CHAPTER 2

LITERTURE REVIEW

Endophytic fungi, at the beginning were applied for any organism found within the plant by De Bary in 1866. Then the term endophyte was refered to the diverse group of fungi which live asymptomatic within photosynthetic tissue of plant for all or some part of their life cycle (Petrini, 1986; Maheshwari, 2006; Arnold, 2007). In the broadest sense, endophytic fungi are fungi that colonize or habit in healthy living inter plant tissue without causing any disease (Figure 1) (Stone *et al*., 2004). Moreover, endophytic fungi also refer to a group of primarily ascomycetous fungi (Arnold, 2007). This definition includes virtually the entire spectrum of symbiotic interactions in which fungi and plants participate: parasitism, commensalism and mutualism (Stone *et al*., 2004). These ubiquitous fungi can be found in every plant species and all higher plant is host to one or more (Arnold *et al*., 2000; Strobel and Daisy, 2003). Communities of these stupendous diversity microfungi are ranging from the arctic to the tropics (Arnold, 2007). The relationship between fungal endophyte and their host plant has been discovered in fossilized tissue (Taylor, 1999) and can be symbiotic to pathogenic (Clay and Holah, 1999; Strobel, 2002a.). For grass (primary Poaceae) the word endophyte has been used to denote a particular type of systemic, nonpathogenic symbiosis. Grass endophytes provide their hosts with a number of benefits, such as protection against herbivory and pathogens, that increase their fitness (Saikkonen *et al*., 2000).

Figure 1 Hypha of endophytic fungi; A: *Rhabdocline parkeri* (arrows) in Douglas fir (*Pseudotsuga taxifolia*) needles (×400), B: Hypha of *Stagonospora innumerosa* in an epidermal cell of *Juncus effuses* var *pacificus* (Stone *et al*., 2004).

2.1 Mode of endophytic infection and colonization

The colonization of plant tissues by endiphytes, plant pathogens and mycorrhizae involves several steps involving host recognition, spore germination, penetration of the epidermis (Figure 1) and tissue colonization (Petrini, 1991, Petrini, 1996). The inoculum source of fungal endophytes widely considered to be the airborne spore and also seed transmission and transmission of propagules by insect vectors (Petrini, 1991). A high level of genetic diversity of endophyte isolates suggests that infection foci arise from different strins of derived from constant new inocula (Hammerli *et al*., 1992; Rodrigues, 1994). In terms of mechanical and enzymatic of penetration by endophytic fungi, it can be assumed that endophytes adopt the same strategy for penetration of host tissue as pathogens (Petrini *et al*., 1992b). Fungi can invade plant tissues by direct cuticular penetration, *via* appressoria formed on the cuticle, after which penetration occurs through the cuticle and epidermal cell wall or *via* natural like stomata (O'Donnell and Dickinson, 1980; Viret and Petrini, 1994). Following

penetration the infection may be inter-cellular or intra- cellular and may be limited to one cell or in a limited area around the penetration site. Limited cytological work on nonclavicipitaceous endophytes has shown that infection of these endophytes in host plants may be inter or intra- cellular and often localized in single cells (Cabral *et al*., 1993).

Some endophytic fungi including those which are latent pathogens are host-specific, whereas other seems to invade any available hosts (Petrini *et al*., 1992a). The study on the infection of *Juncus* spp. with the endophytes *Stagnospora innumerosa* and *Drecheslera* spp., Cabral et al. (1993) observed callose formation in the individual cells as a host defense response.

Schulz *et al*. (1999) studied the secondary metabolites produced by the endophytes and their host interaction in order to understand why endophytic infections are symptomless. The production of herbicidal active substances was three times that of soil isolates and twice that of phytopathigenic fungi, whereas the the phenolic metabolites in the host were higher in the roots of plants infected with an endophyte than infected with pathogens. Their study hypothesized that both the pathogen- host and endophyte- host interaction involved constant mutual antoganism, at least in part based on the secondary metabolite which the partners produce. The pathogen-host interaction was thought to be imbalanced and resulted in disease while that of the endophytes and its host is a balanced antagonism.

2.2 Biodiversity and distribution of endophytic fungi

An endophytic habit apparently has evolved independently numerous times and represented by fungi in various orders of the Ascomycetes (Tables 1). Species composition of endophyte assemblages and infection frequencies vary according to host species; site characteristics, such as elevation, exposure and associated vegetation; tissue type; and tissue age (Stone *et al*., 2004). Arnold (2007) used molecular sequence data of 1403 endophyte strains which collected from arctic to tropical sites and the result showed that endophytes increase in incidence, diversity, and host breadth from arctic to tropical sites. Endophyte communities from higher latitudes are characterized by relatively few species from many different classes of Ascomycota, whereas tropical endophyte assemblages are dominated by a small number of classes with a very large number of endophytic species.

Table 1 Genera of endophytes commonly isolated from the foliage of woody perennials (Stone *et al*., 2004)

Holomorph order	Endophyte genera
Leotiales	Pezicula, Cryptosporiopsis, Phlytema, Chloroscypha, Sirodothis, Gremmeniella, Brunchorstia, Phragmopycnis, Rhabdocline
Dothidiales	Hormonema, Stagonospora, Phyllosticta
Pleosporales	Pleospora, Alternaria, Curvularia, Sporormia, Sporormiella, Stemphyllium
Diaporthales	Diaporthe, Phomopsis, Apiognomonia, Discula, Cytospora, Gnomonia, Ophiognomonia
Diatrypales	Libertella, Diatrypella, Diatrype, Eutypa
Rhytismatales	Ceuthospora, Lophiodermium, Tryblidiopsis, Cyclaneusma
Xylariales	Coniochaeta, Hypoxylon, Biscogniauxia, Camillea, Geniculosporium, Nodulisporium, Virgariella, Periconiella, Xylaria
Sordariales	Chaetomium, Sordaria, Gelasinospora
Hypocreales	Clonostachys, Cylindrocarpon, Dendrodochium, Fusarium, Gibberella, Gliocladium, Nectria, Trichoderma, Stilbella, Volutella

Table 1 (continued)

Holomorph order	Endophyte genera
Amphisphaeriales	Pestalotiopsis, Seiridium, Pestalotia, Seimatosporium
Polystigmatales	Glomerella, Colletotrichum
Uncertain	Phialocephala, Cryptocline, Gelatinosporium, Acremonium, IdriellaFoeostoma, Kabatina, Sirococcus

Lin *et al*. (2007) isolated endophytic fungi from the pharmaceutical plant, *Camptotheca acuminate* and obtained 174 isolated in 18 taxa. Non-sporulating fungi (48.9%), Alternaria (12.6%), Phomopsis (6.9%), Sporidesmium (6.3%), Paecilomyces (4.6%) and Fusarium. ITS rDNA assay indicated that most of the nonsporulating fungi belonged to the Pyrenomycetes and Loculoascomycetes ascomycetes or their anamorph Coelomycetes (4.6%) were dominant. The endophytic fungi with bioactivities were distributed in more than 12 taxa including nonsporulating fungi, which are reliable sources for bioactive agents.

Rakotoniriana *et al*. (2007) isolated fungal endophyte from leaves of *Centella asiatica* (Apiaceae) in Madagascar. Forty- five different taxa were recovered and the overall foliar colonization rate was 78%. The most common endophytes were the nonsporulating 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 non-sporulating species 2 (IF 3.7%) and *Phialophora* sp. (IF 3.5%). Using sequences of the ribosomal DNA internal transcribed spacer (ITS) regions, major endophytes (IF > 7%) were identified as xylariaceous taxa or as *Colletotrichum higginsianum*, *Guignardia mangiferae* and *Glomerella cingulata*.

Wang *et al*. (2007) found 17 genera of fungal endophytes from 4 native Gossypium species. Phoma, Alternaria, Fusarium, Botryosphaeria, Dichomera and Phomopsis were common.

Larran *et al*. (2006) isolated the endophytic fungi from leaves, stems, glumes and grains of 5 wheat cultivars and 722 isolates of endophytic fungi recovered were identified as 30 fungal genera. The fungi which showed the highest colonization frequency (CF%) in all the tissues and organs analysed were *Alternaria alternata, Cladosporium herbarum, Epicoccum nigrum, Cryptococcus* sp., *Rhodotorula rubra, Penicillium* sp. and *Fusarium graminearum*.

Naik *et al*. (2006) isolated fungal endophyte from *Oryza sativa* found 19 fungal taxa and *Chaetomium globosum*, *Penicillium chrysogenum*, *Fusarium oxysporum* and *Cladosporium cladosporioides* were dominant.

Suryanarayanan *et al*. (2003) studied the distribution, diversity and host recurrence in 24 tree hosts of endophytic fungi of two dry tropical forests. From 3600 tissue segments were yield 81 endophyte taxa and suggest that dry tropical forests are not hyperdiverse with reference to endophytes and the generalists among endophytes be identified before extrapolating data to calculate global fungal diversity.

Santos *et al*. (2003) collected endophytic fungi, obtained from living apparently symptomless roots, stems, leaves and fruits of *Melia azedarach*, an exotic tree introduced into Brazil from Asia and was a producer of insecticidal compounds. A total of 55 fungal isolates were recovered. Hyphomycetes were more prevalent than Ascomycetes, Coelomycetes and Basidiomycetes. The genera Aspergillus and Penicillium were the most common in this plant.

Canon and Simmons (2002) isolated endophytic fungi from 12 trees species from two locations in the Iwokrama Forest Reserve, Guyana. Sixty-four fungal morphotaxa were characterized from 2,492 cultures, which were derived from a total of 2520 sample units. Species of Colletotrichum, Nodulisporium, Pestalotiopsis and Phomopsis were most frequently isolated. Colonization was greater in samples from the midrib than from laminar tissue and slightly greater at the tip of the lamina compared with the base of the leaf. In contrast to studies in temperate ecosystems, no distinct fungal communities were identified for individual plant species.

Hata *et al*. (2002) isolated fungal endophyte from *Pasania edulis* leaves, one of the most important trees of the warm temperate forests in Japan. Phyllosticta, Colletotrichum an ascomycetous fungus (Ascomycete species. 1) and Phomopsis were frequently isolated. Phyllosticta was isolated more frequently from petiole segments and leaf segments with midrib and *Phomopsis* sp. from petiole segments and leaf-base segments with midrib than other segments. *Colletotrichum* spp. were isolated less frequently from petioles and Ascomycete species. 1 from petiole segments and leaf-base segments with midrib than other segments. As possible causes of such biases in within-leaf distributions of the endophytes, differences in infection modes and negative interactions of major endophytes within leaves are suggested.

Gamboa and Bayman (2001) screened for diversity and composition of endophytic fungal communities from two populations of *Guarea guidonia* trees (Meliaceae) in the Luquillo region, Puerto Rico. From the survey, Thirty-eight morphospecies of endophytes were found. Phomopsis, Colletotrichum, Xylaria, and Rhizoctonia-like fungi were the most abundant taxa and over *95* percent of the leaf pieces had endophytes. Fungal communities were stratified according to height within a tree, but no difference was found between blade, petiolule, and rachis.

Fungal endophytes have been studied for over 70 years (Lewis, 1924). From 1971- 1990, the journal publication about the diversity of fungal endophytes associated with above-ground tissue of non-grass ca 1.2 papers per year and from 2001 to early 2007 is increase to more than 15 papers per year (Arnold, 2007). After the diversity study, the scientist found out that the endophytic fungi was the great source novel natural products (Li *et al*., 1998; Strobel *et al*., 2002b) which were useful in medicine, agriculture and industry, research focusing on the interaction with host plant, ultra structural and colonization, characterization of novel metabolites and other topics related to endophytic symbioses has been dramatically increase from 0.8 papers per years to more than 200 papers per years in over the past six years (Arnold, 2007).

2.3 Isolation of endophytic fungi

By definition, endophytic fungi are the fungi which live inside plant tissue without the appearance of any symptom (Petrini, 1996; Maheshwari, 2006; Arnold, 2007) so it is important to understand the methods and rationale used to provide the best opportunities to isolate novel endophytic microorganisms as well as ones making

novel bioactive products. A specific rationale for the collection of each plant for endophyte isolation and natural-product discovery is used. Several reasonable hypotheses govern this plant selection strategy and these are as follows (Strobel and Daisy, 2003). (i) Plants from unique environmental settings, especially those with an unusual biology and possessing novel strategies for survival are seriously considered for study. (ii) Plants that have an ethnobotanical history (use by indigenous people) that are related to the specific uses or applications of interest are selected for study. Ultimately, it may be learned that the healing powers of the botanical source, in fact, may have nothing to do with the natural products of the plant, but of the endophyte (inhabiting the plant). (iii) Plants that are endemic, that have an unusual longevity, or that have occupied a certain ancient land mass are also more likely to lodge endophytes with active natural products than other plants. (iv) Plants growing in areas of great biodiversity also have the prospect of housing endophytes with great biodiversity.

Bacon and White (1994) have written a review on staining, media and procedure for analyzing endophytes. Endophytes can be isolated from various plant parts such as seeds, leaf and stem or direct isolation of ascospores is also in practice. The plant and the plant parts collected for studying should look apparently healthy. A young tissue is appropriate for isolation as older tissues containing many additional fungi that make isolation of slow growing fungi difficult. The sample should be processed in the shortest time possible after collection. Plant part of investigation should be cut into small pieces to facilitate sterilization and isolation process. Bill (1996) discussed various surface sterilization techniques in details. Any method (Table 2) used for surface sterilization provided that it could eliminate most of the epiphytic fungi from

the exterior tissues and encourage the growth of the internal mycobiota. The method used by Petrini *et al*. (1992a) has been used extensively and found very successful in studying endophytes (Rodrigues and Samuels, 1990; Schulz *et al*., 1993). This method comprises dipping sample in 96% ethanol for 2 min then in 65 % commercial Clorox (final concentration 3.25% aqueous sodium hypochlorite) for 10 min and finally in 96% ethanol for 30 sec. Malt extract agar is considered the most suitable medium for the growth and sporulation of endophytic fungi (Bill and Polishook, 1992 and Bill, 1996). Amendment of medium with streptomycin sulfate is practical to prevent bacterial contamination. To prevent the fast growing fungi overgrowing the plate, growth inhibitors, rose bengal is added to the medium.

Surface-sterilized plant tissues are plated in an appropriate medium amended with antibiotics and rose bengal then incubated at room temperature with periodic light and darkness. Incubated plates are checked after 1 week of incubation at regular internals for fungal development. If the colony is very small and there is a risk of engulfment by other colonies, it needs to be subcultured. Subculture isolates are generally maintained at room temperature for many weeks to study morphological and other characteristics. Some isolates may fail to produce reproductive structures even after several months. Subculture of these isolates onto medium with autoclaved host tissue strips (Matsushima, 1971) or filter paper (Dhingra and Sinclair, 1994) can promote sporulation. In general, sterile isolates should be checked regularly for fruiting bodies over a period of 3-4 months and the isolates failing to produce fruiting body are refered as a sterile.

Table 2 Surface sterilization materials and protocols (Mueller *et al*., 2004)

Disinfectant, concentration and duration	Host/tissue	Reference
Formaldehyde 37–40%, 1–5 min. NaOCl, 10% available Cl, 5 min.	Various hosts leaves *Festuca* leaves *and culms, Anemone, Crataegus, Glechoma, Potentilla, Salix, Sorbus, Teucrium, Vaccinium* leaves	Schulz *et al*., 1993 Schulz *et al*., 1993
Ethanol 96%, 1 min.; NaOCl, 10 % available Cl, 5 min., ethanol 96%, 30 sec.	*Crataegus, Glechoma, Potentilla, Salix* leaves	Schulz *et al*., 1993
Ethanol 96%, 1 min.; NaOCl, 2% available Cl, (1:2 bleach), 7 min; ethanol 96%, 30 sec.	Conifer twigs	Petrini and Müller, 1979
Ethanol 99%, 1 min.; NaOCl 8.7% available Cl, 5–120 min.; ethanol 99%, 30 sec.	*Castanaea* shoots	Bissegger and Sieber, 1994
Ethanol 96%, 1 min.; NaOCl 3% available Cl, 10 min.; ethanol 70%, 30 sec.	*Sequoia* leaves	Espinosa-Garcia and
Ethanol 96%, 30 sec.; NaOCl 2.5% available Cl, 1–3 min.; ethanol 96%, 30 sec.	Lichens, mosses, ferns	Langenheim, 1990 Petrini, 1986
Ethanol 96%, 30 sec.; sterile water, 30 sec.; NaOCl 5% available Cl, 5 min.; ethanol, 3 sec.; sterile water, 30 sec.	*Crataegus, Glechoma, Salix, Sorbus Teucrium, Vaccinium* leaves	Schulz *et al*., 1993
Ethanol 95%, 1 min.; NaOCl 20% available Cl, 3 min.; ethanol 95%, 30 sec.	*Pteridium* rhizomes, rachis, pinnules	Petrini *et al*., 1992a
Ethanol 75–96%, 1 min.; NaOCl 2–4% available Cl, 3–5 min.	Conifer needles *Quercus* leaves and twigs	Carroll and Carroll 1978 Halmshlager *et al*., 1993

Table 2 (Continued)

Disinfectant, concentration and duration	Host/tissue	Reference
Ethanol 75–96%, 30 sec., rinse with sterile water	*Ulex* twigs	Fisher *et al*., 1986
	Pinus, Fagus twigs	Petrini and Fisher, 1988
	Salix, Quercus twigs	Petrini and Fisher, 1990
	Quercus leaves, twigs	Fisher *et al*., 1994
	Acer, Betula, Picea roots	Sridhar and Bärlocher, 1992
	Fagus leaves, twigs	Sieber and Hugentobler, 1987
	Alnus leaves, twigs	Sieber *et al*., 1991
	Fagus buds, twigs	Toti *et al*., 1993
	Chamaecyparis leaves, twigs	Bills and Polishook, 1992
	Pinus needles	Helander *et al*., 1994
	Abies, Larix, Picea, Pinus, Acer	Kowalski and Kehr, 1992
	Alnus, Betula,Carpinus, Fagus, Fraxinus, Quercus branch bases, *Acer, Quercus, Tilia* leaves	Pehl and Butin, 1994
Ethanol 99%, 1 min.; H_2O_2 35% available Cl, 5–120 min.	*Castanea* shoots	Bissegger and Sieber, 1994
Ethanol 99%, 30 sec.	*Abies, Fagus, Picea, Pinus* roots	Ahlich and Sieber, 1996
Ethanol 70%, 1 min.; H_2O_2 15% available Cl, 15 min.; ethanol 70%, 1 min; sterile water, 2 rinses	*Pinus* needles	Hata and Futai, 1995
Ethanol 96%, 1 min.; per acetic acid 0.35%, 3–5 min.; ethanol 96%, 30 sec.	*Alnus* stems	Fisher and Petrini, 1990
$HgCl_2$ 0.01%, 3 min.	*Picea* roots	Summerbell, 1989
$HgCl_2$ 0.1%, 1 min.; ethanol 5%, 1 min.	*Eucalyptus* leaves	Cabral, 1985
	Acer leaves	Pugh and Buckley, 1971
$HgCl_2$ 0.001%, 1–5 min; ethanol 70%, 1 min; sterile water, 1 min.	Plant meterial	Booth, 1971

2.4 Endophytes of tropical plants

It is thought that endophytes of tropical plants are an important component of global fungal biodiversity (Hawksworth and Rossman, 1997; Fröhlich and Hyde, 2000). Why? First, the tropics are rich in undescribed plant species. Second, the ratio of plant-associated fungal species to plant species has been estimated at 6:1 in Britain. (Britain is used as a point of reference because its floras are well sampled.) If this ratio holds for the tropics, it would imply a vast number of undescribed endophyte species. However, it is not clear what the fungus: plant ratio is. The plant species richness in the tropics may select against highly specific plant-associated microorganisms (May, 1988) in which case the ratio would be lower than 6:1. This controversy has stimulated research on biodiversity and host specificity of tropical endophytes. Results are more mixed than for temperate trees: while some studies have found evidence of host specificity (Arnold *et al*., 2000), others studies have not (e.g. Bayman *et al*., 1998; Cannon and Simmons, 2002). Taxonomic studies support the view that many of the 'missing fungi' are endophytes, especially in tropical plants. Many new species of endophytes and saprophytes of palms have been described by Kevin Hyde and coworkers in Hong Kong (Hyde, 2001). Based on the number of fungi they could identify from a single palm tree, they argue that the 6:1 ratio of fungal species to plant species is too low. They propose a 33:1 ratio, which would greatly increase the extrapolated total number of fungal species (Fröhlich and Hyde, 2000). Comparisons of endophyte communities in neighbouring plant species usually show quantitative, rather than qualitative differences. For this reason, it may be more accurate to speak of endophyte host preferences, rather than host specificity (Lodge, 1997), at least for tropical plants. Specificity aside, themost frequent endophytes in the tropics often

differ from those of temperate plants. *Xylaria* and its anamorphs have been isolated as endophytes in a wide range of tropical plants, including important crops, but in temperate areas they are saprotrophs and wood-rotters rather than endophytes (Rodrigues and Petrini, 1997; Rogers, 2000 and Bayman, 2006). *Xylaria* spp. is special interest because they produce several types of bioactive secondary metabolites. Like many tropical endophytes, *Xylaria* is difficult to fruit in culture, making identification difficult. Studies on tropical endophytes often group sterile fungi together in morphospecies, on the basis of morphology in culture (Arnold *et al*., 2000; Gamboa and Bayman, 2001). However, a comparison of morphospecies and DNA sequencing showed that morphospecies data are a fairly accurate way to estimate the number of species in a sample (Arnold *et al*., 2000). Other genera such as *Guignardia* fit the general pattern described here for *Xylaria*.

2.5 Natural products form endophytic fungi with use in Agriculture

The world's first billion dollar anticancer drug; taxol was found in yew (Taxus) species. This compound is used to treat a number of human and tissue-proliferating diseases as well (Strobel, 2002b). In fact that endophytes are virtually present in all of the world's higher plants, it was reasoned that yew trees conceivably might support certain endophytic microorganisms that also make taxol (Stierle *et al*., 1993). These compounds encourage the scientist to study endophytic fungi more with the expectation of the other useful compound. This following paragraphs show some example of the natural product with potential in agricultural.

2.5.1 Endophytic fungi products as antibiotics

Cryptosporiopsis quercina is the imperfect stage of *Pezicula cinnamomea* which isolated as an endophyte from *Tripterigeum wilfordii*, a medicinal plant native to Eurasia. *In situ*, *C. quercina* demonstrated excellent antifungal activity against some important human fungal pathogens. A unique peptide antimycotic, termed cryptocandin, was isolated and characterized from *C. quercina* (Strobel., 2002b) This compound contains a number of peculiar hydroxylated amino acids and a novel amino acid: 3-hydroxy-4-hydroxy methyl proline. The bioactive compound is related to the known antimycotics (Walsh, 1992). As well as other antifungal agents related to cryptocandin are also produced by *C. quercina.* Cryptocandin is also active against a number of plant-pathogenic fungi, including *Sclerotinia sclerotiorum* and *Botrytis cinerea* (Li *et al*., 2000). This unusual compound possesses potent activity against *Pyricularia oryzae* as well as a number of other plant pathogenic fungi.

Pestalotiopsis microspora is a common rainforest endophyte (Strobel, 2003; Strobel *et al*., 1996) which enormous biochemical diversity does exist in this endophytic fungus, and as such there seem to be many secondary metabolites produced by a myriad of strains of this widely dispersed fungus. Ambuic acid is the one of secondary metabolite from several isolates of *P. microspora* found as representative isolates in many of the world's rainforests (Li *et al*., 2000). In fact, this compound and another endophyte product, terrein, have been used as models to develop new solid-state nuclear magnetic resonance tensor methods to assist in the characterization of molecular stereochemistry of organic molecules (Harper *et al*., 2001). A strain of *P. microspora* was also isolated from the endangered tree *Torreya taxifolia* and produces several compounds that have antifungal activity, including

pestaloside, an aromatic *β*-glucoside, and two pyrones: pestalopyrone and hydroxypestalopyrone (Lee *et al*., 1995). Other newly isolated secondary products obtained from *P. microspora* (endophytic on *T. brevifolia*) include two new caryophyllene sesquiterpenes-pestalotiopsins A and B (Pulici *et al*., 1996). Other novel sesquiterpenes produced by this fungus are 2-α-hydroxydimeninol and a highly functionalized humulane. A newly described species of *Pestalotiopsis*, namely, *Pestalotiopsis jesteri*, from the Sepik River area of Papua New Guinea, produces jesterone and hydroxy-jesterone, which exhibit antifungal activity against a variety of plant-pathogenic fungi (Li, 2001).

Colletotric acid, a metabolite of *Colletotrichum gloeosporioides*, an endophytic fungus in *Artemisia mongolica*, displayed antimicrobial activity against bacteria as well as against *Helminthsporium sativum* (Zou *et al*., 2000). Another *Colletotrichum* sp. isolated from *Artemisia annua*, produces bioactive metabolites that showed varied antimicrobial activity as well. *A. annua* is a traditional Chinese herb. The *Colletotrichum* sp. in *A. annua* produced not only metabolites with activity against human pathogenic fungi and bacteria but also metabolites that were fungistatic to plant pathogenic fungi (Lui *et al*., 2001). *Acremonium zeae*; fungal endophyte which is antagonistic to kernel rotting and mycotoxin producing from *Aspergillus flavus* and *Fusarium verticillioides* in cultural. The chemical analysis from maize kernel fermentations of *A. zeae* displayed significant antifungal activity against *Aspergillus flavus* and *F. verticillioides*, revealed that the metabolites accounting for this activity were two newly reported antibiotics pyrrocidines A and B. Pyrrocidines were detected in fermentation extracts (Wicklow *et al*., 2005).

2.5.2 Volatile Antibiotics from Endophytes

Muscodor albus is a newly described endophytic fungus obtained from limbs of *Cinnamomum zeylanicum* (cinnamon tree) (Worapong *et al*., 2001). This Xylariaceaous effectively inhibits and kills certain other fungi and bacteria by producing a mixture of volatile compounds (Strobel *et al*., 1996). The five classes of volatile compounds produced by the fungus had some inhibitory effect against the test fungi and bacteria but none was lethal. However, collectively they acted synergistically to cause death in a broad range of plant- and human- pathogenic fungi and bacteria. The most effective class of inhibitory compounds was the esters, of which isoamyl acetate was the most biologically active. The ecological implications and potential practical benefits of the "mycofumigation" effects of *M. albus* are very promising given the fact that soil fumigation utilizing methyl bromide will soon be illegal in the United States. The potential use of mycofumigation to treat soil, seeds and plants may soon be a reality. In fact, this organism is already on the market for the decontamination of human wastes. The newly described *Muscodor roseus* was twice obtained from tree species growing in the Northern Territory of Australia. This fungus is just as effective in causing inhibition and death of test microbes in the laboratory as *M. albus* (Worapong *et al*., 2002)

2.5.3 Products of Endophytes with Insecticidal Activities

Several endophytes have anti-insect properties. Nodulisporic acids, novel indole diterpenes that exhibit potent insecticidal properties against the larvae of the blowfly, work by activating insect glutamate-gated chloride channels. The first nodulisporic compounds were isolated from an endophyte, a *Nodulisporium* sp. from the plant

Bontia daphnoides. This discovery has since resulted in an intensive search for more *Nodulisporium* spp. or other producers of more-potent nodulisporic acid analogues (Demain, 2000). Insect toxins have also been isolated from an unidentified endophytic fungus from wintergreen (*Gaultheria procumbens*). The two new compounds show toxicity to spruce budworm and the latter is also toxic to the larvae of spruce budworm (Findlay *et al*., 1997). Another endophytic fungus, *Muscodor vitigenus*, isolated from a liana (*Paullina paullinioides*), yields naphthalene as its major product. Naphthalene, the active ingredient in common mothballs, is a widely exploited insect repellant. *M. vitigenus* shows promising preliminary results as an insect deterrent and has exhibited potent insect repellency against the wheat stem sawfly (*Cephus cinctus*) (Daisy *et al*., 2002a; Daisy *et al*., 2002b)

Beside those potential which describe above, endophytic fungi also have other properties as an antiviral compound (Guo *et al*., 2000), anticancer (Li *et al*., 1998; Strobel *et al*., 1996; Stierle *et al*., 1993), antidiabetic (Zhang *et al*., 1999), antioxidants (Harper *et al*., 2001) and immunosuppressive compound (Lee *et al*., 1995). These are the potential which apply for medical and pharmaceutical arenas.

2.5.4 Study of Endophytic fungi for agricultural application

Arnold *et al*. (2003) used the foliar endophytes associated with *Theobroma cacao*. The inoculation of endophyte-free leaves with endophytes isolated frequently from naturally infected significantly decreases both leaf necrosis and leaf mortality when *T. cacao* seedlings are challenged with a major pathogen (*Phytophthora* sp.).

Santos *et al*. (2003) obtained endophytic fungi from living symptomless roots, stems, leaves and fruits of *Melia azedarach*. This is an exotic tree introduced into

Brazil from Asia and is a producer of limonoids which are terpenoidic natural substances with great insecticidal activities but exhibit no fungicide properties. A total of 55 fungal isolates were recovered and showed the resistance to many pathogens. From the identification, Hyphomycetes were more prevalent than Ascomycetes, Coelomycetes and Basidiomycetes. The genera Aspergillus and Penicillium were the most common in the plant.

Rubuni *et al*. (2005) were studied the diversity of endophytic fungal community of cacao (*Theobroma cacao* L.) and biological control of *Crinipellis perniciosa,* causal agent of witches' broom disease in Brazil. The indentified by molecular technique use rDNA sequencing as belonging to the genera Acremonium, Blastomyces, Botryosphaeria, Cladosporium, Colletotrichum, Cordyceps, Diaporthe, Fusarium, Geotrichum, Gibberella, Gliocladium, Lasiodiplodia, Monilochoetes, Nectria, Pestalotiopsis, Phomopsis, Pleurotus, Pseudofusarium, Rhizopycnis, Syncephalastrum, Trichoderma, Verticillium and Xylaria. These fungi were evaluated both *in vitro* and *in vivo* by their ability to inhibit *C. perniciosa.* Among these, some were identified as potential antagonists but only one fungus (*Gliocladium catenulatum*) reduced the incidence of Witches' Broom Disease in cacao seedlings to 70%.

Li *et al*. (2005) screened for antifungal activity of 130 endophytic fungi isolated from 12 Chinese traditional medicinal plants. From the growth inhibition test against 7 phytopathogenic fungi showed fermentation broths from 30% of isolates exhibited antifungal activity and some of them exhibited broad-spectrum antifungal activity. Endophytic fungi of Chinese traditional medicinal plants are promising sources of novel bioactive compounds.

Dhingra *et al* (2006) selected endophytic non-pathogenic isolate of *Fusarium oxysporum* to suppress Fusarium yellow of bean. Mixing chlamydospores of *Fusarium oxysporum* reduced percentage of root colonized from pathogen. In the field experiment, Mixing chlamydospores of *F. oxysporum* increased area under the chlorophyll retention curve and grain yield about 100% over control. The suppressive of *F. oxysporum* was related to its high saprophytic competitiveness with the pathogen in the soil matrix and parasitic competitiveness by reducing the availability of infection sites and internal root colonization by the pathogen.

Kim *et al*. (2006) isolated endophytic fungi from vegetable plants and examine their *in vivo* anti Oomycete activity against *Phytophthora infestans*. *F. oxysporum* EF119 showed the most potent *in vivo* anti-oomycete activity against tomato late blight and *in vitro* anti-oomycete activity against several oomycete pathogens.

Inácio *et al*. (2006) screened for Antifungal metabolites from endophytic fungi living in plants from Cerrado and Atlantic Forest, healthy leaves of *Cryptocarya mandioccana* were submitted to isolation of endophytes and 15 strains were isolated. Among them *Colletotrichum gloeosporioides* was selected for chemical and biological investigation because of the strong antifungal activity of the crude ethyl acetate extract against the phytopathogenic fungi *Cladosporium cladosporioides* and *Cladosporium sphaerospermum*. After chemical analyse, two new compounds were investigated and suggests that the endophytic fungi *C. gloeosporioides* could protect the host by producing metabolites, which may be toxic or even lethal to phytopathogens.

Campanile *et al*. (2007) isolated *Trichoderma viride*, *Epicoccum nigrum*, *Fusarium tricinctum*, *Alternaria alternata*, *Sclerotinia sclerotiorum* and Cytospora from epigeous declining oak tissues and evaluated for its ability to control *Diplodia*

corticola; causal agent of cankers, vascular necrosis and dieback on various oak species. *T. viride* and *F. tricinctum*. showed maximum *in vitro* inhibition of mycelial growth of *D. corticola*. Then evaluated for their ability to reduce mortality caused by *D. corticola* of *Quercus cerris* and *Q. pubescens* seedlings. *F*. t*ricinctum* and *A*. *alternate* significantly reduced mortality caused by *D. corticola*.

Hussain *et al*. (2007) studied on endophytic fungus *Acremonium* sp. isolated from *Plantago lancelota* and an unidentified Ascomycetes, isolated from *Erica arborea* were selected for chemical and biological investigations because of the strong antifungal activity of the crude ethyl acetate extract against *Botrytis cinerea*, as well as good algicidal and antibacterial activities. After that, analyzed by chromatography found the bioactive substance; Djalonensone (Onocha *et al*., 1995) which tested for herbicidal, antibacterial, and antifungal activities and showed strong antifungal activity against the fungus *Microbotryum violaceum*.

2.6 Study of endophytic fungi in Thailand

Fungal endophytes have been report from Thailand. Lanyong *et al*. (1998) were surveyed for the distribution of endophytic fungi among plant species in Doi Suthep-Pui area. Thirty nine indigenous dicotyledonous plant seedling speices and two mature plants (*Shorea roxburgii* and *Mesua ferrea*) were examined for endophytic fungi. Four hundred fungal isolates were obtained and 30% of those were Mycelia sterilia. Many of isolated fungi belonged to the typical genera; *Phomopsis* sp., *Colletotrichum* sp., *Phoma* sp., *Fusarium* sp., *Curvalaria* sp. And several of isolates are sterile Xylariaceous fungi. Rare species were *Apiosordaria striatispora* and *Guignardia* sp. Bussaban (2005) studied the biodiversity of endophytic fungi in wild

and cultivated Zingiberaceae and obtained 55 endophytic fungi, 36 fungal texa including three new species of *Pyricularia*, *Gaeumannomyces* and *Letospharella*. Wiyakrutta *et al*. (2003) collected 81 Thai medicinal plant species from forests in four geographical regions of Thailand were examined for the presence of endophytic fungi with biological activity. Extracts of 92 isolates could inhibit *Mycobacterium tuberculosis* assay, while extracts of six inhibited *Plasmodium falciparum*. Strong anti-viral activity against *Herpes simplex virus* type 1 was observed in 40 isolates. The results suggested that Thai medicinal plants can provide a wide variety of endophytes that might be a potential source of novel bioactive compounds.

Jailae (2003) reported 90 taxa of fungal endophyte were obtained from Nutgrass, cogon grass and common reed. Then 163 isolates were test against *Bipolaris sorokiniana* by dual culture and soaking seed in endophyte suspension, Mycelia sterilia (4) T_5UL033 show the highest percentages of inhibition in both experiment.

Likhittragulrung (2002) isolated 611 endophytic fungi from tomato and identified into 20 taxa. From the *in vitro* test, found *Xylaria* sp., *Colletotrichum* sp., Mycelia Sterilia and *Fusarium* sp. showed the high potential to against *Alternarai solani*. Seed germination in blotter method was introduced on tomato seed, *Vigaria* sp. show the highest percentage of seed germination.

Chareprasert *et al*. (2005) studied fungal endophyte from teak (*Tectona grandis*) and rain tree (*Samanea saman* Merr.) in Thailand. The fungal genera found in both young and mature teak leaves were *Alternaria*, *Colletotrichum*, *Nigrospora*, *Phomopsis* and Mycelia sterilia. *Phomopsis* was the dominant genus in both young (newly emerged) and mature leaves. *Fusarium*, *Penicillium*, *Schizophyllum* commune and members of the Xylariaceae were found only in mature leaves. A total of 37

isolates of endophytic fungi isolated from teak and rain tree leaves were tested for the production of antimicrobial activities.

Boonmakad *et al*. (2007) investigated the new biocompound from endophytic fungi associated with *Stemona* spp. Total 170 fungal endophyte were isolated from *Stemona* spp which collected from 4 provinces. The result from antibacterial activity against bacterial plant pathogens; *Erwinia carotovora*, *Pseudomonas solanacearum* subvar.1, *Pseudomonas solanacearum* subvar.2 and *Xanthomonas citrii* show that 15 isolates had potential and some isolates showed broad spectrum of antibacterial activity.

2.7 The endophytic fungi associated with palm

There were many palm endophytes studied in tropical forest. In 1990, Samuels and Rodrigues did the preliminary study of endophytic fungi in tropical palm tree (*Licula ramsayi*). Rodrigues (1994) surveyed for endophytic fungi in *Euterpe oleracea* or Amazonian palm and found that over all colonization was positively correlated with leaf age, plant growth stage and the interaction of these three factors. Hyde *et al*. (1997) were analyzed the occurrence of fungi on *Licuala* sp. and *Archontophoenix alexandriae* in North Queensland gave conservative estimates of the total numbers of palm fungi in Australia. The ratio of palm host to fungi species (1:26) appears to be higher than the generally accepted ratio 1:6 for other plants. For palm species in the tropics to host specific fungal 1 to 33 would be a more accurate estimate (Hyde *et al*., 1997)

Taylor *et al*. (1999; 2000) studied fungal endophyte from 3 temperate and tropical palms; *Trachycarpus fortune*, *Archontophoenix alexandrae* and *Cocos*

nucifera. Different assemblages of fungi were found in association with palms in temperate regions as compared to those in tropical regions and the statuses of the hosts at the site were also influential.

Petrini *et al*. (1992a) isolated endophyte from *Licula* sp. in Brunei Darussalam and Australia with high colonization rate (81-90%). Differences of endophytic fungi were observed between different palm tissues. Xylariaceous was the dominant species. The results presented suggest that most of the endophytes entered the petiole *via* the leaf and that transmission of palm endophytes is likely to be horizontal (*via* airborne propagules) rather than vertical (*via* seed).

2.8 Thai dwarf fishtail palm: *Wallichia siamensis*

Thai dwarf fishtail palm or *Wallichia* is a genus of 10 currently recognized species (Govaerts and Dransfield, 2005), occurring in Nepal, Bhutan, northeastern India, Myanmar, Thailand, Laos, Vietnam, and China (Figure 2). The genus is placed in the tribe Caryoteae, along with *Caryota* and *Arenga* (Uhl and Dransfield, 1987). There are 8 species present in this genus; *W. disticha*, *W. oblongifolia*, *W. caryotoides* *W. triandra. W. nana*, *W. lidiae*, *W. marianneae* and *W. gracilis*.

Wallichia siamensis (Thai dwarf fishtail palm, local name: taou-rung-nu) is a native palm to Thailand. The palm is small, and densely clustered up to 4.5 m in high and widespread in the northern part of Thailand but is never common. The palm grows in moist areas at 500-1200 m elevation (Gardner *et al*., 2003). The palm is classified as follows:

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Liliopsida*

Order: *Arecales*

Family: *Arecaceae*

Subfamily: *Arecoidae*

Tribe: Caryotae

Genus: *Wallichia* Roxb.

Species: *Wallichia siamensis* Becc. (Henderson, 2007)

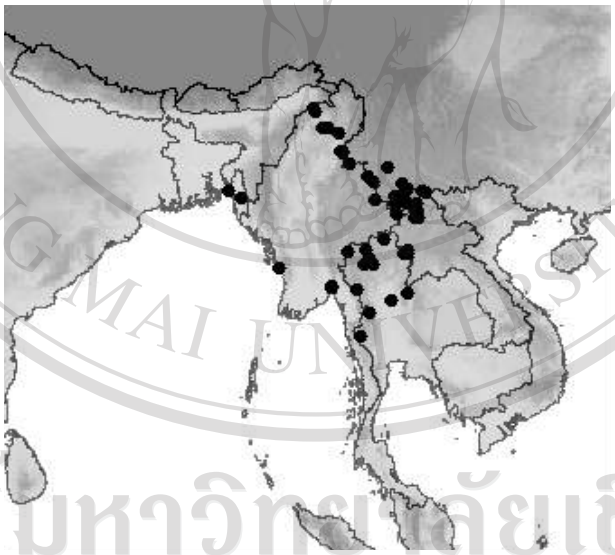

Figure 2 Distribution map of Thai dwarf fishtail palm (Henderson, 2007)