
Isolation and Identification of Endophytic Fungi from 10 Species Palm Trees

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Abstract Endophytic fungi are those living inside the host plant without causing any apparent negative effect on host plant. Endophytic fungi from palm trees in King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand, were isolated and identified by morphology. 105 isolates were isolated from leaves, petioles and roots of 10 species healthy palms. Among them, 45 unidentified isolates were group to mycelia sterilia fungi. Other 60 isolates were identified as 15 species belong to *Fusarium* spp., *Xylaria* spp, *Cladosporium* spp, *Phialophora* spp, *Pestalotiopsis* spp, *Rhizoctonia* spp., *Colletotrichum* spp., *Chaetomium globosum*, *Chaetomium aureum*, *Giberrella* spp, *Emericella* spp, *Aspergillus* spp., *Curvalaria lunata*, *Phoma* spp and *Nigrospora* spp by morphological characters.

Keywords: Endophytic fungi, Identification, palm trees

Introduction

Endophytes are microorganisms that live within plants for at least a part of their life cycle without causing any visible manifestation of disease (Bacon and white, 2000). A wide range of plants have now been examined for endophytes, and endophytes have been found in nearly all of them, including trees, grass, algae and herbaceous plants. Evidence of plant-associated microorganisms found in the fossilized tissues of stems and leaves has revealed that endophyte-plant associations may have evolved from the time higher plants first appeared on the earth (Redecker *et al.*, 2000). Hawksworth and Rossmann estimated that nearly one million species of endophytes may exist in the unexplored plants (Strobel and Daisy, 2003; Arnold, 2005). Endophytes microorganisms were discovered including fungi, bacteria, and actinomycetes. And fungal endophytes are the most frequently encountered endophytes (Staniek *et al.*, 2008). It have great promise with diverse potential for exploitation (Li *et al.*, 2012; Staniek *et al.*, 2008). An enormous number of different fungi can be isolated from plants growing in their native habitat. Most of the fungi are uncommon and narrowly distributed, taxonomically and geographically. However a few

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fungi are widely distributed with the host, suggesting a long standing, close and mutually beneficial interaction. Ever since the discovery of the rich diversity of the endophytic fungi, their population dynamics, their role in improving plant growth, plant health (Hallmann *et al.*, 2007), their distribution in the plant, the metabolites they secrete and their potency to produce novel compounds within the plants (Tan and Zou, 2001), have formed an important aspect of present day research sources of bioactive substances with agricultural and/or pharmaceutical potential, as exemplified by taxol (Stierle *et al.*, 1993; Wang *et al.*, 2000), subglutinol A and B (Lee *et al.*, 1995) and peptide leucinostatin A (Stroble and Hess, 1997). Endophytic fungi are thus expected to be potential sources of new bioactive agents.

Nowadays, many endophytic fungi associated with palms had reported, including temperature palms and tropic palms (Rodrigues and Samuels 1990; Fröhlich and Hyde 2000; Hyde *et al.*, 2000). Jane Frohlich *et al.*, (2000) reported endophytic fungi from three unidentified *Licuala* sp. palms in Brunei Darussalam and from three *L. ramsayi* palms in Australia and got 75 fertile species in 48 genera and 60 sterile morphospecies including 10 *Xylaria* anamorphs, *Phomopsis* sp., *Phoma* sp., *Trichoderma* sp., *Colletotrichum* sp., *Pestalotiopsis palmarum.*, *Lasiodiplodia* sp. *Hyphomycete* sp., *Nodulisporium* sp., *Dictyochaeta* sp., *Phyllosticta* sp., *Distocercospora* sp., *Verticillium* sp., *Coelomycete* sp., *Aspergillus niger*, *Beltraniella* spp., *Botrytis allii*. The endophyte communities of both palms were composed of a single, dominant xylariaceous species.

The aim of this research focused on isolate endophytic fungi from leaves, petioles and roots of palm trees and identified by morphological characters.

Materials and methods

Plant Sample Collection

Plant samples were randomly collected from 10 species healthy palms at King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok 10520, Thailand. Species of palms were identified followed by Andrew Henderson *et al.*, (1995), Donald R. Hodel (1998), Langlois, Arthur C (1902) and David L. Jones (1995). All the samples from healthy leaves, petioles and roots of randomly select plants were cut and taken to the laboratory, processed within 24 h. 10 species palms were collected as follows:

1. *Ptychosperma macarthuri* (MacArthur Palm);
2. *Rhapis humilis* (Slender Lady Palm);
3. *Wodyetia bifurcata* (Foxtail Palm);
4. *Chrysalidocarpus lotescens* ;
5. *Veitchia merrillii* (Manila Palm)

6. *Phoenix roebelenii*;
7. *Rhapis Laosensis* (Thailand Lady Palm);
8. *Licula spinosa*;
9. *Livistona chinensis* (Chinese Fan Palm);
10. *Mascarena Lagencuulis* (Bottle Palm).

Isolation of Endophytic Fungi

Plant specimens were thoroughly washed in running tap water for 5 minutes remove dust and debris and then air dried. The cleaned leaves, petioles and roots were surface sterilized with 75% ethanol 1 min and sodium hypochlorite (3%available chlorine) 3-5min and then were remove outer epidermal tissues and cuticle before cut under sterile conditions into small pieces of 3×3×3 mm. Briefly, fragments were cleaned in sterilized water and sterilized in 75% ethanol 30s and then cleaned in sterilized water again and placed on water agar (WA) medium, incubate at room temperature waiting endophytic fungi grow out. The endophytic fungi growing out from the plant tissue were transferred into potato dextrose agar (PDA) plates and incubate for two to six days. Continuous plates were subculture until get pure culture.

Identification of Endophytic Fungi

The isolates of endophytic fungi were identified by the morphology of the fungal culture, including colony and medium color, Colony characters, Spore characters, Mycelium characters, Fruiting structures by following the standard mycological manuals (e.g. Ellis, 1971; Barnett and Hunter, 1987; Domsch and Games, 1993; Sutton, 1980; Nag Raj, 1993). The sterile isolates were grown on PDA with decoction of host leaves medium to observe sporulation. For tentative identification, microscopic slides of each endophytic fungi were prepared and examined under binocular compound microscope for morphological identification.

Results and Discussion

From leaves, petioles and roots of 10 species palm trees in this study yielded 105 isolates (Table. 1). More isolates were obtained from *Wodyetia bifurcate* (Foxtail Palm) than other palm trees. And, Root of palms can harbor more endophytics than leaves and petioles.

Table. 1 Number of isolates from leaf, petiole and root of 10 species palm trees

Host Plant	Leaf samples	Petiole samples	Root samples	Total
<i>Ptychosperma macarthurii</i>	2	4	5	11
<i>Rhapis humilis</i>	3	2	3	8
<i>Wodyetia bifurcate</i>	5	4	8	17
<i>Chrysalidocarpus lotescens</i>	3	1	7	11
<i>Veitchia merrillii</i>	3	3	6	12
<i>Phoenix roebelenii</i>	5	3	5	13
<i>Rhapis Laosensis</i>	4	1	3	8
<i>Licula spinosa</i>	3	2	5	10
<i>Livistona chinensis</i>	1	2	3	6
<i>Mascarena Lagencuulis</i>	2	3	4	9
Total	31	25	49	105

Total 105 isolates obtained from palm tissues in this study yielded 15 identified taxa (Table. 2). These identifiable cultures represented 60 (57.14%) of the total isolates. The remaining 45(42.86%) isolates did not sporulate and were grouped into Mycelia Sterilia fungi (MSF). The morphology characters of 15 identified endophytic fungi (Fig. 1) are as follows:-

1. *Cladosporium* spp.

The colonies range from olive or deep green to black color in PDA media. They are relatively slow-growing. Mycelium immersed and superficial. Conidiophores macronematous and micronematous, sometimes up to 350 μ long but generally much shorter, 2-6 μ thick, phal to mid olivaceous brown, smooth or verruculose. Conidia formed in long branched chains, mostly 0-septate, ellipsoidal or limoniform, pale olivaceous brown, most commonly smooth but verruculose in some strains.

Habit: *Ptychosperma macarthurii*; *Wodyetia bifurcate*

Luiz *et al.*, (2012) study evaluates the diversity of microbial community associated with healthy *E. purpurea* clones and their ability to produce defense compounds. They recovered and identified thirty-nine fungal endophytes, which were closely related to species of the following genera *Ceratobasidium*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Glomerella*, and *Mycoleptodiscus*.

2. *Phialophora* spp.

Colonies slow-growing and white color with purple. Mycelium partly superficial, partly immersed. Stroma none. Setae and hyphopodia absent. Conidiogenesis phialidic; phialides arising solitarily from vegetative hyphae or on branched conidiophores. Conidia aggregated in slimy heads,

one-celled, straight or curved, ellipsoidal or oblong rounded at the ends, courless, smooth, 0-septate.

Habit: *Phoenix roebelenii*

Fungal endophytes associated with the palms, *Calamus kerrianus* (rattan) and *Wallichia caryotoides* (taorang) were investigated at two sites within Doi Suthep-Pui National Park, Thailand. Thirty-five endophytic fungi isolated included xylariaceous taxa (20 morphotypes), sterile mycelia, one unidentified and 13 mitosporic fungi including *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Corynespora*-like sp., *Fusarium* sp., *Guignardia coccaicola*, *Paecilomyces* sp. *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phomopsis* sp., *Phyllosticta* sp., and *Sarcopodium* sp. (Saisamorn Lumyong *et al.*, 2009).

3. *Pestalotiopsis* sp1.

Colonies grow fast with white yellow color. Acervuli dark, discoid or cushion-shaped, subepidermal. Conidiophores short, simple; conidia dark, several-celled, with hyaline, pointed end cells, ellipsoid to fusoid, with two or more hyaline, apical appendages.

Habit: *Rhapis humilis*

Pestalotiopsis sp2.

Colonies slow-growing, with white and grey felty aerial mycelium. Pycnidia brown to pale, in spots, erumpent to subsuperficial, globose to flattened, with small ostiole. Conidia hyaline, 2-celled, cylindrical, with 3 to 4 hyaline setate at one end.

Habit: *Wodyetia bifurcate*

Endophytic fungi were isolated from three unidentified *Licuala* sp. palms in Brunei Darussalam and from three *L. ramsayi* palms in Australia. *Xylaria* spp., *Phomopsis* spp., *Pestalotiopsis* spp., *Nodulisporium* spp., *Colletotrichum* sp. and *Distocercospora* sp. were obtained as endophytic fungi (Jane Frohlich, 1999).

4. *Phoma* spp.

Colonies grow fast on PDA with abundant floccose, whitish to olivaceous-grey aerial mycelium; reverse uncoloured. Pycnidia abundantly produced in the centre of the colonies, olivaceous-brown, dark around the ostiole. Pseudosclerotia absent; conidia oblong, two-celled.

Habit: *Rhapis humilis*; *Wodyetia bifurcate*; *Chrysalidocarpus lotescens*; *Veitchia merrillii*; *Phoenix roebelenii*

The diversity of endophytic fungi in leaves, stems and roots from transgenic (Bt) and its isoline (non-Bt) cotton was evaluated during different plant developmental stages to investigate possible non-target effects of genetically modified cotton on endophytic fungal communities. A total of 17 genera of endophytic fungi were isolated. The most frequently isolated species were *Phomopsis archeri* from leaves and stems and *Phoma destructiva* from roots (P. D. de Souza Vieira *et al.*, 2011).

5. *Nigrospora* spp.

The white woolly colonies grow fairly rapidly. Colonies at first white with small, shining black conidia easily visible under a lower-power dissecting microscope, later brown when sporulation is abundant. Mycelium all immersed or partly superficial. Stroma none. Setae and hyphopodia absent. Conidiophores semi-macronematous, branched, flexuous, colourless to brown, smooth. Conidia solitary, with a violent discharge mechanism, acrogenous, simple, spherical or ellipsoidal, compressed dorsiventrally. Black, shining, smooth, 0-septate.

Habit: *Wodyetia bifurcate*; *Mascarena Lagencuulis*

Endophytic fungi were isolated from living symptomless leaves of 12 tree species from two locations in the Iwokrama Forest Reserve, Guyana. Species of *Colletotrichum*, *Nodulisporium*, *Pestalotiopsis* and *Phomopsis* were most frequently isolated, *Nigrospora* sp. also were identified as endophytic fungi. (Paul F. Cannon *et al.*, 2002).

6. *Xylaria* spp.

The colonies grow quite slow on PDA medium. Mycelium white color. The stromata are finger-like. ascocarps (fruitbodies) are black at the base but white and branched towards the top, Didn't produce ascospore on PDA medium.

Habit: *Wodyetia bifurcate*; *Veitchia merrillii*; *Phoenix roebelenii*

The occurrence of *Xylaria* species as endophytes in tropical plants is well documented (Bacon CW *et al.*, 1994; Bayman P *et al.*, 1997; Bussaban B. *et al.*, 2001; Clay K, 1988; Frohlich J *et al.*, 2000; Photita W *et al.*, 2001; Tomita F, 2003). Cladistic analyses in the study of Itthayakorn Promputtha *et al.* (2007) also show that more than one species of *Xylaria* can coexist within living leaf tissues of *M. liliifera*. In this study, most *Xylaria* species are endophytic in origin.

7. *Fusarium* sp1.

The colonies fast growing and moist, floccose, granulose, cream colour. Aerial mycelium floccose and whitish. Macro-conidia abundant and more-celled, slightly curved or bent at the pointed ends, typically canoe-shaped. Microconidia 1-celled, ovoid or oblong. Chlamydospores often sparse, globose, intercalary, pale, formed singly or in chains.

Habit: *Chrysalidocarpus lotescens*

***Fusarium* sp2.**

The colonies fast growing with discrete sporodochia and white-ochraceous colour. Aerial mycelium floccose. Macro-conidia abundant and more-celled, slightly curved or bent at the pointed ends; central part straight, cylindrical, typically canoe-shaped. Phialides bearing micro-conidia very long.

Habit: *Rhapis Laosensis*; *Mascarena Lagencuulis*; *Livistona chinensis*; *Licula spinosa*; *Ptychosperma macarthuri*; *Rhapis humilis*

The study of (Masroor Qadri *et al.*, 2013) was conducted to characterize and explore the endophytic fungi of selected plants from the Western Himalayas for their bioactive potential. A total of 72 strains of endophytic fungi were isolated and characterized morphologically as well as on the basis of ITS1-5.8S-ITS2 ribosomal gene sequence acquisition and analyses. Samples of *Platanus orientalis* were found to harbor only *Fusarium* spp. representing 4 different strains. *Artemesia annua* also possessed several strains of *Fusarium* as endophytes (5/22) whereas almost half of the endophytes of *Withania somnifera* (4/9) were also *Fusarium* spp.

8. *Rhizoctonia* spp.

Colonies grow quite fast on agar surface. The mycelium at first are white and later becoming black. The culture didn't produce spores, but are composed of hyphae and sclerotia (hyphal propagules). The cells of mycelium long, septa of branches set off from the main hyphae.

Habit: *Wodyetia bifurcate*; *Phoenix roebelenii*; *Licula spinosa*; *Livistona chinensis*

Harvais G. and Hadley G. (1967) Extensive isolation of root endophytes from *Orchis* {*Dacylorchis*} *pitrpurella* and other British orchids yielded a variety of strains of *Rhizoctonia* and of other fungi. *R. repens*, a common orchid endophyte, occurred in several host species and habitats. *R. solani* was uncommon, being obtained only from *Orchis piirpiirella* and *Coeloglossum viride* in certain situations.

9. *Colletotrichum* spp.

The colony growth slow, and mycelia initially white-grey and then become black-brown. Acervuli disc-shaped, subepidermal, typically with dark, spines at the edge or among the conidiophores. Conidia 1-celled, ovoid or oblong.

Habit: *Ptychosperma macarthuri*; *Veitchia merrillii*; *Licula spinosa*; *Mascarena Lagencuulis*

Fungal endophytes were isolated from leaves of *Centella asiatica* (Apiaceae) collected at Mangoro (E. F. Rakotoniriana *et al.*, 2007). The most common endophytes were the non-sporulating species 1 (isolation frequency IF 19.2%) followed by *Colletotrichum* sp.1 (IF 13.2%), *Guignardia* sp. (IF 8.5%), *Glomerella* sp. (IF 7.7%), an unidentified ascomycete (IF 7.2%), the nonsporulating species 2 (IF 3.7%) and *Phialophora* sp. (IF 3.5%).

10. *Chaetomium globosum*

Colonies slow growing with little superficial mycelium and a dense olivaceous layer os ascomata. Phialoconidia absent; ascomata dark brown or black, globose to subglobose; lateral hairs dark brown with paler tips, minutely roughened; terminal hairs dark olive brown with paler tips, wavy or loosely coiled and intertwined. Spores pale greenish to dark olive-brown, flattened lemon-shaped, hardly apiculate.

Habit: *Veitchia merrillii*; *Ptychosperma macarthuri*; *Rhapis Laosensis*

Endophytic fungi from the Chinese medicinal plant *Actinidia macrosperma* were isolated and identified for the first time (Yin Lu *et al.*, 2012). In total, 17 fungal isolates were obtained. Five different taxa were represented by 11 isolates (*Acremonium furcatum*, *Cylindrocarpon pauciseptatum*, *Trichoderma citrinoviride*, *Paecilomyces marquandii*, and *Chaetomium globosum*).

11. *Chaetomium aureum*

Colonies slow growing with immersed mycelium and little superficial mycelium, red and white colors. Phialoconidia absent; ascomata red, globose; lateral hairs dark brown with paler tips, minutely roughened; terminal hairs dark olive brown with paler tips and intertwined. Ascospores halfmoon-shaped with two apical germ pores and brown colour.

Habit: *Livistona chinensis*

In Pablo Martínez-Álvarez *et al.* (2015) study, 154 endophyte isolates were selected from a collection of 546 fungi tested in a preliminary confrontation assay. In total, 138 endophytes displayed antagonistic activity towards *F. circinatum* in the dual cultures of the in vitro experiment. In the field test, the endophytes *Chaetomium aureum* and *Alternaria* sp. reduced the area under disease progress curve (AUDPC) for the *P. radiata* seedlings, indicating that they may therefore be suitable for use as biological control agents (BCAs) of the disease.

12. *Gibberella* spp.

Colonies slow-growing and white colours. The ascocarp is globose with smooth walls. Ascomata abundantly. The asci are globose or ovoid-shaped, lenticular, valves smooth or slightly roughened, with a broad equatorial furrow, with very low ridges but no wings.

Habit: *Livistona chinensis*

Masroor Qadri *et al.* (2013) conducted to characterize and explore the endophytic fungi of selected plants from the Western Himalayas for their bioactive potential. A total of 72 strains of endophytic fungi were isolated. *Gibberella moniliformis*, *Chaetomium globosum*, *Alternaria* spp., *Fusarium* spp., *Cladosporium cladosporioides* and so on were collected and identified based on morphology and molecular methods.

13. *Emericella* spp.

Colonies growing restrictedly, with few green conidial heads and abundant purple-brown ascomata formed in several layers. Ascomata surrounded by dark brown, globose hulle cells. Ascospores purple-red, lenticular, rugulose, with two sinuate equatorial crests.

Habit: *Mascarena Lagencuulis*

Chemical investigation of the endophytic fungus *Emericella* sp. (HK-ZJ) isolated from the mangrove plant *Aegiceras corniculatum* led to

isolation of six isoindolones derivatives termed as emerimidine A and B and emeriphenolicins A and D, and six previously reported compounds named aspernidine A and B, austin, austinol, dehydroaustin, and acetoxydehydroaustin, respectively (Zhang, G.J. *et al.*, 2011). In this paper, the first isolation, structural elucidation and biological evaluation of novel isoindolone derivatives from the fungal endophyte *Emericella* sp. (HK-ZJ) was reported.

14. *Aspergillus* sp.

Colonies growing rather slow on PDA with creamy-yellow color. Mycelium partly immersed, partly superficial. Stroma none; Setae and hyphopodia absent. Vesicles small, variable in shape. Conidial heads globose and bright yellow. Conidia globose to subglobose, smooth-walled, uninucleate, the chains sometimes sliming down.

Habit: *Wodyetia bifurcate*; *Veitchia merrillii*

Nur Amin *et al.* (2014) isolate and identify fungal endophyte from clones cocoa resistant VSD M.05 and clones cocoa susceptible VSD M.01. A total of 10 isolates of fungal endophytes were isolated from clones cocoa resistant VSD M.05. The isolates belonged to 6 genera namely: *Curvularia* sp., *Fusarium* sp., *Geotrichum* sp., *Aspergillus* sp., *Gliocladium* sp., *Colletotrichum* sp.. The fungal endophytes were isolated from clones cocoa susceptible VSD M.01, that as 4 genera identified as *Aspergillus* sp., and *Gliocladium* sp..

15. *Curvularia lunata*

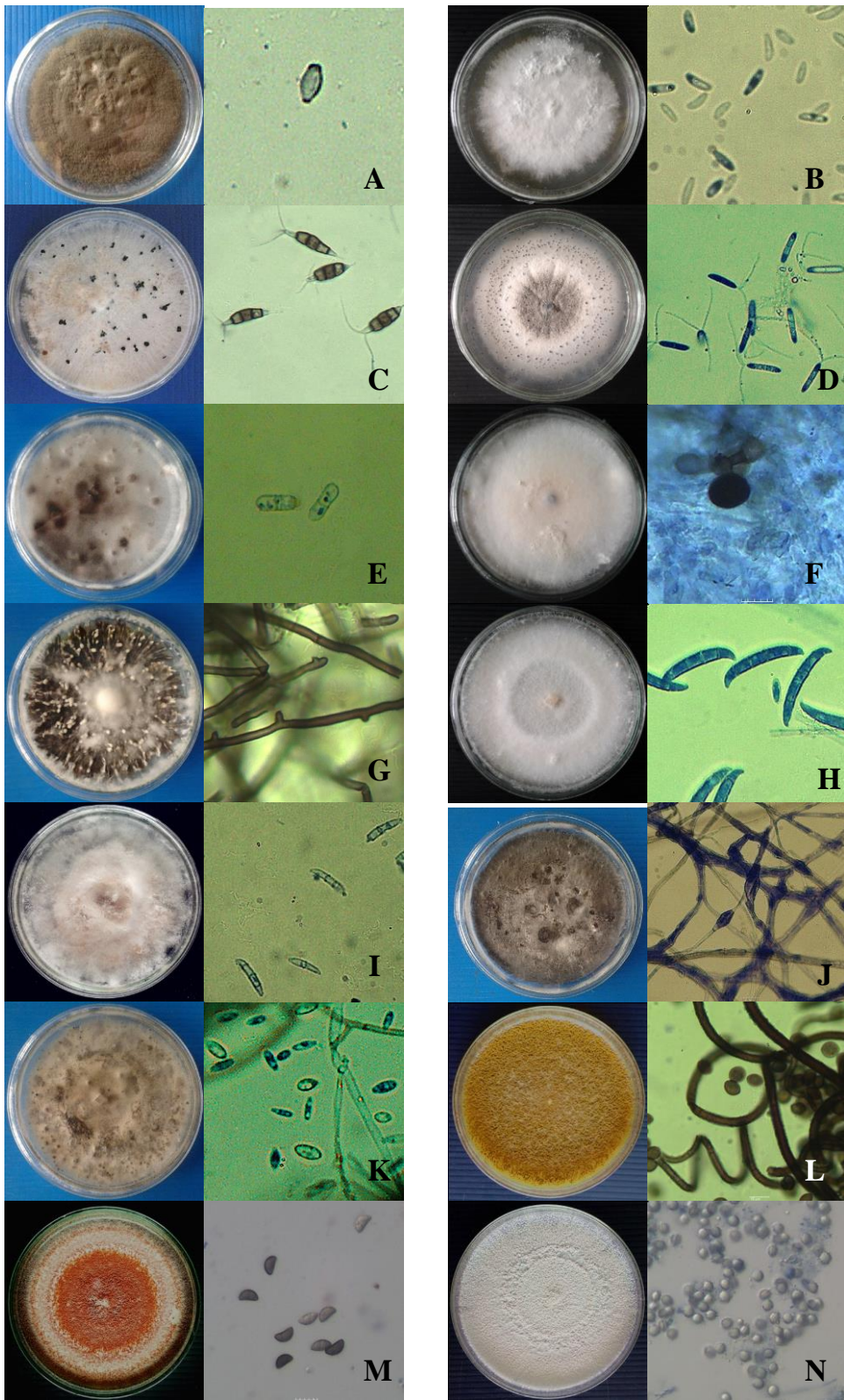
Colonies effuse, hairy and black color on PDA medium. Mycelium immersed in natural substrata. Conidia with hilum scarcely, remaining smooth-walled, dark brown color. Conidia predominantly 3-septate, the middle septum below the centre and the third cell strongly curved, tapering gradually towards the base.

Habit: *Phoenix roebelenii*

The present study of Ines Ben Chobba *et al.* (2013) investigating the diversity of both cultivable and non-cultivable endophytic fungal floras in the internal tissues (roots and leaves) of Tunisian date palm trees (*Phoenix dactylifera*). Accordingly, 13 isolates from both root and leaf samples, exhibiting distinct colony morphology, were identified by a sequence match search where in their 18S–28S internal transcribed spacer (ITS) sequences were compared to those available in public databases. The cultivable root and leaf isolates mostly fell into *Alternaria* spp., *Fusarium* spp., *Curvularia* spp., *Cladosporium* spp. and *Pythium* spp..

Table 2. Taxon of endophytic isolates

Taxon	Class and order	Family and Genus	Reference
<i>Cladosporium</i> spp.	Dothideomycetes; Capnodiales	Davidiellaceae; <i>Cladosporium</i>	Luiz H. Rosa <i>et al.</i> , 2012; Ines Ben Chobba <i>et al.</i> 2013
<i>Phialophora</i> spp.	Eurotiomycetes Chaetothyriales	Herpotrichiellaceae <i>Phialophora</i>	E. F. Rakotoniriana <i>et al.</i> , 2007
<i>Pestalotiopsis</i> spp.	Sordariomycetes Xylariales	Amphisphaeriaceae <i>Pestalotiopsis</i>	Jane Frohlich, 1999
<i>Phoma</i> spp.	Dothideomycetes Pleosporales	Incertae sedis <i>Phoma</i>	Luiz H. Rosa <i>et al.</i> , 2012
<i>Nigrospora</i> spp.	Sordariomycetes; Trichosphaeriales	Trichosphaeriaceae; <i>Nigrospora</i>	Paul F. Cannon <i>et al.</i> , 2002
<i>Xylaria</i> spp.	Sordariomycetes; Xylariales	Xylariaceae; <i>Xylaria</i>	Archana Nath <i>et al.</i> , 2012; E. F. Rakotoniriana <i>et al.</i> , 2007;
<i>Fusarium</i> spp.	Sordariomycetes Hypocreales	Nectriaceae <i>Fusarium</i>	Luiz H. Rosa <i>et al.</i> , 2012; Masroor Qadri <i>et al.</i> , 2013; Ines
<i>Rhizoctonia</i> spp.	Agaricomycetes; Cantharellales	Ceratobasidiaceae; <i>Rhizoctonia</i>	Harvais G. and Hadley G., 1967
<i>Colletotrichum</i> spp.	Sordariomycetes Glomerellales	Glomerellaceae <i>Colletotrichum</i>	Luiz H. Rosa <i>et al.</i> , 2012; E. F. Rakotoniriana <i>et al.</i> , 2007
<i>Chaetomium globosum</i>	Sordariomycetes Sordariales	Chaetomiaceae <i>Chaetomium</i>	Yin Lu <i>et al.</i> , 2012
<i>Chaetomium aureum</i>	Sordariomycetes Sordariales	Chaetomiaceae <i>Chaetomium</i>	Pablo Martínez-Álvarez <i>et al.</i> , 2015;
<i>Giberrella</i> spp.	Sordariomycetes Hypocreales	Nectriaceae <i>Giberrella</i>	Masroor Qadri <i>et al.</i> , 2013
<i>Emericella</i> spp.	Eurotiomycetes Eurotiales	Trichocomaceae <i>Emericella</i>	Zhang, G.J. <i>et al.</i> , 2011
<i>Aspergillus</i> spp.	Eurotiomycetes Eurotiales	Trichocomaceae <i>Aspergillus</i>	Nur Amin <i>et al.</i> 2014;
<i>Curvalaria lunata</i>	Euascomycetes Pleosporales	Pleosporaceae <i>Curvalaria</i>	Ines Ben Chobba <i>et al.</i> 2013; Nur Amin <i>et al.</i> 2014



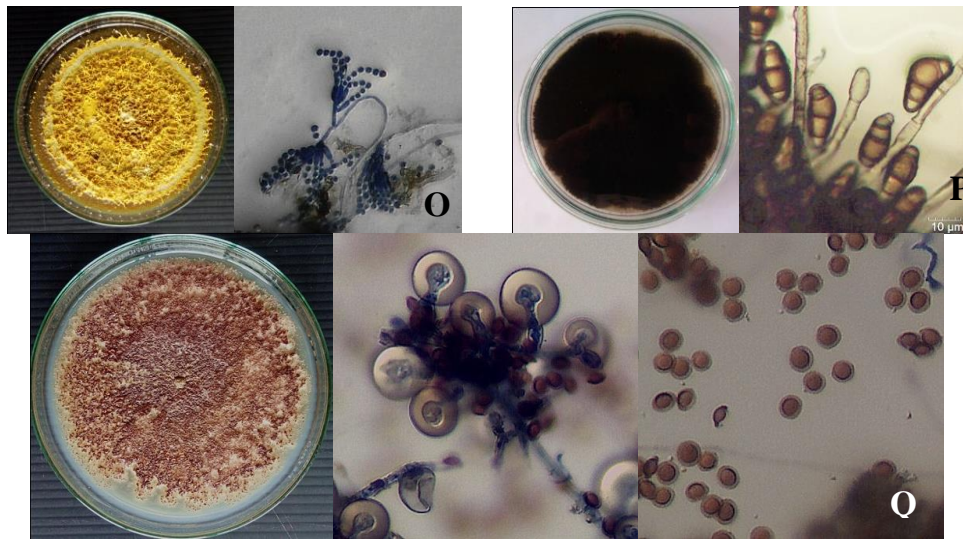


Figure 1. Endophytic isolates

A: *Cladosporium* spp.; B: *Phialophora* spp.; C: *Pestalotiopsis* sp1.; D: *Pestalotiopsis* sp2.; E: *Phoma* spp.; F: *Nigrospora* spp; G: *Xylaria* spp.; H: *Fusarium* sp1; I: *Fusarium* sp2.; J: *Rhizoctonia* spp.; K: *Colletotrichum* spp.; L: *Chaetomium globosum*; M: *Chaetomium aureum*; N: *Giberrella* spp.; O: *Aspergillus* spp.; P: *Curvalaria lunata*; Q: *Emericella* spp.

Table 3. Isolate Numbers of each species

Endophytic fungi	Isolate from			total
	leaf	petiole	root	
<i>Cladosporium</i> spp.	-	2	1	3 (2.85%)
<i>Phialophora</i> spp.	1	-	3	4 (3.80%)
<i>Pestalotiopsis</i> spp.	1	-	1	4 (3.80%)
<i>Phoma</i> spp.	2	1	2	5 (4.76%)
<i>Nigrospora</i> spp.	3	-	2	5 (4.76%)
<i>Xylaria</i> spp.	5	3	1	9 (8.57%)
<i>Fusarium</i> spp.	3	4	6	13(12.38%)
<i>Rhizoctonia</i> spp.	1	-	2	3 (2.85%)
<i>Colletotrichum</i> spp.	2	1	3	6(5.71%)
<i>Chaetomium globosum</i>	-	1	-	1(1.05%)
<i>Chaetomium aureum</i>	-	1	-	1(1.05%)
<i>Giberrella</i> spp.	-	1	-	1(1.05%)
<i>Emericella</i> spp.	-	-	1	1(1.05%)
<i>Aspergillus</i> sp.	-	-	1	1(1.05%)
<i>Curvalaria lunata</i>	3	-	-	3(2.85%)
Mycelia Sterilia	12	13	20	45(42.85%)
Total	33	27	45	105

In this study, mycelia sterilia had the highest relative frequency (42.86%) (Table.2). *Fusarium* spp. (12.38%) which are frequently identified as endophytes (Masroor Qadri *et al.*, 2013) was the second most frequent

endophytic group. Followed by *Xylaria* spp.; *Colletotrichum* spp.; *Phoma* spp. and *Nigrospora* spp. These were the dominant genera or order of endophytic fungi found in this study, similar to the findings reported previously for many tropical endophytic fungi (Luiz H. Rosa *et al.*, 2012; Nur Amin *et al.*, 2014; Jane Frohlich, 1999).

Conclusion

Fifteen species of endophytic fungi were isolated and identified from leaves, petioles and roots of 10 species palm trees which belong to *Fusarium* spp., *Xylaria* spp., *Cladosporium* spp., *Phialophora* spp., *Pestalotiopsis* spp., *Rhizoctonia* spp., *Colletotrichum* spp., *Chaetomium globosum*, *Chaetomium aureum*, *Giberrella* spp., *Emericella* spp., *Aspergillus* spp., *Curvalaria lunata*, *Phoma* spp. and *Nigrospora* spp.. 45 unidentified isolates were group to mycelia sterilia fungi which was morphological fungal types, but not forming true spores. Mycelia sterilia fungi had the highest relative frequency (42.86%) in this study and *Fusarium* was the second most frequent endophytic group, followed by *Xylaria* spp.; *Colletotrichum* spp.; *Phoma* spp. and *Nigrospora* spp.

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References

- Andrew, H., Galeano, H. and Bernal, R. (1995). Field guide to the palms of the Americas. *Princeton University Press*, Princeton, New Jersey 08540.
- Arnold, A. E. (2005). Diversity and ecology of fungal endophytes in tropical forests. In: *Current trends in mycological research*, Deshmukh, D. (ed.). pp. 49-68.
- Bacon, C. W. and White, J. F. (2000). *Microbial Endophytes*. (New York: Marcel Deker Inc.).
- Bacon, C. W. and White, J. F. (1994). *Biotechnology of Endophytic Fungi of Grasses*. CRC Press, Boca Raton, FL.
- Barnett, H. L. and Hunter, B. B. (1987). *Illustrated Genera of Imperfect Fungi*. Macmillan Publishing Company 866 Third Avenue, New York, New York. 10022.
- Bayman, P, Lebro, L. L., Tremblay, R. L. and Lodges, D. J. (1997). Variation in endophytic fungi from roots and leaves of *Lepanthes* (Orchidaceae). *The New Phytologist* 135:143-149.
- Ben, C., Amine, E., Imen, A., Lamia, K., Ahmed, N., Frederique, C., Noureddine, D., Néji, G. and Tatiana, V. (2013). Fungal diversity in adult date palm (*Phoenix dactylifera* L.) revealed by culture-dependent and culture-independent approaches. *Journal of Zhejiang University* 14:1084-1099.

- Bussaban, B., Lumyong, S., Lumyong, P., McKenzie, E. H. C. and Hyde, K. D. (2001). Endophytic fungi from *Amomum siamense*. *Canadian Journal of Microbiology* 47: 943-948.
- Clay, K. (1988). Fungal endophytes of grasses: a defensive mutualism between plants and fungi. *Ecology* 69:10-16.
- Domsch, K. H., Gams, W. and Anderson, T. H. (1980). *Compendium of soil fungi*. Volume 1. Academic Press (London) Ltd.
- Donald R. H. (1998). *The palms and Cycads of Thailand*. Kampon Tansacha, Nong Nooch Tropical Garden, Thailand. *Allen Press*, Lawrence, Kansas, USA.
- Ellis M. B. (1971). Dematiaceous Hyphomycetes. *Principal Mycologist, Commonwealth Mycological Institute, Kew*. pp. 312-318.
- Froehlich, J. and Petrini, O. (2000). Endophytic fungi associated with palms. *Mycological Research* 104:1202-1212.
- Froehlich J. and Hyde K. D. (2000). Palm Microfungi. *Fungal Diversity Research Series*.
- Froehlich, J., Hyde, K. D. and Petrini, O. (2000). Endophytic fungi associated with palms. *Mycological Research* 104:1202-1212.
- Hallmann, J., Berg, G. and Schulz, B. (2007). *Isolation procedures for endophytic microorganisms*. Springer Berlin Heidelberg, New York.
- Harvais, G. and Hadley, G. (1967). The Relation Between Host and Endophyte in Orchid Mycorrhiza. *New Phytology* 66:205-215.
- Ithayakorn, P., Lumyong, S., Dhanasekaran, V., McKenzie, E. H. C., Hyde, K. D. and Jeewon, R. (2007). A Phylogenetic Evaluation of Whether Endophytes Become Saprotrophs at Host Senescence. *Microbial Ecology* 53:579-590.
- Jones, D. L. (1995). *Palms throughout the world*. Smithsonian Institution Press. Washington, D.C.
- Langlois, A. C. (1902). *Supplement to palms of the world*. Horticultural Books, Inc. P.O. Box 107,129 Martin Ave. Stuart, Florida 33494.
- Lee, J. C., Lobkovsky, E., Pliam, N. B., Stroble, G. A. and Clardy, J. (1995). Subglutinol A and B: immunosuppressive compounds from the endophytic fungus *Fusarium subglutinans*. *The Journal of Organic Chemistry* 60:7076-7077.
- Li, H. Y., Wei, D. Q., Shen, M. and Zhou, Z. P. (2012). Endophytes and their role in phytoremediation. *Fungal Diversity* 54:1-18.
- Rosa, L. H., Tabanca, N., Tehen, N., Wedge, D. E., Pan, Z., Bernier, U. R. and Moraes, R. M. (2012). Diversity and Biological Activities of Endophytic Fungi Associated with Micropropagated Medicinal Plant *Echinacea purpurea* (L.) Moench. *American Journal of Plant Sciences* 3:1105-1114.
- Martínez-Álvarez, P., Fernández-González, R. A., Sanz-Ros, A. V., Pando, V., and Diez, J. J. (2016). Two fungal endophytes reduce the severity of pitch canker disease in *Pinus radiata* seedlings. *Biological control* 94:1-10.
- Masroor, Q., Johri, S., Shah, B. A., Khajuria, A., Sidiq, T., Lattoo, S. K., Abdin, M. Z. and Riyaz-Ul-Hassan, S. (2013). Identification and bioactive potential of endophytic fungi isolated from selected plants of the Western Himalayas. *SpringerPlus* 2:8 <http://www.springerplus.com/content/2/1/8>.
- Raj, N. (1993). *Coelomycetous Anamorphs with Appendage Bearing Conidia*. Edwards Brothers Publishing Co., Ann Arbor, Michigan, USA.
- Amin, N., Salam, M., Junaid, M., Asman and Baco, M. S. (2014). Isolation and identification of endophytic fungi from cocoa plant resistente VSD M.05 and cocoa plant Susceptible VSD M.01 in South Sulawesi, Indonesia. *International Journal of Current Microbiology and Applied Sciences* 3:459-467.
- Paul, F. C. and Simmons, C. M. (2002). Diversity and host preference of leaf endophytic fungi in the Iwokrama Forest Reserve, Guyana. *Mycologia*. pp. 210-220.

- Photita, W., Lumyong, S. and Lumyong, P. (2001). Endophytic fungi of wild banana (*Musa acuminata*) at doi Suthep Pui National Park, Thailand. *Mycological Research* 105:1508-1513.
- Rakotoniriana, E. F., Munaut, F., Decock, C., Randriamampionona, D., Andriambololoniaina, M., Rakotomalala, T. and Mahillon, J. (2008). Endophytic fungi from leaves of *Centella asiatica*: occurrence and potential interactions within leaves. *Antonie van Leeuwenhoek* 93:27-36.
- Redecker, D., Kodner, R. and Graham, L. E. (2000). Glomalean fungi from the Ordovician. *Science* 289:1920-1921.
- Rodrigues, K. F. and Samuels, G. J. (1990). Preliminary study of endophytic fungi in a tropical palm. *Mycological Research* 94:827-830.
- Saisamorn, L., Techa, W., Lumyong, P., McKenzie, E. H. C. and Hyde, K. D. (2009). Endophytic Fungi from *Calamus kerrianus* and *Wallichia caryotoides* (Arecaceae) at Doi Suthep-Pui National Park, Thailand. *Chiang Mai Journal of Science* 36:158-167.
- Staniek, A., Woerdenbag, H. J. and Kayser, O. (2008). Endophytes: exploiting biodiversity for the improvement of natural product-based drug discovery. *Journal Plant Interact* 3:75-93.
- Stierle, A., Strobel, G. A. and Stierle, D. (1993). Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungi of Pacific yew. *Science* 260:214-216.
- Strobel, G. A. and Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews* 67:491-502.
- Stroble, G. A. and Hess, W. M. (1997). Glucosylation of the peptide leucinostatin A, produced by an endophytic fungus of European yew, may protect the host from leucinostatin toxicity. *Chemistry & Biology* 4:529-536.
- Sutton, B. C. (1980). *The Coelomycetes - Fungi Imperfecti with pycnidia, acervuli and stromata*. Commonwealth Mycological Institute, Kew, UK.
- Tan, R. X. and Zou, W. X. (2001). Endophytes: a rich source of functional metabolites. *National Product Reports* 18:448-459.
- Tomita, F. (2003). Endophytes in Southeast Asia and Japan: their taxonomic diversity and potential applications. *Fungal Divers* 14:187-204.
- Wang, J., Li, G., Lu, H., Zheng, Z., Huang, Y. and Su, W. (2000). Taxol from *Tubercularia* sp. strain TF5, an endophytic fungus of *Taxus mairei*. *Federation of European Microbiological Societies* 193:249-253.
- Yin, L., Chen, C., Chen, H., Zhang, J. and Chen, W. (2012). Isolation and Identification of Endophytic Fungi from *Actinidia macrosperma* and Investigation of Their Bioactivities. Hindawi Publishing Corporation, Evidence-Based Complementary and Alternative Medicine. doi:10.1155/2012/382742.
- Zhang, G., Sun, S., Zhu, T., Lin, Z., Gu, J., Li, D. and Gu, Q. (2011). Antiviral isoindolone derivatives from an endophytic fungus *Emericella* sp. associated with *Aegicerus corniculatum*. *Phytochemistry* 72:436-442.

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