CY.OS 150	CY.OS 112, 132, 150	Antifungal
13	CY.OS 151	CY.OS 151	Antifungal
14	CY.OS 153	CY.OS 153	Antifungal
15	CY.OS 162	CY.OS 162, 163,164	Antifungal

Table 4.3 Grouping of endophytic fungi isolates by PCR-RFLP and their activities (cont.)

Group	Representative	Isolates	Activities
16	CY.OS 185	CY.OS 145, 185	IAA production
17	CY.OS 188	CY.OS 121, 154, 188, 190	IAA production
18	CY.OS 197	CY.OS 197, 200, 217	IAA production
19	CY.OS 209	CY.OS 209, 225, 228, 231	IAA production
20	CY.OS 211	CY.OS 102,106, 135, 144, 149, 172, 203, 211, 213, 214, 223	Antifungal

Results showed that five species of yeast were identified by molecular technique, as *Aureobasidium* sp. (CY.OS 01, 02, 03, 10, 11 and CY.OS 13), *Candida* sp. (CY.OS 07, 09), *Cryptococcus laurentii* (CY.OS 08), *Hanseniaspora* sp. (CY.OS 05, 06, 12), and *Rhodotorula* sp. (CY.OS 04), respectively (Table 4.4). For endophytic filamentous fungi were grouped into 6 groups which were identified as *Colletotrichum* sp. (group: 6, 7, 8, 9, 15, and 19), *Lasiodiplodia* sp. (group: 11, 12, 13 and 18), *Aspergillus* sp. (group 14 and 20), *Rhytidhysteron* sp. CY.OS 69, and *Penicillium* sp. groups 16, and *Phyllosticta* sp. groups 17, respectively. The details were shown in Table 4.5.

Table 4.4 Molecular identification of endophytic yeasts

Group	Representative isolate	Yeast species	% Identity
1	CY.OS 03	*Aureobasidium* sp.	97
2	CY.OS 04	*Rhodotorula* sp.	98
3	CY.OS 05	*Hanseniaspora* sp.	99
4	CY.OS 07	*Candida* sp.	99
5	CY.OS 08	*Cryptococcus laurentii*	96

Table 4.5 Molecular identification of endophytic filamentous fungi

Group	Representative isolate	Yeast species	% Identity
1	CY.OS 20	*Colletotrichum* sp.	99
2	CY.OS 22	*Colletotrichum* sp.	99
3	CY.OS 33	*Colletotrichum* sp.	100
4	CY.OS 64	*Colletotrichum* sp.	99
5	CY.OS 69	*Rhytidhysteron* sp.	99
6	CY.OS 75	*Lasiodiplodia* sp.	99
7	CY.OS 150	*Lasiodiplodia* sp.	99
8	CY.OS 151	*Lasiodiplodia* sp.	100
9	CY.OS 153	*Aspergillus* sp.	99
10	CY.OS 162	*Colletotrichum* sp.	99
11	CY.OS 185	*Penicillium* sp.	99
12	CY.OS 188	*Phyllosticta* sp.	99
13	CY.OS 197	*Lasiodiplodia* sp.	99
14	CY.OS 209	*Colletotrichum* sp.	99
15	CY.OS 211	*Aspergillus* sp.	99

CHAPTER V
CONCLUSIONS AND DISCUSSION

5.1. Isolation and identification of endophytic fungi from mango trees

Total of 239 endophytic fungi isolated from 6 mango plants of 3 varieties, 2 Okrong (OR1 and OR2), 2 Talapnak (TN1 and TN2), and 2 Khiaosawoey (KW1 and KW2). Different parts of healthy organically grown mango trees: leaves, twigs, roots, and fruits were collected for isolation of the endophytes. Thirteen endophytic yeasts could only be isolated from ripen fruit season, and 226 endophytic filamentous fungi were isolated from raw fruit season (79 isolates) and from ripen fruit season (147 isolates). The number of isolates from PDA medium and ripen fruit season were higher than that from YM medium, and the isolation in raw fruit season. The number of endophytic yeasts isolated from ripen fruit season was much lower than those of endophytic filamentous fungi isolates. The Talapnak variety of mango trees showed the highest number of isolates than the other varieties studied. The results suggested that endophytic fungi are able to grow and spread depending on environmental factors including humidity, pH, nutrients, location, and host. In the ripen fruit season, mango fruits are rich of nutrients as increasing of sugar content which might be a factor to promote growth of endophytic fungi. For isolation media (YM and PDA), PDA seems to be more complex in composition which can supplement growth of fungi, while in YM medium could not support growth of some fungi. Compant et al. (2010) suggested that the stability or variability of interaction between plants and endophytic fungi depends on several factors such as environmental stress, endophytes ability, hosts senescence, and the host defense response (Compant et al. 2010).

The endophytic fungi isolates with their outstanding activities were characterized and identified according to their characteristics on morphologies of colony and conidia or spore along with molecular techniques identification. Thirteen endophytic yeast isolates could be divided into 5 groups and identified as *Aureobasidium* sp., *Candida* sp., *Cryptococcus laurentii, Hanseniaspora* sp., and *Rhodotorula* sp. For endophytic filamentous fungi isolates, 20 representatives from each group were collected for identification from 72 isolates. Their molecular

identification was high % identity to *Aspergillus* sp., *Colletotrichum* sp., *Lasiodiplodia* sp., *Penicillium* sp., *Phyllosticta* sp., and *Rhytidhysteron* sp. Most taxa were common endophytic fungi that have been previously reported (Chareprasert et al. (2006); Gond et al. (2007), and Huang et al. (2007)). Many previous reports showed that endophytic fungi could be isolated from various host plants (Table 2.1 and 2.2 of Chapter II). Most endophytic fungi isolates were non-sporulating fungi which were classified into mycelia sterilia (not produce sexual spores or asexual spores). Amirita et al. (2012) reported that 48% of endophytic fungi isolated from four medicinal plants were in sterile form when compared to 25% Hypomycete, 14% Coelomycetes, and 13% Xylariales. This study was in agreement with those previous reports that most of the isolated endophytic fungi did not produce conidia or spores (Saikkonen et al. 1998, Huang et al. 2007, Lin et al. 2007, Gong and Gou 2009) when cultured on common mycological media (Wiyakrutta et al. 2004).

5.2. Antifungal phytopathogens activity assay

5.2.1. Evaluation of the *in vitro* antagonism between endophytic fungi and phytopathogenic fungi

In vitro antagonism between endophytic fungi and *C. gloeosporioides* and *L. theobromae* was determined by dual culture method. Results showed that endophytic yeast *Candida* sp. CY.OS 09, and *Aureobasidium* sp. CY.OS 13 showed the highest growth inhibition activity against *C. gloeosporioides* at 15.78% and 12.63%, respectively. However, none of the endophytic yeast isolates could inhibit *L. theobromae* in this report. Endophytic filamentous fungi composed of 226 isolates showed growth inhibition against *C. gloeosporioides* ranging from 0.74% - 81.48%. The *Colletotrichum* sp. CY.OS 162, *Lasiodiplodia* sp. CY.OS 132, and *Colletotrichum* sp CY.OS 160 showed the highest observed activity at 81.84%, 79.63%, and 77.41%, respectively as comparing to other isolates. There were 162 filamentous fungi isolates that showed antagonism, while *Lasiodiplodia* sp. CY.OS 132 showed the strongest growth inhibition at 50.37%. From the results, it could be concluded that most of endophytic fungi isolates possess none to mild antagonistic activities against

phytopathogens such as *C. gloeosporioides* and *L. theobromae*. *Colletotrichum* sp., and *Lasiodiplodia* sp. exhibited anti-phytopathogens activity higher than the other isolates.

According to Motaal et al. (2010) whose reported that *Aspergillus fumigatus* and *Penicilium citrinum* isolated from *Hyoscyamus muticus* L. showed antagonistic activities against plant pathogenic fungi *Gibberella zeae* and *Thanatephorus cucumeri*. Especially, *P. citrinum* showed more than 50% reduction in growth of *N. dimidiatum*. Besides, *Colletotrichum* sp., *C. gloeosporioides, and L. theobromae* isolated from *Theobroma cacao* tissues showed *in vitro* antagonism against *Moniliophthora roreri* (causing agent of frosty pod rot), *Phytophthora palmivora* (corresponding to black pod rot) and *Moniliophthora perniciosa* (corresponding to witches broom) (Vieira et al. 2012).

In addition, the antagonistic activity of three endophytic fungi such as *Robillarda sessilis, Phomopsis mangiferae* and *P. guepinii* isolated from *M. indica* against plant pathogenic fungi, *Gloeosporium mangiferae, Fusarium moniliforme* and *Lasiodiplodia theobromae* which cause leaf spot diseases in mango, from the results showed that *R. sessilis* showed growth inhibition against three plant pathogens at 40%, 53% and 65% respectively, *P. mangiferae* showed 64%, 48% and 66% respectively, and *P. guepinii* showed growth inhibition 51%, 63% and 68% respectively Chaudhary (2012)

5.2.2. Antifungal volatile compounds of endophytic fungi

Antifungal volatile compounds against *C. gloeosporioides* and *L. theobromae* of endophytic fungi isolates was assayed using double dishes method. Endophytic yeasts *Cryptococcus laurentii* CY. OS 08, and *Aureobasidium* sp. CY. OS 10 showed 40%, and 46% growth inhibition against *C. gloeosporioides*, respectively. None of endophytic yeasts exhibited antifungal activity against *L. theobromae*. From 226 endophytic filamentous fungi isolates, only 88 isolates showed antagonistic activity against *C. gloeosporioides* ranging from 1.11% - 14.81% growth inhibition. *Colletotrichum* sp. CY.OS 161 showed the strongest activity on anti-fungal volatile compound isolates. Moreover, there were 50 isolates that had activity of anti-fungal volatile compound against *L. theobromae* ranging from 1.85% - 34.44% growth inhibition. The isolate *Colletotrichum* sp. CY.OS 33 showed the best activity among other isolates. It could be concluded that all of isolated endophytic fungi from this study

possesed low activities on production of antifungal volatile compounds against *C. gloeosporioides* and *L. theobromae.* Previous reports stated on volatile compounds against postharvest decay fungi such as *Aspergillus niger, Alternaria alternata, C. gloeosporioides, L. theobromae, Phomopsis viticola* and *Rhizopus stolonifer* were tested by clove and cinnamon oil extracted from *Syzygium aromaticum* (Linn.) Merr & Perry (dried bud) and *Cinnamonum zeylanicum* (dried bark) by inverted petriplate method. The results showed that Cinnamon oil at 10 µl showed 100% antifungal activities against all the tested fungi except *R. stolonifer*. Clove oil also showed complete inhibition against all of tested fungi. However it had relatively low inhibitory effect on *C. gloeosporioides* and *L. theobromae* whose inhibition indices were 59.12 and 74.07 %, respectively (Sukatta et al. 2008).

Furthermore, endophytic *Fusarium oxysporum* CanR-46 isolated from *Brassica napus* had been reported to exhibit 91% inhibition against growth of *Botrytis cinerea* (gray rot pathogen) on PDA (Zhang et al. 2014). However, there was no report on antifungal volatile compounds production from endophytic fungi isolated from mango against *C. gloeosporioides* and *L. theobromae*.

5.3. Evaluation on indole acetic acid production activities

Total of 239 endophytic fungi were tested for IAA production. However, only 54 isolates could produce IAA ranging from 3.64 mg/g DCW – 96.22 mg/g DCW. For endophytic yeast, *Aureobasidium* sp. CY.OS 13, and endophytic filamentous fungi *Colletotrichum* sp. CY.OS 22 showed the highest IAA production at 53.64 mg/g DCW (0.35 mg/ml) and 96.22 mg/g DCW (0.42 mg/ml), respectively. There were several reports on IAA production by endophytic fungi as follows: *Williopsis saturnus* isolated from maize roots produced IAA up to 22.51 µg/ml and it helped increase shoots and roots length (Nassar et al. 2005). Endophytic yeasts *Rhodotorula graminis* and *Rhodotorula mucilaginosa* isolated from poplar plants also produced IAA at 20 mg/g and 40 mg/g dry cells (Xin et al. 2009). Moreover, *Rhodotorula mucilaginosa* from poplar plants showed strong growth promoting activity on crop plants *e.g.* bell pepper with a 60% increase in root and shoot mass, earlier flowering and fruit set which significantly increased fruit yields (Khan et al. 2012). Nutaratat et al. (2014) reported that *Rhodotorula paludigenum* could produce IAA, yielding at 29.3 mg/g DCW. *Phoma*

glomerata and *Penicillium* sp. isolated from roots of field-grown cucumber plants could produce IAA at 3.89 μg/mL and 29.8 μg/mL, respectively (Waqas et al. 2012). The findings in this study were in consistent with the previous reports that *Rhodotorula* sp, and *Penicillium* sp. were able to produce IAA. However, in this study *Rhodotorula* sp. CY.OS 04 and *Penicillium* sp. CY.OS 185 could produce IAA at 16.13 mg/g DCW (0.133 mg/ml) and 11.54 mg/g DCW (0.103 mg/ml), respectively. IAA producted by endophytic fungi in this study were higher than those in previous reports, especially by endophytic filamentous fungi isolates. In this study, endophytic yeast *Aureobasidium* sp. CY.OS 13 could produce IAA at 53.64 mg/g DCW when cultivating for 7 days while the endophytic filamentous fungus *Colletotrichum* sp. CY.OS 22 could produce IAA at 96.22 mg/g DCW when cultivating for 30 days. Therefore, considering on the basis of cultivation time, endophytic yeast *Aureobasidium* sp. CY.OS 13 should be the highest IAA producer of this study.

Finally, although the isolated endophytic fungi showed rather weak activities against phytopathogens, *C. gloeosporioides* and/or *L. theobromae*. This will limit the use of them in biological control and post-harvesting in agricultural field. However, most endophytic fungi isolates have potential on IAA production, which play an important role in plant growth promotion. Further study will focus on plant growth promoting activities of endophytic fungi candidates for IAA production. Furthermore, siderophore production, phosphate solubilization, and other plant growth promoter such as gibberellins or cytokinin will be screened and *in vivo* assays will be performed with the aim of application in agriculture field. In addition, study on extracellular enzymes production by these endophytic fungi isolates will also be an interesting aspect.

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APPENDIX

APPENDIX A
Media preparation

(1) Potato Dextrose Agar/Broth (PDA or PDB)

Potato	200 g
Dextrose or D-Glucose	20 g
Agar	15 g
Distilled water	1000 ml

Potato infusion can be made by boiling sliced (washed and peeled) potatoes in 1 liter distilled water for 30 minutes and then decanting or straining the broth though cheesecloth. Distilled water is added such that the total volume of the suspension is 1 liter. Mixed all of components and the medium is sterilized by autoclaving at 15 min, 121ºC. For PDB, agar is not added.

(2) Yeast Malt (YM)

Yeast extract	3 g
Malt extract	3 g
Peptone	5 g
Dextrose or D-Glucose	10 g
Agar	15 g
Distilled water	1000 ml

Dissolved in distilled water and autoclaved at 121°C, 15 psi for 15 min.

(3) Yeast extract Peptone Dextrose (YPD)

Yeast extract	10 g
Peptone	20 g
Dextrose or Glucose	20 g
Agar	15 g
Distilled water	1000 ml

Dissolve all components in distilled water and autoclaved at 121°C, 15 psi for 15 min. For broth, it does not contain agar.

APPENDIX B

Reagent and Chemical preparation

(1) Salkowski reagent

35% $HClO_4$

0.5 M $FeCl_3$

The freshly prepare before using with 50 ml of 35% $HClO_4$ and 1 ml of 0.5 M $FeCl_3$.

(2) 100 mM Indole acetic acid (IAA) Stock

IAA	0.01 g or 10 mg
Ethanol	500 µl
Distilled water	500 µl

IAA was dissolved in ethanol 500 µl, then distilled water 500 µl was added, mixed and stored at -20ºC.

(3) L-Tryptophan

L-Tryptophan	1 g
Distilled water	100 ml

Dissolved in distilled water and autoclaved at 121ºC, 15 psi for 15 min. For working solution, 100 ml Tryptophan solution per 1000 ml medium.

(4) Antibiotics (Ampicillin and Kanamycin**)**

Antibiotic	1000 mg
Distilled water	10 ml

For 100 µg/ml final concentration, the antibiotic was dissolved in distilled water to 100 mg/ml then was mixed by vortexing, filtered through sterile Millipore membranes (pore size 0.22 µm) and collected in sterile tubes stored at -20ºC for preservation. For using, 1 ml antibiotic solution is added to 1000 ml medium.

(5) 50x TAE buffer

Trisma base	242 g
Acetic acid	57.1 ml

Dissolved Trisma base and acetic acid in 0.5 M pH 8.0 100 ml and adjust to 1000 ml by ddH2O. Autoclaved at 121°C, 15 psi for 15 min. Dilute to 1x for use in electrophoresis.

(6) Ethanol 70%

Ethanol 99%	707 ml
ddH_2O	293 ml

(7) 2% Agar gel

1X TAE buffer	20 ml
Agarose gel	0.4 g

(8) Ethidium bromide solution

Dissolve ethidium bromide in 1X TAE buffer at final concentration of 10 µg/ml and store in dark container where electro phoresed agar gel is stained.

(9) Lysis buffer for CTAB

Mixed 2% CTAB, 100 mM TRIS-HCl pH 8, 1.4 M NaCl, 25 mM EDTA pH 8

(10) Lysis buffer for using glass bead

2% Triton X-100, 1% SDS, 100 mM NaCl, 10 Tris pH 8, 1 mM EDTA

APPENDIX C

Endophytic fungi isolated from *Mangifera indica* L.

Table S1 Source of isolation of endophytic yeasts isolated from ripen fruit season.

Isolates No.	Source of isolation		
	Mango variety	Part	Isolation medium
CY.OS 01	OR1	Fruit	PDA
CY.OS 02	OR1	Fruit	PDA
CY.OS 03	TN2	Twig	PDA
CY.OS 04	TN2	Fruit	PDA
CY.OS 05	TN1	Fruit	YM
CY.OS 06	TN1	Fruit	YM
CY.OS 07	KW2	Root	YM
CY.OS 08	KW2	Root	YM
CY.OS 09	KW2	Root	YM
CY.OS 10	OR1	Fruit	PDA
CY.OS 11	OR1	Fruit	YM
CY.OS 12	TN1	Fruit	YM
CY.OS 13	TN2	Fruit	PDA

YM= Yeast extract Malt extract medium, PDA= Potato Dextrose Agar

OR1= Okrong 1, OR2= Okrong 2, TN1= Talapnak 1, TN2= Talapnak 2

KW1= Khiaosawoey 1, KW2= Khiaosawoey 2

Table S2 Source of isolation of endophytic filamentous fungi isolated from raw fruit season.

Isolates No.	Source of isolation		
	Mango variety	Part	Isolation medium
CY.OS 14	OR1	Twig	YM
CY.OS 15	OR1	Twig	YM
CY.OS 16	OR1	Twig	PDA
CY.OS 17	OR1	Root	PDA
CY.OS 18	OR1	Twig	PDA
CY.OS 19	OR2	Leaf	YM
CY.OS 20	OR1	Twig	PDA
CY.OS 21	OR1	Twig	PDA
CY.OS 22	OR1	Twig	YM
CY.OS 23	OR1	Twig	YM
CY.OS 24	OR1	Twig	YM
CY.OS 25	OR1	Twig	PDA
CY.OS 26	OR2	Twig	YM
CY.OS 27	OR2	Twig	YM
CY.OS 28	OR2	Twig	PDA
CY.OS 29	OR2	Twig	YM
CY.OS 30	OR2	Twig	YM
CY.OS 31	OR2	Twig	PDA
CY.OS 32	OR2	Twig	YM
CY.OS 33	OR2	Twig	YM
CY.OS 34	OR2	Root	YM
CY.OS 35	OR2	Twig	YM
CY.OS 36	OR2	Twig	YM
CY.OS 37	OR2	Root	YM

YM= Yeast extract Malt extract medium, PDA= Potato Dextrose Agar

OR1= Okrong 1, OR2= Okrong 2, TN1= Talapnak 1, TN2= Talapnak 2

KW1= Khiaosawoey 1, KW2= Khiaosawoey 2

Table S2 Source of isolation of endophytic filamentous fungi isolated from raw fruit season (cont.).

Isolates No.	Source of isolation		
	Mango variety	Part	Isolation medium
CY.OS 38	OR2	Root	PDA
CY.OS 39	OR2	Twig	PDA
CY.OS 40	OR2	Twig	PDA
CY.OS 41	OR2	Twig	PDA
CY.OS 42	OR2	Twig	PDA
CY.OS 43	OR2	Twig	PDA
CY.OS 44	OR2	Twig	YM
CY.OS 45	OR2	Root	PDA
CY.OS 46	OR2	Twig	PDA
CY.OS 47	OR2	Twig	PDA
CY.OS 48	OR2	Root	PDA
CY.OS 49	OR2	Twig	PDA
CY.OS 50	OR2	Twig	PDA
CY.OS 51	OR2	Leaf	PDA
CY.OS 52	OR2	Leaf	PDA
CY.OS 53	OR2	Twig	PDA
CY.OS 54	KW1	Twig	YM
CY.OS 55	TN1	Twig	YM
CY.OS 56	KW1	Twig	PDA
CY.OS 57	KW1	Twig	PDA
CY.OS 58	KW2	Twig	PDA
CY.OS 59	KW2	Twig	PDA
CY.OS 60	KW2	Twig	YM
CY.OS 61	KW2	Root	PDA

YM= Yeast extract Malt extract medium, PDA= Potato Dextrose Agar

OR1= Okrong 1, OR2= Okrong 2, TN1= Talapnak 1, TN2= Talapnak 2

KW1= Khiaosawoey 1, KW2= Khiaosawoey 2

Table S2 Source of isolation of endophytic filamentous fungi isolated from raw fruit season (cont.).

Isolates No.	Source of isolation		
	Mango variety	Part	Isolation medium
CY.OS 62	TN2	Root	YM
CY.OS 63	OR1	Leaf	PDA
CY.OS 64	OR2	Twig	PDA
CY.OS 65	TN1	Twig	PDA
CY.OS 66	TN1	Twig	PDA
CY.OS 67	OR2	Twig	YM
CY.OS 68	KW1	Root	PDA
CY.OS 69	KW1	Twig	PDA
CY.OS 70	KW1	Twig	PDA
CY.OS 71	KW1	Twig	PDA
CY.OS 72	KW2	Twig	YM
CY.OS 73	KW2	Twig	PDA
CY.OS 74	KW2	Twig	YM
CY.OS 75	TN2	Twig	YM
CY.OS 76	TN2	Root	YM
CY.OS 77	TN2	Root	YM
CY.OS 78	TN2	Root	YM
CY.OS 79	TN2	Root	YM
CY.OS 80	TN2	Twig	PDA
CY.OS 81	TN2	Twig	YM
CY.OS 82	TN1	Twig	PDA
CY.OS 83	TN1	Root	PDA
CY.OS 84	TN1	Twig	PDA
CY.OS 85	TN1	Leaf	PDA

YM= Yeast extract Malt extract medium, PDA= Potato Dextrose Agar

OR1= Okrong 1, OR2= Okrong 2, TN1= Talapnak 1, TN2= Talapnak 2

KW1= Khiaosawoey 1, KW2= Khiaosawoey 2

Table S2 Source of isolation of endophytic filamentous fungi isolated from raw fruit season (cont.).

Isolates No.	Source of isolation		
	Mango variety	**Part**	**Isolation medium**
CY.OS 86	TN1	Root	PDA
CY.OS 87	TN1	Leaf	PDA
CY.OS 88	TN1	Twig	PDA
CY.OS 89	TN1	Twig	PDA
CY.OS 90	TN1	Twig	PDA
CY.OS 91	TN1	Twig	PDA
CY.OS 92	TN1	Twig	PDA

YM= Yeast extract Malt extract medium, PDA= Potato Dextrose Agar

OR1= Okrong 1, OR2= Okrong 2, TN1= Talapnak 1, TN2= Talapnak 2

KW1= Khiaosawoey 1, KW2= Khiaosawoey 2

Table S3 Source of isolation of endophytic filamentous fungi isolated from ripen fruit season.

Isolates No.	Source of isolation		
	Mango variety	Part	Isolation medium
CY.OS 93	KW1	Twig	PDA
CY.OS 94	KW1	Twig	PDA
CY.OS 95	KW2	Fruit	PDA
CY.OS 96	KW2	Fruit	PDA
CY.OS 97	KW2	Twig	PDA
CY.OS 98	KW2	Twig	PDA
CY.OS 99	KW2	Twig	PDA
CY.OS 100	TN1	Twig	PDA
CY.OS 101	TN1	Fruit	PDA
CY.OS 102	TN1	Root	PDA
CY.OS 103	TN1	Fruit	PDA
CY.OS 104	TN1	Root	PDA
CY.OS 105	TN1	Twig	PDA
CY.OS 106	TN1	Root	PDA
CY.OS 107	TN1	Twig	PDA
CY.OS 108	TN2	Leaf	PDA
CY.OS 110	TN2	Root	PDA
CY.OS 111	TN2	Leaf	PDA
CY.OS 112	TN2	Twig	PDA
CY.OS 113	TN1	Root	PDA
CY.OS 114	TN2	Leaf	PDA
CY.OS 115	TN2	Root	PDA
CY.OS 116	TN2	Root	PDA
CY.OS 117	TN2	Twig	PDA
CY.OS 118	TN2	Twig	PDA

YM= Yeast extract Malt extract medium, PDA= Potato Dextrose Agar

OR1= Okrong 1, OR2= Okrong 2, TN1= Talapnak 1, TN2= Talapnak 2

KW1= Khiaosawoey 1, KW2= Khiaosawoey 2

Table S3 Source of isolation of endophytic filamentous fungi isolated from ripen fruit season (cont.).

Isolates No.	Source of isolation		
	Mango variety	Part	Isolation medium
CY.OS 119	TN2	Root	PDA
CY.OS 120	TN2	Twig	PDA
CY.OS 121	OR2	Leaf	PDA
CY.OS 122	OR2	Leaf	PDA
CY.OS 123	OR2	Leaf	PDA
CY.OS 124	OR2	Leaf	PDA
CY.OS 125	OR2	Twig	PDA
CY.OS 126	OR2	Twig	PDA
CY.OS 127	OR2	Twig	PDA
CY.OS 128	OR2	Twig	PDA
CY.OS 129	OR2	Twig	PDA
CY.OS 130	OR2	Twig	PDA
CY.OS 131	OR2	Root	PDA
CY.OS 132	KW2	Twig	YM
CY.OS 133	KW2	Twig	YM
CY.OS 134	KW2	Leaf	YM
CY.OS 135	KW2	Leaf	YM
CY.OS 136	KW2	Leaf	YM
CY.OS 137	KW2	Leaf	YM
CY.OS 138	KW2	Root	YM
CY.OS 139	KW2	Root	YM
CY.OS 140	KW2	Fruit	YM
CY.OS 141	KW2	Fruit	YM
CY.OS 142	KW2	Fruit	YM

YM= Yeast extract Malt extract medium, PDA= Potato Dextrose Agar

OR1= Okrong 1, OR2= Okrong 2, TN1= Talapnak 1, TN2= Talapnak 2

KW1= Khiaosawoey 1, KW2= Khiaosawoey 2

Table S3 Source of isolation of endophytic filamentous fungi isolated from ripen fruit season (cont.).

Isolates No.	Source of isolation		
	Mango variety	Part	Isolation medium
CY.OS 143	KW1	Twig	YM
CY.OS 144	KW1	Twig	YM
CY.OS 145	KW1	Twig	YM
CY.OS 146	KW1	Twig	PDA
CY.OS 147	KW1	Leaf	PDA
CY.OS 148	KW1	Leaf	PDA
CY.OS 149	KW1	Fruit	PDA
CY.OS 150	KW1	Root	PDA
CY.OS 151	KW1	Root	PDA
CY.OS 152	OR1	Fruit	YM
CY.OS 153	OR1	Fruit	YM
CY.OS 154	OR1	Leaf	YM
CY.OS 155	KW2	Root	YM
CY.OS 156	OR1	Leaf	YM
CY.OS 157	OR1	Leaf	YM
CY.OS 158	OR1	Twig	YM
CY.OS 159	OR1	Twig	YM
CY.OS 160	OR1	Twig	YM
CY.OS 161	OR1	Twig	YM
CY.OS 162	OR1	Root	YM
CY.OS 163	OR1	Root	YM
CY.OS 164	OR1	Root	YM

YM= Yeast extract Malt extract medium, PDA= Potato Dextrose Agar

OR1= Okrong 1, OR2= Okrong 2, TN1= Talapnak 1, TN2= Talapnak 2

KW1= Khiaosawoey 1, KW2= Khiaosawoey 2

Table S3 Source of isolation of endophytic filamentous fungi isolated from ripen fruit season (cont.).

Isolates No.	Source of isolation		
	Mango variety	Part	Isolation medium
CY.OS 165	OR1	Root	YM
CY.OS 166	OR1	Root	YM
CY.OS 167	OR1	Root	YM
CY.OS 168	OR1	Twig	YM
CY.OS 169	KW1	Twig	YM
CY.OS 170	KW1	Root	YM
CY.OS 171	KW1	Root	YM
CY.OS 172	KW1	Root	YM
CY.OS 173	KW1	Fruit	PDA
CY.OS 174	KW1	Fruit	PDA
CY.OS 175	KW1	Fruit	PDA
CY.OS 176	KW1	Leaf	PDA
CY.OS 177	KW1	Root	PDA
CY.OS 178	KW1	Root	PDA
CY.OS 179	OR1	Twig	YM
CY.OS 180	OR1	Twig	YM
CY.OS 181	OR1	Leaf	YM
CY.OS 182	OR1	Leaf	YM
CY.OS 183	OR1	Leaf	YM
CY.OS 184	OR1	Leaf	YM
CY.OS 185	TN1	Leaf	YM
CY.OS 186	TN1	Leaf	YM
CY.OS 187	TN1	Leaf	YM
CY.OS 188	TN1	Leaf	YM

YM= Yeast extract Malt extract medium, PDA= Potato Dextrose Agar

OR1= Okrong 1, OR2= Okrong 2, TN1= Talapnak 1, TN2= Talapnak 2

KW1= Khiaosawoey 1, KW2= Khiaosawoey 2

Table S3 Source of isolation of endophytic filamentous fungi isolated from ripen fruit season (cont.).

Isolates No.	Source of isolation		
	Mango variety	**Part**	**Isolation medium**
CY.OS 189	TN1	Fruit	YM
CY.OS 190	TN1	Leaf	PDA
CY.OS 191	TN2	Root	YM
CY.OS 192	TN2	Root	YM
CY.OS 193	TN1	Root	YM
CY.OS 194	TN1	Twig	YM
CY.OS 195	TN1	Twig	YM
CY.OS 196	TN1	Twig	YM
CY.OS 197	TN1	Twig	YM
CY.OS 198	TN1	Root	YM
CY.OS 199	TN1	Twig	PDA
CY.OS 200	TN1	Twig	PDA
CY.OS 201	TN1	Twig	PDA
CY.OS 202	TN1	Root	PDA
CY.OS 203	TN1	Leaf	YM
CY.OS 204	TN2	Twig	PDA
CY.OS 205	TN2	Twig	PDA
CY.OS 206	TN2	Twig	PDA
CY.OS 207	TN2	Twig	PDA
CY.OS 208	TN2	Twig	PDA
CY.OS 209	TN2	Twig	PDA
CY.OS 210	TN2	Root	YM
CY.OS 211	TN2	Fruit	YM
CY.OS 212	TN2	Root	PDA

YM= Yeast extract Malt extract medium, PDA= Potato Dextrose Agar

OR1= Okrong 1, OR2= Okrong 2, TN1= Talapnak 1, TN2= Talapnak 2

KW1= Khiaosawoey 1, KW2= Khiaosawoey 2

Table S3 Source of isolation of endophytic filamentous fungi isolated from ripen fruit season (cont.).

Isolates No.	Source of isolation		
	Mango variety	**Part**	**Isolation medium**
CY.OS 213	TN2	Twig	PDA
CY.OS 214	TN2	Root	PDA
CY.OS 215	OR2	Root	YM
CY.OS 216	OR2	Root	YM
CY.OS 217	OR2	Twig	YM
CY.OS 218	OR2	Leaf	YM
CY.OS 219	OR2	Leaf	YM
CY.OS 220	OR2	Leaf	YM
CY.OS 221	OR2	Root	YM
CY.OS 222	OR2	Root	YM
CY.OS 223	TN2	Fruit	YM
CY.OS 224	TN1	Twig	YM
CY.OS 225	TN1	Leaf	YM
CY.OS 226	TN1	Root	YM
CY.OS 227	TN1	Root	YM
CY.OS 228	TN1	Root	PDA
CY.OS 229	TN1	Fruit	PDA
CY.OS 230	TN1	Fruit	PDA
CY.OS 231	TN2	Root	YM
CY.OS 232	TN2	Twig	PDA
CY.OS 233	OR2	Leaf	YM
CY.OS 234	KW1	Root	YM
CY.OS 235	KW1	Leaf	YM
CY.OS 236	KW1	Twig	YM

YM= Yeast extract Malt extract medium, PDA= Potato Dextrose Agar

OR1= Okrong 1, OR2= Okrong 2, TN1= Talapnak 1, TN2= Talapnak 2

KW1= Khiaosawoey 1, KW2= Khiaosawoey 2

Table S3 Source of isolation of endophytic filamentous fungi isolated from ripen fruit season (cont.).

Isolates No.	Source of isolation		
	Mango variety	**Part**	**Isolation medium**
CY.OS 237	KW1	Twig	YM
CY.OS 238	KW1	Leaf	YM
CY.OS 239	KW1	Root	YM

YM= Yeast extract Malt extract medium, PDA= Potato Dextrose Agar

OR1= Okrong 1, OR2= Okrong 2, TN1= Talapnak 1, TN2= Talapnak 2

KW1= Khiaosawoey 1, KW2= Khiaosawoey 2

APPENDIX D

Antifungal phytopathogens of endophytic fungi

Table S4 Growth inhibition of *C. gloeosporioides* and *L. theobromae* by endophytic yeast isolates.

Isolates No.	% Inhibition against phytopathogens			
	Antagonism		Volatile compound production	
	C. gloeosporioides	***L. theobromae***	***C. gloeosporioides***	***L. theobromae***
CY.OS 01	11.05	_	42.00	_
CY.OS 02	8.42	_	18.00	_
CY.OS 03	10.52	_	38.80	_
CY.OS 04	12.63	_	18.00	_
CY.OS 05	7.36	_	17.20	_
CY.OS 06	8.95	_	25.20	_
CY.OS 07	13.68	_	20.00	_
CY.OS 08	3.15	_	40.00	_
CY.OS 09	15.78	_	15.20	_
CY.OS 10	10.53	_	46.80	_
CY.OS 11	12.63	_	12.00	_
CY.OS 12	7.37	_	28.00	_
CY.OS 13	12.63	_	_	_

– No activity

Table S5 Growth inhibition of *C. gloeosporioides* and *L. theobromae* by endophytic filamentous fungi isolates.

Isolates No.	% Inhibition with phytopathogens			
	Antagonism		Volatile compound production	
	C. gloeosporioides	*L. theobromae*	*C. gloeosporioides*	*L. theobromae*
CY.OS 14	38.89	_	_	_
CY.OS 15	41.48	_	_	_
CY.OS 16	54.81	1.85	14.81	_
CY.OS 17	52.96	_	12.96	_
CY.OS 18	37.78	_	_	_
CY.OS 19	14.81	_	9.26	_
CY.OS 20	52.96	_	3.70	_
CY.OS 21	43.70	_	_	_
CY.OS 22	37.41	_	_	_
CY.OS 23	33.33	_	_	_
CY.OS 24	48.15	0.74	_	_
CY.OS 25	41.48	1.48	_	_
CY.OS 26	35.56	5.19	_	_
CY.OS 27	35.56	3.70	_	_
CY.OS 28	30.37	1.48	_	_
CY.OS 29	27.04	_	_	_
CY.OS 30	12.59	_	_	_
CY.OS 31	16.30	4.07	_	_
CY.OS 32	8.89	2.59	_	_
CY.OS 33	41.48	0.74	_	34.44
CY.OS 34	17.41	_	_	_
CY.OS 35	50.00	18.89	6.67	_
CY.OS 36	28.52	5.56	_	_
CY.OS 37	27.78	10.37	_	_

– No activity

Table S5 Growth inhibition of *C. gloeosporioides* and *L. theobromae* by endophytic filamentous fungi isolates (cont.).

Isolates No.	% Inhibition with phytopathogens			
	Antagonism		Volatile compound production	
	C. gloeosporioides	*L. theobromae*	*C. gloeosporioides*	*L. theobromae*
CY.OS 38	31.48	21.48	_	_
CY.OS 39	7.04	_	_	_
CY.OS 40	0.74	_	_	_
CY.OS 41	12.96	_	_	_
CY.OS 42	52.22	18.52	5.56	_
CY.OS 43	39.26	15.19	1.48	_
CY.OS 44	15.56	_	_	_
CY.OS 45	4.81	_	_	_
CY.OS 46	28.89	7.41	_	_
CY.OS 47	32.22	11.11	2.96	_
CY.OS 48	32.96	4.81	_	3.70
CY.OS 49	35.93	13.70	_	_
CY.OS 50	41.48	15.56	_	_
CY.OS 51	5.19	_	_	_
CY.OS 52	6.30	_	_	_
CY.OS 53	32.22	15.56	_	_
CY.OS 54	27.41	15.56	_	_
CY.OS 55	57.78	10.37	_	_
CY.OS 56	24.44	10.00	_	_
CY.OS 57	26.67	15.56	_	_
CY.OS 58	26.30	4.07	2.59	_
CY.OS 59	66.67	41.85	3.70	_
CY.OS 60	28.89	18.52	_	_
CY.OS 61	24.07	9.63	_	_

– No activity

Table S5 Growth inhibition of *C. gloeosporioides* and *L. theobromae* by endophytic filamentous fungi isolates (cont.).

Isolates No.	% Inhibition with phytopathogens			
	Antagonism		Volatile compound production	
	C. gloeosporioides	*L. theobromae*	*C. gloeosporioides*	*L. theobromae*
CY.OS 62	30.37	11.11	_	_
CY.OS 63	3.70	_	_	_
CY.OS 64	57.41	1.85	14.81	_
CY.OS 65	30.74	6.30	2.59	_
CY.OS 66	25.56	3.70	_	_
CY.OS 67	37.78	9.26	_	_
CY.OS 68	22.96	17.04	12.59	_
CY.OS 69	32.22	5.56	_	_
CY.OS 70	32.22	12.96	3.70	_
CY.OS 71	37.41	18.89	4.44	_
CY.OS 72	25.93	20.37	4.44	_
CY.OS 73	31.85	7.04	1.85	_
CY.OS 74	9.63	_	_	_
CY.OS 75	48.15	38.15	5.56	_
CY.OS 76	26.67	11.11	_	_
CY.OS 77	34.07	17.41	14.81	_
CY.OS 78	32.22	10.37	15.56	_
CY.OS 79	31.11	18.15	2.59	_
CY.OS 80	30.74	10.00	1.11	_
CY.OS 81	12.96	8.89	10.74	_
CY.OS 82	8.52	_	_	_
CY.OS 83	21.48	10.37	_	_
CY.OS 84	28.52	12.22	4.81	_
CY.OS 85	46.67	9.63	8.89	_

– No activity

Table S5 Growth inhibition of *C. gloeosporioides* and *L. theobromae* by endophytic filamentous fungi isolates (cont.).

Isolates No.	% Inhibition with phytopathogens			
	Antagonism		Volatile compound production	
	C. gloeosporioides	*L. theobromae*	*C. gloeosporioides*	*L. theobromae*
CY.OS 86	22.96	9.26	1.11	_
CY.OS 87	34.07	9.26	5.93	_
CY.OS 88	66.67	35.19	3.70	12.96
CY.OS 89	18.89	_	_	_
CY.OS 90	30.74	11.48	_	_
CY.OS 91	34.81	4.44	_	_
CY.OS 92	32.22	6.67	1.11	_
CY.OS 93	34.07	5.19	_	_
CY.OS 94	73.33	50.74	3.70	25.93
CY.OS 95	37.78	7.78	_	_
CY.OS 96	65.93	8.15	13.70	29.63
CY.OS 97	31.85	_	_	_
CY.OS 98	38.52	_	_	_
CY.OS 99	41.48	20.00	11.85	_
CY.OS 100	62.96	26.30	10.37	_
CY.OS 101	4.44	_	_	_
CY.OS 102	28.52	64.81	3.70	_
CY.OS 103	52.22	1.11	_	_
CY.OS 104	54.81	_	_	_
CY.OS 105	48.89	3.70	_	_
CY.OS 106	34.44	48.15	_	_
CY.OS 107	59.63	7.41	_	_
CY.OS 108	54.81	40.37	8.89	_
CY.OS 109	64.81	42.59	7.41	_
CY.OS 110	41.48	7.78	_	_

– No activity

Table S5 Growth inhibition of *C. gloeosporioides* and *L. theobromae* by endophytic filamentous fungi isolates (cont.).

Isolates No.	% Inhibition with phytopathogens			
	Antagonism		Volatile compound production	
	C. gloeosporioides	*L. theobromae*	*C. gloeosporioides*	*L. theobromae*
CY.OS 111	31.48	_	_	_
CY.OS 112	60.37	41.85	14.81	_
CY.OS 113	42.96	22.22	_	_
CY.OS 114	55.56	28.89	_	_
CY.OS 115	31.85	5.19	_	_
CY.OS 116	3.70	_	_	_
CY.OS 117	42.96	11.48	_	_
CY.OS 118	35.19	_	_	_
CY.OS 119	5.56	_	_	_
CY.OS 120	54.81	2.96	_	_
CY.OS 121	15.56	_	_	_
CY.OS 122	61.11	11.85	_	18.52
CY.OS 123	50.00	4.44	_	_
CY.OS 124	8.15	_	_	_
CY.OS 125	31.48	_	_	_
CY.OS 126	56.30	_	_	_
CY.OS 127	75.93	42.96	7.41	11.11
CY.OS 128	77.04	48.89	9.26	17.78
CY.OS 129	61.85	5.56	14.81	17.41
CY.OS 130	55.56	2.59	_	_
CY.OS 131	66.30	14.81	3.70	7.78
CY.OS 132	79.63	50.37	7.78	24.07
CY.OS 133	71.48	40.37	11.11	1.85
CY.OS 134	68.89	39.26	8.89	1.85

– No activity

Table S5 Growth inhibition of *C. gloeosporioides* and *L. theobromae* by endophytic filamentous fungi isolates (cont.).

Isolates No.	% Inhibition with phytopathogens			
	Antagonism		Volatile compound production	
	C. gloeosporioides	*L. theobromae*	*C. gloeosporioides*	*L. theobromae*
CY.OS 135	39.63	43.33	_	_
CY.OS 136	8.15	_	_	_
CY.OS 137	11.11	_	_	_
CY.OS 138	50.74	_	_	_
CY.OS 139	55.56	34.07	7.41	_
CY.OS 140	72.59	40.74	3.70	22.59
CY.OS 141	30.74	_	_	_
CY.OS 142	60.00	40.37	7.41	1.85
CY.OS 143	44.44	_	_	_
CY.OS 144	34.81	46.30	_	_
CY.OS 145	12.96	_	_	_
CY.OS 146	56.30	11.11	7.41	8.52
CY.OS 147	75.93	42.22	11.11	23.33
CY.OS 148	39.26	7.41	_	_
CY.OS 149	29.26	46.30	_	11.48
CY.OS 150	70.74	12.96	_	_
CY.OS 151	67.78	50.74	12.59	22.22
CY.OS 152	32.59	41.48	11.85	_
CY.OS 153	34.07	3.70	8.52	3.70
CY.OS 154	3.70	_	_	_
CY.OS 155	67.41	11.85	_	5.56
CY.OS 156	48.15	5.56	3.70	_
CY.OS 157	53.70	6.30	_	12.96
CY.OS 158	75.56	49.26	11.11	22.59

– No activity

Table S5 Growth inhibition of *C. gloeosporioides* and *L. theobromae* by endophytic filamentous fungi isolates (cont.).

Isolates No.	% Inhibition with phytopathogens			
	Antagonism		Volatile compound production	
	C. gloeosporioides	*L. theobromae*	*C. gloeosporioides*	*L. theobromae*
CY.OS 159	52.96	41.48	_	_
CY.OS 160	77.41	48.89	9.26	24.07
CY.OS 161	54.81	1.85	14.81	_
CY.OS 162	81.48	12.96	7.41	22.96
CY.OS 163	65.19	11.11	7.41	6.30
CY.OS 164	54.81	7.41	_	_
CY.OS 165	50.74	1.85	_	_
CY.OS 166	55.56	1.85	_	_
CY.OS 167	54.81	1.85	_	_
CY.OS 168	5.56	41.85	13.70	27.78
CY.OS 169	67.41	_	_	_
CY.OS 170	61.11	42.59	14.81	_
CY.OS 171	52.78	8.15	_	_
CY.OS 172	26.67	45.19	_	_
CY.OS 173	58.52	3.70	3.70	_
CY.OS 174	33.33	4.81	_	_
CY.OS 175	41.48	11.48	_	_
CY.OS 176	23.33	_	_	_
CY.OS 177	22.22	_	_	_
CY.OS 178	50.37	_	4.44	_
CY.OS 179	11.85	_	_	_
CY.OS 180	41.48	2.96	_	_
CY.OS 181	9.63	_	_	_
CY.OS 182	48.89	_	_	_

– No activity

Table S5 Growth inhibition of *C. gloeosporioides* and *L. theobromae* by endophytic filamentous fungi isolates (cont.).

Isolates No.	% Inhibition with phytopathogens			
	Antagonism		Volatile compound production	
	C. gloeosporioides	*L. theobromae*	*C. gloeosporioides*	*L. theobromae*
CY.OS 183	26.30	_	_	_
CY.OS 184	40.00	_	_	_
CY.OS 185	47.41	22.22	_	10.00
CY.OS 186	35.56	3.70	_	_
CY.OS 187	47.41	22.22	_	14.81
CY.OS 188	3.70	_	_	_
CY.OS 189	22.22	_	_	_
CY.OS 190	14.81	_	_	_
CY.OS 191	22.22	_	_	_
CY.OS 192	14.81	37.04	8.15	3.70
CY.OS 193	66.30	14.81	11.85	_
CY.OS 194	49.63	_	_	_
CY.OS 195	62.22	9.26	7.41	6.30
CY.OS 196	62.22	9.26	8.52	14.81
CY.OS 197	62.22	42.59	11.48	16.67
CY.OS 198	69.63	9.26	11.85	7.41
CY.OS 199	59.26	_	_	_
CY.OS 200	60.74	5.56	3.70	12.96
CY.OS 201	60.74	7.41	7.41	7.41
CY.OS 202	59.26	11.11	_	11.11
CY.OS 203	30.37	46.30	_	_
CY.OS 204	55.19	11.11	13.70	11.11
CY.OS 205	55.19	9.26	11.11	19.26
CY.OS 206	59.26	9.26	9.26	14.07

– No activity

Table S5 Growth inhibition of *C. gloeosporioides* and *L. theobromae* by endophytic filamentous fungi isolates (cont.).

Isolates No.	**% Inhibition with phytopathogens**			
	Antagonism		**Volatile compound production**	
	C. gloeosporioides	***L. theobromae***	***C. gloeosporioides***	***L. theobromae***
CY.OS 207	55.19	45.56	3.70	18.15
CY.OS 208	22.22	9.26	12.22	11.85
CY.OS 209	56.30	0.37	11.11	20.37
CY.OS 210	46.67	30.74	1.48	5.19
CY.OS 211	35.19	46.30	_	_
CY.OS 212	39.63	16.67	1.48	_
CY.OS 213	35.56	46.30	_	_
CY.OS 214	23.33	46.30	_	_
CY.OS 215	62.59	7.41	3.70	6.30
CY.OS 216	51.11	34.07	_	_
CY.OS 217	51.11	5.56	3.70	17.41
CY.OS 218	46.30	2.22	_	_
CY.OS 219	57.41	_	_	_
CY.OS 220	22.22	_	_	_
CY.OS 221	60.00	3.70	4.81	6.67
CY.OS 222	62.59	7.41	10.74	11.11
CY.OS 223	28.15	41.85	3.70	14.81
CY.OS 224	28.52	_	_	_
CY.OS 225	52.59	35.56	_	_
CY.OS 226	22.96	_	_	_
CY.OS 227	61.11	32.96	14.81	_
CY.OS 228	57.41	44.07	10.37	_
CY.OS 229	19.26	_	_	_
CY.OS 230	30.37	22.96	_	_

– No activity

Table S5 Growth inhibition of *C. gloeosporioides* and *L. theobromae* by endophytic filamentous fungi isolates (cont.).

Isolates No.	**% Inhibition with phytopathogens**			
	Antagonism		**Volatile compound production**	
	C. gloeosporioides	***L. theobromae***	***C. gloeosporioides***	***L. theobromae***
CY.OS 231	61.85	45.56	3.70	_
CY.OS 232	54.81	9.26	12.96	12.96
CY.OS 233	19.63	_	_	_
CY.OS 234	26.67	9.26	_	_
CY.OS 235	48.89	_	_	_
CY.OS 236	68.52	41.85	_	_
CY.OS 237	61.11	3.70	8.52	_
CY.OS 238	64.81	41.48	_	_
CY.OS 239	48.89	41.85	7.78	_

– No activity

APPENDIX E

IAA production of endophytic fungi

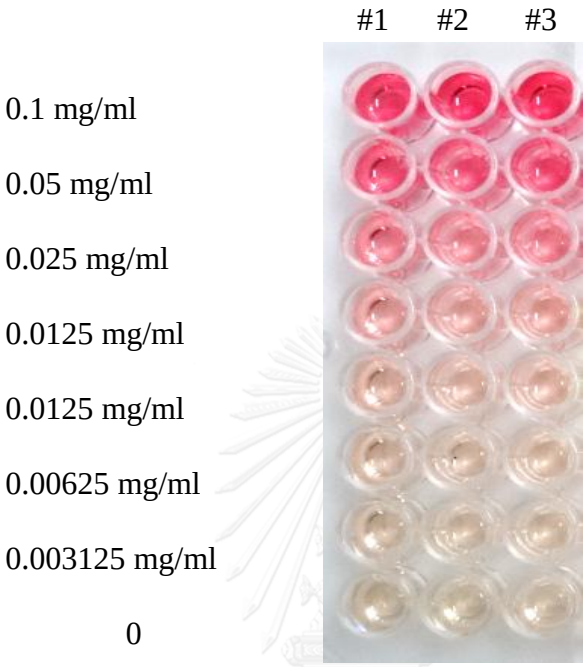

Figure S1 Colorimetric assay for indole acetic acid using Salkowski reagent. The experiments were performed in triplicate (#1, #2 and #3).

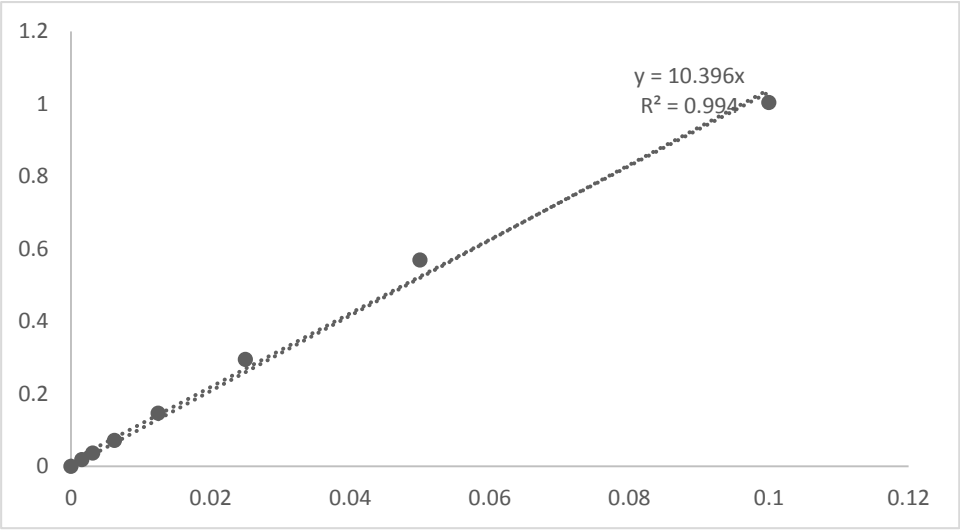

Figure S2 Standard curve of IAA stock was used to calculate IAA produced by endophytic fungi.

Table S6 Indole acetic acid (IAA) production by endophytic yeast isolates

Isolates No.	IAA production (mg/g DCW*)
CY.OS 01	29.95 ± 0.053
CY.OS 02	41.20 ± 0.103
CY.OS 03	50.22 ± 0.006
CY.OS 04	16.13 ± 0.078
CY.OS 08	20.33 ± 0.107
CY.OS 10	30.36 ± 0.113
CY.OS 11	43.19 ± 0.045
CY.OS 13	53.64 ± 0.054

*DCW= Dry Cell Weight

Table S7 Indole acetic acid (IAA) production by endophytic filamentous fungi isolates

Isolates No.	IAA production (mg/g DCW*)
CY.OS 16	38.38 ± 0.027
CY.OS 17	88.56 ± 0.119
CY.OS 20	77.18 ± 0.093
CY.OS 21	86.37 ± 0.019
CY.OS 22	96.22 ± 0.029
CY.OS 25	52.98 ± 0.013
CY.OS 33	65.69 ± 0.022
CY.OS 64	78.96 ± 0.018
CY.OS 69	15.38 ± 0.003
CY.OS 75	24.22 ± 0.020
CY.OS 98	95.47 ± 0.052
CY.OS 104	31.19 ± 0.007

*DCW= Dry Cell Weight

Table S7 Indole acetic acid (IAA) production by endophytic filamentous fungi isolates (cont.)

Isolates	IAA production (mg/g DCW*)
CY.OS 110	40.21 ± 0.015
CY.OS 112	21.49 ± 0.008
CY.OS 113	51.87 ± 0.006
CY.OS 114	15.88 ± 0.026
CY.OS 117	16.13 ± 0.001
CY.OS 121	19.68 ± 0.001
CY.OS 122	94.69 ± 0.013
CY.OS 124	75.92 ± 0.024
CY.OS 133	28.25 ± 0.010
CY.OS 134	20.53 ± 0.010
CY.OS 140	30.84 ± 0.005
CY.OS 141	33.03 ± 0.017
CY.OS 145	3.64 ± 0.022
CY.OS 150	28.48 ± 0.008
CY.OS 154	14.61 ± 0.003
CY.OS 155	20.86 ± 0.018
CY.OS 161	92.53 ± 0.024
CY.OS 162	8.01 ± 0.004
CY.OS 163	7.51 ± 0.003
CY.OS 164	8.73 ± 0.002
CY.OS 165	5.56 ± 0.002
CY.OS 166	5.03 ± 0.003
CY.OS 167	7.22 ± 0.002
CY.OS 185	11.54 ± 0.002
CY.OS 188	10.73 ± 0.003
CY.OS 190	11.04 ± 0.001

*DCW= Dry Cell Weight

Table S7 Indole acetic acid (IAA) production by endophytic filamentous fungi isolates (cont.)

Isolates	IAA production (mg/g DCW*)
CY.OS 197	11.99 ± 0.003
CY.OS 200	4.71 ± 0.002
CY.OS 204	9.14 ± 0.060
CY.OS 209	77.87 ± 0.168
CY.OS 212	10.23 ± 0.012
CY.OS 225	15.98 ± 0.002
CY.OS 228	39.15 ± 0.007
CY.OS 231	38.65 ± 0.010

*DCW= Dry Cell Weight

APPENDIX F

The internal transcribed spacer (Compant et al.) region of rDNA

CY.OS 01, 02, 03, 10, 11 & 13 (*Aureobasidium* sp.) 97%

AAGGTTTCAGTCGGCAGAAGTCCTCTCCTTTGACAGACGTTCGAATAAATT
CTACTACGCCTAAAGCCGGTGAGGCCTCGCCGAGGTCTTTAAGGCGCGCC
CAACTAAGGACGGCACCCAATACCAAGCATAGCTTGAGTGGTGTAATGAC
GCTCGAACAGGCATGCCCCTCGGAATACCAAGGGGCGCAATGTGCGTTCA
AAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTC
GCTGCGTTCTTCATCGATGCGAGAACCAAGAGATCCGTTGTTGAAAGTTTT
GATTTATTCAAAATTTTAACTCAGACGACCGGTTTAATAACAAGAGTTTGG
TTTAACTCTGGCGGGCGCTCGCCTGGGACGAATCCCCAGCGGCTCGAGAC
CGAGCGGTCCCGCCAAAGCAACAAGGTAGTTTTAACAACAAAGGGTTGGA
GGTCGGGCGCTGAGCACCC

CY.OS 04 (*Rhodotorula* sp.) 98%

TTGCATTAATTGCACCACATGTGTTTTTTATTGAACAAATTTCTTTGGTGGC
GGGAGCAATCCTACCGCCAGAGGTTATAACTAAACCAAACTTTTTATTTAC
AGTCAAACTTGATTTATTATTACAATAGTCAAAACTTTCAACAACGGATCT
CTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATATG
AATTGCAGATATTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTT
TGGTATTCCAAAGGGCATGCCTGTTTGAGCGTCATTTCTCCCTCAAACCCC
CGGGTTTGGTGTTGAGCAATACGCTAGGTTTGTTTGAAAGAATTTAACGTG
GAAACTTATTTTAAGCGACTTAGGTTTATCCAAAACGCTTATTTTGCTAGT
GGCCACCACAATTTATTTCATAACTTTGACCTCA

CY.OS 05, 06 & 12 (*Hanseniaspora* sp.) 99%

CTGATTTGAGGTCAAACTTGATGAATATTAMAAGCAACCCTTTGCCTAAG
GTACATTACCATTTCCCTTGTAAAGTAAAACGAATAAATCCATAAATACAT
CACAGCGAGAACAGCGTCTCCAAAGAAGCTAAGTGTTGAATTAAAAAAG
ACTGAAACAGTCTCCAATTTCAAGCTAACCCTGAGTATCGCCCACAACCA
AAAAATAAAAAATTATCTTTTGAGAAGGAAATGACGCTCAAACAGGCATG
CCCTTGAGAATGCTCAAGGGCGCAATGTGCGTTCAAAAATTCAATGATTC
ACGAGTATCTGCAATTCACATTACTTATCGCAATTCGCTACGTTCTTCATC
GATGCGAGAACCAAGAGATCCGTTGTTGAAAGTTTTAAATTATTTTAAAA
TTTCCGTTAGGAATTTTGGTTTAGTTTAAAAAATTATAATAAAATAAAATT
GTTTGTGTTTGTTTTTTGCCTTGAACCTTTCGATTCAAAGCAGAAAGAATT
AAATTAAAGTAAAAAACTCCAATGTGTGTAAACGTTGACTGAGATTCAAG
CAAGACTACTTCTACTGCGACACTCTAATGAAGCAGCGCAATTAAGCGCA
CATCTCCAATAAAGATACACATTATTGTAAAAGATCTAAACAAGAACTCG
ACGCAACTGAATGATAA

CY.OS 07 & 09 (*Candida* sp.) 99%

GTATCTTTTTCGGTCATTGCCAGCGCTTTCACTAGCGCGGCGACCAAACCT
TACACACAGTGCTTAGTTTTTTCTAACATTGCTTTGGGTTGAGGCCTTGTGT
CTCGGCCCAGAGGATCAAAAACACAAGATTTTAATATCTTTTTCTTTAAAA
ACCTTTAGTCAAGAAGTTTTCTTAAAAACAAATCTTCAAAACTTTCAACAA
CGGATCTCTTGGTTCTCGCAACGATGAAGAACGCAGCGAAATGCGATACG
TAATGTGAATTGCAGGTTTTCGTGAATCATCGAATCTTTGAACGCATATTG
CGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTC
AAACCCTCGGGTTTGGTATTGAGTGATACTCTGTTAAACTGGGTTAACTTG
AAATAATGTTGGCAAGAGCTGCTTCAGCAGATCTTCCTACTGAACTAACG
TTTCTAGGTTCTACCAATTCGTTGTGTTCTATAGGACATGTTCTCTCTAGCT
CTGGGCTCGGCCTAACAATTTCACACAAGTTTGACCTC

CY.OS 08 (*Cryptococcus laurentii*) 96%

ACGGACTGATYTGAGGWCAGAGTCTAMAAGTGCGCGATGGCAGGTTATG
AGCAGACTTCATAAGCAAGGGACAAGACGAAACTTATTACGTCTTGTCAC
GGATGCAGAGAGATCCACTAAGTCATTTTGAGGCGAGCCGTGTGACCGGC
AGACGCCCATATCCAAGTCCGACCCAGATCATAAACCCAGGGGGATTGAG
ATTTCATGACACTCAAACAGGCAYGCCTTTCGGAATACCAAAAGGCGCAA
GGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTT
ATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGT
TGAAAGTTTTGTTTTGTTTAATGATATATACGTTCATTACACTGATGTTTGT
TAAATAGACCCGGAGGTCCACAGTTCACAGAGGTGAGGGATATAGAGAT
AAGACCYTTCGSYCA

CY.OS 20 (*Colletotrichum* sp. 99%)

GGGCTCTGACTAGCTCTACACCCTTTGTGACATACCTATAACTGTTGCTTC
GGCGGGTAGGGTCTCCGCGACCCTCCCGGCCTCCCGCCTCCGGGCGGGTC
GGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGA
GTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTG
GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA
ATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCT
GGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTG
TTGGGGCCCTACAGCTGATGTAGGCCCTCAAAGGTAGTGGCGGACCCTCT
CGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGAGGG
ACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTA
GGAATACCCGCTGAACTTAAGCATATCA

CY.OS 22 (*Colletotrichum* sp. 99%)

GTGGCTCTGACTAGCTCTACACCCTTTGTGACATACCTATAACTGTTGCTT
CGGCGGGTAGGGTCTCCGCGACCCTCCCGGCCTCCCGCCTCCGGGCGGGT
CGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTG
AGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCT
GGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAG
AATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTC

TGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGT
GTTGGGGCCCTACAGCTGATGTAGGCCCTCAAAGGTAGTGGCGGACCCTC
TCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGAGG
GACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGT
AGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGGAGGGAG

CY.OS 33 (*Colletotrichum* sp. 100%)

GGGCTCTGACTAGCTCTATACCCTTTGTGACATACCTATAACTGTTGCTTC
GGCGGGTAGGGTCTCCGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTC
GGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGA
GTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTG
GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA
ATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCT
GGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTG
TTGGGGCCCTACAGCTGATGTAGGCCCTCAAAGGTAGTGGCGGACCCTCC
CGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGAGGG
ACTCTTGCCGTAAAACCCCCAATTCTCCAAAGGTTGACCTCGGATCAGGTA
GGAATACCCGCTGAACTTAAGCATATCA

CY.OS 64 (*Colletotrichum* sp. 99%)

GGGGCCTTCTGAGTGAGCACTATACCCTTTGTGACATACCTATAACTGTTG
CTTCGGCGGGTAGGGTCTCCGTGACCCTCCCGGCCTCCCGCCCCCGGGCG
GGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTT
CTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGT
TCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGC
AGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCA
TTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTT
GGTGTTGGGGCCCTACAGCTGATGTAGGCCCTCAAAGGTAGTGGCGGACC
CTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGG
AGGGACTCTTGCCGTAAAACCCCCAATTCTCCAAAGGTTGACCTCGGATC
AGGTAGGAATACCCGCTGAACTTAAGCATATCAAAGCCCGGGAGGAA

CY.OS 69 (*Rhytidhysteron* sp. 99%)

CTACGCAGCATGCGAGACGCGCGAGGGGGGTAGGGCAACCTAACCCACC
CGACTGGTCGGACCCGTGCCCACATACTGCGGGGAGCCTTGCGGCTCTCT
CGAGGGGGGCGTGGCGGCTCGTCCGCCCGCCTGTAGACGGGGCCTTCCCT
TTAGGTGGGGCTTCCCTCCGCCGTTCTTGCCTTCCACAACCTTCACCCTTG
ATTACCCTAGCCTTTGTTGCCTCGGCAGGTTCGCCTGCCAGAGGACACAAC
CTAAACTACTGTTGTTAACAGCGAAGTCTGAGCTACAAAGCAATTGTTTA
AAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC
GAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCT
TTGAACGCACATTGCGCCCTTTGGCATCCCGAAGGGCATGCCTGTTCGAGC
GTCATTTCACCAATCAAGCCTGGCTTGGTGTTGGGTGCCGTCCCGCCTCTG
GTGCGCGGACGCTCCCTAAAATCATCGGCGGTGCAGCACCGGCTTCGAGC
GCAGCAGATGTTCGCTCTGTGGGTCTGGTGAGGCAGCGGCCGCGACGACA
ACCTCTAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCA
TATAAA

CY.OS75 (*Lasiodiplodia* sp. 99%)

GGCTCGGGCTTGGGTCTCTTCCCGAGCCCACTCTCCAACCCTTTGTGTACC
TACCTCTGTTGCTTTGGCGGGCCGCGGTTCTCCGCGGCCGGCCCCCTGATC
GGGGCTGGCCCGCGCCCGCCAGAGGACCACAAAACTCCAGTCAGTGAACT
TTGCTGTCTGATATAAATTCAATAAACTAAAACTTTCAACAACGGATCTCT
TGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAA
TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTG
GTATTCCGAAGGGCATGCCTGTTCGAGCGTCATTACACCCCTCAAGCTCTG
CTTGGTGTTGGGCAGCGTCCTCTCGGACGCGCCTCAAAGACCTCGGCGGT
GGCGTCTTGCCTCAAGCGTAGTAGAAAACACCTCGCTTTGGAGGACGGGA
CGACCGCTCGCCGGACGAACCTTTGAATTCATTTTCCTAGGTTGACCTCGG
ATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAA

CY.OS 150 (*Lasiodiplodia* sp. 99%)

GGCTCGGAGCTTGGGTCTCTTCCCGAGCCCACTCTCCAACCCTTTGTGTAC
CTACCTCTGTTGCTTTGGCGGGCCGCGGTTCTCCGCGGCCGGCCCCCTGAT
CGGGGCTGGCCCGCGCCCGCCAGAGGACCACAAAACTCCAGTCAGTGAA
CTTTGCTGTCTGATATAAATTCAATAAACTAAAACTTTCAACAACGGATCT
CTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTG
AATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTT
TGGTATTCCGAAGGGCATGCCTGTTCGAGCGTCATTACACCCCTCAAGCTC
TGCTTGGTGTTGGGCAGCGTCCTCTCGGACGCGCCTCAAAGACCTCGGCG
GTGGCGTCTTGCCTCAAGCGTAGTAGAAAACACCTCGCTTTGGAGGACGG
GACGACCGCTCGCCGGACGAACCTTTGAATTCATTTTCCTAGGTTGACCTC
GGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAA

CY.OS 151 (*Lasiodiplodia* sp.100%)

GGGCTACGACTTCGAGCTTCGGCTCGACTCTCCCACCCTTTGTGAACGTAC
CTCTGTTGCTTTGGCGGCTTCGGCCGCCAAAGGACCTTCAAACTCCAGTCA
GTAAACGCAGACGTCTGATAAACAAGTTAATAAACTAAAACTTTCAACAA
CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAG
TAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTG
CGCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTACAACCCT
CAAGCTCTGCTTGGAATTGGGCACCGTCCTCACTGCGGACGCGCCTCAAA
GACCTCGGCGGTGGCTGTTCAGCCCTCAAGCGTAGTAGAATACACCTCGC
TTTGGAGCGGTTGGCGTCGCCCGCCGGACGAACCTTCTGAACTTTTCTCAA
GGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAA
G

CY.OS 153 (*Aspergilluss* sp. 99%)

GGCTTCGAGCGTGGGTTCTAGCGAGCCCAACCTCCCACCCGTGTTTACTGT
AACCTTAGTTGCTTCGGCGGGCCCGCCTTTAAGGCCGCCGGGGGGCATCA
GCCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTAG
TGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGAT
CTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTG
TGAATTGCAGAATTCCGTGAATCATCGAGTCTTTGAACGCACATTGCGCCC
CCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAG
CACGGCTTGTGTGTTGGGTCGTCGTCCCCTCTTCGGGGGGGACGGGCCCCA
AAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCA
CCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAAACAACCATTCT
TTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATA
TCAAA

CY.OS 162 (*Colletotrichum* sp. 99%)

GTGCTCTGAGTCGCTCTATACCCTTTGTGACATACCTATAACTGTTGCTTC
GGCGGGTAGGGTCTCCGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTC
GGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGA
GTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTG
GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA
ATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCT
GGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTG
TTGGGGCCCTACAGCTGATGTAGGCCCTCAAAGGTAGTGGCGGACCCTCC
CGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGAGGG
ACTCTTGCCGTAAAACCCCCAATTCTCCAAAGGTTGACCTCGGATCAGGTA
GGAATACCCGCTGAACTTAAGCATATCAAAGCGGGAGGAAACT

CY.OS 185 (*Penicillium* sp. 99%)

GGCTTCGGTCGAACATCGGGGCCCACCTCCCACCCGTGTTGCCCGAACCT
ATGTTGCCTCGGCGGGCCCCGCGCCCGCCGACGGCCCCCCTGAACGCTGT
CTGAAGTTGCAGTCTGAGACCTATAACGAAATTAGTTAAAACTTTCAACA
ACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAA
CTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATT
GCGCCCTCTGGTATTCCGGAGGGCATGCCTGTCCGAGCGTCATTGCTGCCC
TCAAGCCCGGCTTGTGTGTTGGGCCCCGTCCCCCCCGCCGGGGGGACGGG
CCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTT
CGTCACCCGCTCTAGTAGGCCCGGCCGGCGCCAGCCGACCCCCAACCTTT
AATTATCTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAA
GCATATCAAAAGCCGGGAGGAA

CY.OS 188 (*Phyllosticta* sp. 99%)

GGCTTTCTGATGTATACTTCTATTGAAGGTTCCAGAGTAGGCGCTACAACG
CCGAAATGACCTTCTCACCCTTGTGTACTCACTATGTTGCTTTGGCGGGTC
GACCTGGTTCCGACCCAGGCGGCCGGCGCCCCCAGCCTTAACTGGCCAGG
ACGCCCGGCTAAGTGCCCGCCAGTATACAAAACTCAAGAATTCATATTGT
GAAGTCCTGATATATCATTTAATTGATTTAAAACTTTCAACAACGGATCTC
TTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA
ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT
GGTATTCCGGAGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCT
GCTTGGTATTGGGCAACGTCCGCTGCCGGACGTGCCTTGAAGACCTCGGC
GACGGCGTCCTAGCCTCGAGCGTAGTAGTAAAATATCTCGCTTTGGAGTG
CTGGGCGACGGCCGCCGGACAATCGACCTTCGGTCTATTTTTCCAAGGTTG
ACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAA

CY.OS 197 (*Lasiodiplodia* sp. 99%)

GGCTTCGAGCTTGGGTCTCTTCCCGAGCCCACTCTCCAACCCTTTGTGTAC
CTACCTCTGTTGCTTTGGCGGGCCGCGGTTCTCCGCGGCCGGCCCCCTGAT
CGGGGCTGGCCCGCGCCCGCCAGAGGACCACAAAACTCCAGTCAGTGAA
CTTTGCTGTCTGATATAAATTCAATAAACTAAAACTTTCAACAACGGATCT
CTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTG
AATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTT
TGGTATTCCGAAGGGCATGCCTGTTCGAGCGTCATTACACCCCTCAAGCTC
TGCTTGGTGTTGGGCAGCGTCCTCTCGGACGCGCCTCAAAGACCTCGGCG
GTGGCGTCTTGCCTCAAGCGTAGTAGAAAACACCTCGCTTTGGAGGACGG
GACGACCGCTCGCCGGACGAACCTTTGAATTCATTTTCCTAGGTTGACCTC
GGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAA

CY.OS 209 (*Colletotrichum* sp. 99%)

GTGCTCTGAGTCGCTCTATACCCTTTGTGACATACCTATAACTGTTGCTTC
GGCGGGTAGGGTCTCCGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTC
GGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGA
GTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTG
GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA
ATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCT
GGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTG
TTGGGGCCCTACAGCTGATGTAGGCCCTCAAAGGTAGTGGCGGACCCTCC
CGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGAGGG
ACTCTTGCCGTAAAACCCCCAATTCTCCAAAGGTTGACCTCGGATCAGGTA
GGAATACCCGCTGAACTTAAGCATATCAAAGCGGGAGGAAACT

CY.OS 211 (*Aspergillus* sp. 99%)

GGCTTCGAGCGTGGGTTCTAGCGAGCCCAACCTCCCACCCGTGTTTACTGT
AACCTTAGTTGCTTCGGCGGGCCCGCCTTTAAGGCCGCCGGGGGGCATCA
GCCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTAG
TGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGAT
CTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTG
TGAATTGCAGAATTCCGTGAATCATCGAGTCTTTGAACGCACATTGCGCCC
CCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAG
CACGGCTTGTGTGTTGGGTCGTCGTCCCCTCTTCGGGGGGGACGGGCCCCA
AAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCA
CCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAAACAACCATTCT
TTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATA
TCAAA

VITA

My name is Orlavanh Sonesouphap was born in Sayaboury Province, Laos PDR on February 05, 1986. After graduation with Bachelor's degree of Science from Department of Biology, Faculty of Science, National University of Laos in 2008. In 2009-2013, I was a teacher and worked for Faculty of Engineering, National University of Laos. Then continued to study Master's degree in Microbiology and Microbial Technology program of Microbiology Department, Faculty of Science, Chulalongkorn University in 2013.

Academic presentations:

Oral presentation

Orlavanh Sonsouphap, Prakitsin Sihanonth, Chulee Yompakdee. Endophytic Yeasts Isolated from Mangifera indica L. with Anti-fungal Activity and Indole Acetic Acid Production. The 1st Rajamagala University of Technology Lanna Chiangrai Conference 2015 (RCCON 2015). On Mach 23-24, 2015 at Wieng Inn Hotel, Chiangrai, Thailand. Proceeding was published on page 508 – 512.

Poster presentation

Orlavanh Sonsouphap, Prakitsin Sihanonth, Chulee Yompakdee. Isolation and Molecular Identification of Endophytic Yeasts from Mangifera indica L. Capable of Producing Plant Growth Hormone, Indole Acetic Acid. THE SCIENCE FORUM 2015. On May 28, 2015 at Faculty of Science, Chulalongkorn University.