CHAPTER 2

LITERATURE REVIEW

2.1 Distribution and habitats of Zingiberaceae

The Zingiberaceae (gingers) is the largest family of the order Zingiberales and comprises 53 genera with about 1,300 plant species. The Zingiberaceae is widely distributed in tropical regions, especially South-East Asia (Figure 2.1) (Dahlgren *et al*., 1985; Mabberley, 1987; Kress, 1990; Griffiths, 1992; Heywood, 1993; Poulsen, 1996; Kress *et al*., 2002). In Thailand, there are 21 genera with about 200 species of Zingiberaceae occurring throughout the country (Larsen, 1980, 1996).

Species of the Zingiberaceae are the ground plants of the tropical forests or found infrequently in secondary forest. They mostly grow in damp and humid shady places. Some species, however, can grow fully exposed to the sun, and grow at high elevation (Poulsen, 1996; Sirirugsa, 1998).

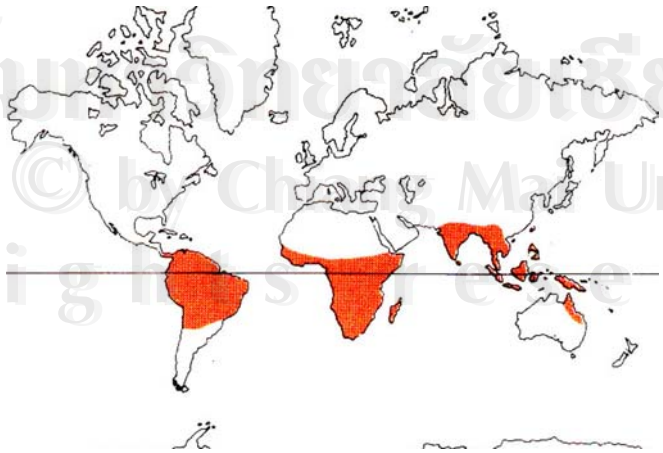

Figure 2.1 Distribution of Zingiberaceae (Heywood, 1993).

2.2 Definition and classification of Zingiberaceae

The Zingiberaceae are perennial herbs usually with creeping horizontal or tuberous rhizomes (Figure 2.2). The leaves are alternate or distichous, the base opened-sheathing and the blade mostly linear to elliptic with penniparallel, strongly ascending veins. The flowers, which are aggregated in inflorescences are bisexual, strongly zygomorphic, and often associated with conspicuous floral bracts in a spike or raceme. The perianth is in two whorls, an herbaceous or membranous 3-lobed or spathaceous tubular calyx and a petaloid tubular corolla with 3 lobes. The androecium typically consists of 1 fertile stamen, a large opposing petaloid labellum representing 2 connate staminodia, and two smaller flanking petaloid staminodia. The gynoecium consists of a single compound pistil of 3 carpels, a single style nestled in a channel of the filament and anther of the fertile stamen and an inferior ovary with typically 3 locules, each containing numerous axile ovules. Rarely the ovary is unilocular with parietal placentation. The fruit is a loculicidal capsule or berry-like. Seeds are round with a red aril and endosperm present (Dahlgren *et al*., 1985; Kress, 1995).

The Zingiberaceae is a member of the order Zingiberales (≡ Scitaminales), which also includes Cannaceae, Costaceae, Heliconiaceae, Lowiaceae, Marantaceae, Musaceae and Strelitziaceae (Dahlgren *et al*., 1985). Morphological synapomorphies for the Zingiberales clade (Figure 2.3) include specialized isomorphic root hair cells, presence of silica bodies in cells, epigynous flowers, pollen grains without distinctive apertures and a reduced exine layer, nuclear endosperm development, and arillate seeds (Smith *et al*., 1993; Kress, 1990, 1995; Chase *et al*., 2000; Kress *et al*., 2001). Zingiberaceae are distinguished by the presence of a labellum, formed by the fusion of two sterile stamens, by the two epigynous nectariferous glands at the base of the

style, and by the presence of essential oils in their tissues (Kress, 1990, 1995). In earlier classification (e.g., Petersen, 1889; Schumann, 1904) the family Costaceae was included in the Zingiberaceae, but with a number of distinctive characters, such as lacking of aromatic oils, branched aerial stems, and spiral monostichous phyllotaxy (Specht *et al*., 2001) it is now accepted as the sister clade to the gingers (Kress, 1990, 1995; Smith *et al*., 1993; Kress *et al*., 2001).

Figure 2.2 General structure of the Zingiberaceae, exemplified by *Zingiber officinale* Rosc: A. Plant with rhizome, B. Inflorescence and leaves, C. Perianth enclosing young flower, D. Flower, E. Asymetrical outer tepal whorl, F. Style apex, G. Labellum and lateral staminodes, H. Pistil, I. Median tepal of inner whorl and anther enclosing style apex, J. Ovary, transverse section, K. Ovary, longitudinal section, with epigynous glands (Wu, 1981).

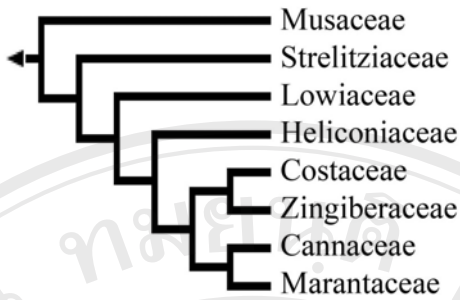

Figure 2.3 Tree diagram of order Zingiberales based on morphological and molecular analyses (Kress *et al*., 2001).

Zingiberaceae previously included four tribes, Alpinieae, Hedychieae, Globbeae and Zingibereae (Burtt and Smith, 1972; Dahlgren *et al*., 1985; Larsen *et al*., 1998). This classification was based on floral and vegetative characters that in most instances were either not unique to any tribe or not universal for all taxa within each tribe. Based on DNA sequence analysis (the plastid *matK* and the nuclear rDNA ITS), Kress *et al*. (2002) proposed a realignment of the genera of the Zingiberaceae into four subfamilies, Alpinioideae, Siphonochiloideae, Tamijioideae and Zingiberoideae.

2.3 Importance of Zingiberaceae

The whole plant of all members of Zingiberaceae contains cells with ethereal oils. Monoterpenoids, sesquiterpenoids and aromatic ketones are characteristic constituents of these oils, and aliphatic compounds occur in considerable amounts in some of the ethereal oils (Luz *et al*., 1984; Dahlgren *et al*., 1985; Lechat-Vahirua *et al*., 1993; Denyer *et al*., 1994). These render a number of species of Zingiberaceae important as sources of spices and perfumes. Furthermore, their colourful inflorescences make them ideal as ornamentals. For example, many species of *Alpinia*

are cultivated as garden plants or pot plants for their attractive, often variegated leaves and striking inflorescences. *Alpinia galanga* is an important spice in Thai cuisine and its rhizomes yield essential oil marketed as 'Essence of Amali' used in perfumery (Mabberley, 1992; Campin, 2004).

Zingiberaceae is the source of ginger root and turmeric, an essential curry ingredient for its yellow colour and distinctive flavour. When the roots of turmeric *Curcuma longa* are dried and ground, the powder produced is yellow with an orange tinge. The powder is often blended with paprika and annatto to produce the desired colour. Its largest use is in prepared mustard, but it is also widely used in curry powder, pickles, relish, sausage and cheese. The extractable colour in turmeric comes from curcumin, which is also a natural antioxidant (Dahlgren *et al*., 1985; Bonte *et al*., 1997; Sirirugsa, 1998). Oleoresin from ginger roots can be found in ginger ale, gingerbread, gingersnap cookies, ginger tea, ginger wine, and many Asian dishes. The volatile oil of ginger contains zingiberene, AR-curcunene and farnesene, while the pungent taste is due to gingeroles and zingerone. In addition to its aromatic contribution to a food, ginger tea is often used to improve circulation, aid digestion, and treat nausea from motion sickness, pregnancy or chemotherapy (Yamahara *et al*., 1990; Kawai *et al*., 1994; Willetts *et al*., 2003).

Many genera in Zingiberaceae are sources of herbal drugs used in Asia and listed in pharmacopedias and other drug compendia (Cantoria, 2003). In Thailand, 58 species from 11 genera (*Alpinia*, *Amomum*, *Boesenbergia*, *Curcuma*, *Etlingera*, *Elletariopsis*, *Gagnepainia*, *Globba*, *Hedychium*, *Kaempferia*, and *Zingiber*) have been recorded with ethnomedicinal use (Table 2.1). Most of them were used for the

Table 2.1 Ethnomedicinal uses of Thai zingiberaceous plants (Chuakul, 2003).

Thai name	Generic name	Part used	Ethnomedicinal uses
Kha daeng (□□ □□□)	*Alpinia blepharocalyx*	rhizome	hematinic
kha pa (□□ □□)	*Alpinia bracteata*	rhizome	abnormal menstruation
Kha ling (□□ □□)	*Alpinia conchigera*	rhizome	diabetes mellitus
Kha (□□)	*Alpinia galanga*	rhizome	flatulence, laxative
Kha nam (□□ □□)	*Alpinia mutica*	rhizome	tonic
Kala (□□□□)	*Alpinia nigra*	rhizome	tonic
Riao (□□□ □)	*Alpinia oxymitra*	rhizome	tonic
Kha khom (□□ □□)	*Alpinia zerumbet*	rhizome	tonic
Krawan (□□□□□□)	*Amomum testaceum*	fruit	carminative, antiflatulent
Reo (□□□)	*Amomum uliginosum*	rhizome	carminative, stomachache
Mak neng (□□□□□□□)	*Amomum villosum* var. *xanthioides*	rhizome	carminative, stomachache
Wan priao (□□ □□□□□ □)	*Boesenbergia longiflora*	rhizome	tonic
Krachai (□□□□□□)	*Boesenbergia rotunda*	rhizome	diuretic
Ngon phaya nark (□□□□□□□□□□□)	*Boesenbergia xiphostachya*	rhizome	flatulence, laxative
Krachiao hin (□□□□□□ □□□)	*Boesenbergia* sp.	rhizome	flatulence
En lueang (□□□ □□□□ □)	*Curcuma aurantiaca*	rhizome	hemostatic
Wan chak motluk (□□ □□□ □□□□)	*Curcuma comosa*	rhizome	treatment of inguinal hernia, for uterine involution
Kha min (□□□)	*Curcuma longa*	rhizome	antipruritic
Dok din (□□□□□)	*Curcuma oligantha*	rhizome	tonic
Krachieo khao (□□□□□□ □□□□)	*Curcuma parviflora*	rhizome	for bodily discomfort
Krachieo daeng (□□□□□□ □□□□)	*Curcuma sessilis*	rhizome	tonic
Chu krachieo (□□ □ □□□□ □)	*Curcuma sparganifolia*	rhizome	asthma
Khamin cheut (□□□ □□)	*Curcuma* sp.	rhizome	food poisoning
Wan fai chai dam (□□ □□□□□□□)	*Curcuma* sp.	rhizome	peptic ulcer
Wan lueat (□□ □□□□ □)	*Curcuma* sp.	rhizome	diarrhoea
Krachieo khao (□□□□□□ □□□□)	*Curcuma* sp.	rhizome	tonic

Kala dok khao (□□□□□□□□□□)	*Etlingera elatior* var. *alba*	rhizome	tonic, for paralysis

Table 2.1 (Continued).

Thai name	Generic name	Part used	Ethnomedicinal uses
Kala dok daeng (□□□□□□□□□□)	*Etlingera elatior* var. *elatior*	rhizome	tonic, for paralysis
Kala dok chomphu (□□□□□□□□□□□)	*Etlingera elatior* var. *elatior*	rhizome	tonic, for paralysis
Dala daeng (□□□□□□□□)	*Etlingera elatior* var. *pileng*	rhizome	tonic
Put khang khok (□□ □□□□□)	*Etlingera littorallis*	seed	carminative, stomachic, heart tonic
Put yai (□□ □□□)	*Etlingera* sp.	seed	carminative, stomachic, heart tonic
Put nok (□□ □□)	*Elletariopsis curtisii*	whole plant	flatulence
Khing nok (□□ □□)	*Elletariopsis triloba*	rhizome	hematinic
Tupmup hu kwai (□□ □□□ □□ □□□)	*Gagnepainia thoreliana*	rhizome	wounds
Khing krathai (□□ □□□□□ □)	*Globba candida*	leaf, rhizome	otorhoea
Khing nuu (□□ □□)	*Globba geoffrayi*	whole plant	antiasthmatic
Kha ling (□□ □□)	*Globba obscura*	rhizome	stomach pain, wounds
Kha dong (□□ □□)	*Hedychium coronarium*	rhizome	tonic
Proh yai (□□□□□□□□)	*Kaempferia elegans*	rhizome	flatulence fever
Ban kham noi (□□□□□□□ □)	*Kaempferia filifolia*	rhizome	leukkorhoea
Proh (□□□□□)	*Kaempferia galanga*	rhizome	detoxicant for poisonous plants
Proh krachieo (□□□□□□□□□□□ □)	*Kaempferia larsenii*	rhizome	intoxication
Thupmup (□□ □□□)	*Kaempferia margianata*	rhizome	oedema
Proh pa (□□□□□□□)	*Kaempferia roscoeana*	rhizome	intoxication
Wan non lap (□□ □□□□□□□)	*Kaempferia rotunda*	rhizome	intoxication
Thupmup bai lae (□□ □□□ □□□□□)	*Kaempferia* sp.	rhizome	oedema
Khing luang (□□ □□□□ □)	*Zingiber chrysostachya*	rhizome	tonic
Phlai (□□□)	*Zingiber montanum*	rhizome	bodily discomfort
Khing dok krachieo yae (□□ □□□ □□□□□ □□□)	*Zingiber niveum*	rhizome	laxative, paralysis
Khing (□□)	*Zingiber officinale*	rhizome	cough

Phlai dam (□□□□□)	*Zingiber ottensii*	rhizome	jaundice
Put (□□)	*Zingiber spectabile*	rhizome	tonic
Put nang hang (□□ □□□ □□□)	*Zingiber wrayi*	rhizome	lagtogogue, bodily discomfort

Table 2.1 (Continued).

Thai name	**Generic name**	**Part used**	**Ethnomedicinal uses**
Kra thue (□□□□□)	*Zingiber zerumbet*	rhizome	tonic
Kha pa (□□ □□)	*Zingiber* sp.	rhizome	tonic
Kha lek, Kha yae (□□ □□□ , □□ □□)	*Zingiber* sp.	rhizome	tonic
Khing dok daeng (□□ □□□□□□)	*Zingiber* sp.	rhizome	antiflatulence

treatment of gastrointestinal diseases (e.g., stomach pain, peptic ulcer) and had carminative or laxative effects (Chuakul, 2003). Because of their medicinally valuable chemical components, they are currently used in many pharmaceutical preparations (Apisariyakul *et al*., 1995; Sirirugsa, 1999; Wuthi-udomlert *et al*., 2000; Koo *et al*., 2001; Trakoontivakorn *et al*., 2001; Tuchinda *et al*., 2002; Ficker *et al*., 2003; Miyoshi *et al*., 2003; Tan and Vanitha, 2004).

2.4 Biology and biodiversity of fungi

Based on cellular morphologies and biochemistries, living organisms are divided into three domains: Eukarya, Bacteria and Archaea (Figure 2.4). The Eukaryotes, are distinguished from the two other domains by their distinctly nucleated cells, and several other features (Table 2.2). Thus, fungi are Eukaryotes but differ from plants as they lack chlorophyll and the subsequent ability to perform photosynthesis. They can be distinguished from animals because of lacking a digestive tract (Gravesen *et al*., 1994). Fungi are heterotrophic, organisms with a filamentous, tubular structure, a single branch of which is called a hypha bound by cell walls (containing chitin and β-glucans). The hypha extends by tip growth, and multiplies by branching, creating a fine network, or mycelium. Fungi employ exoenzymes, form spores, and lack flagella (Jennings and Lysek, 1996; Nicklin *et al*., 1999; Kirk *et al*., 2001).

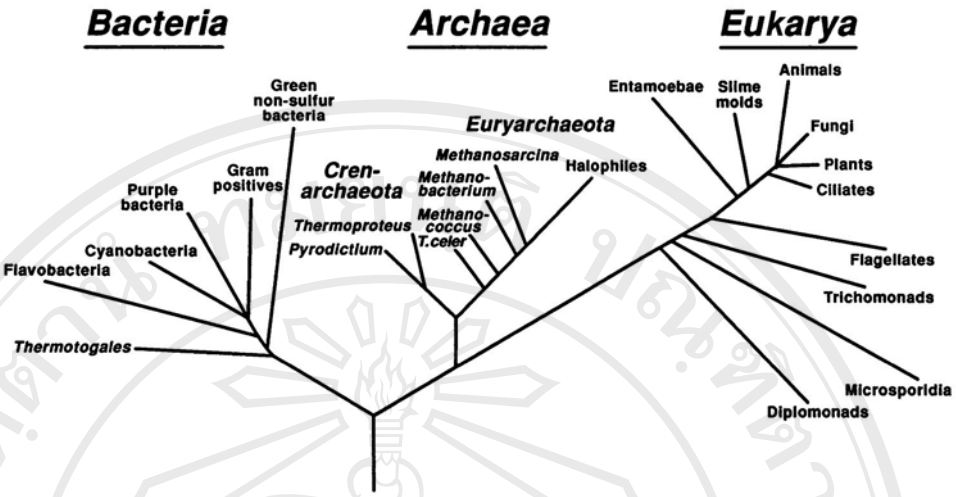

Figure 2.4 Universal phylogenetic tree in rooted form, showing the three domains: Archaea, Bacteria, and Eukarya. Branching order and branch lengths are based upon rRNA sequence comparisons. The position of the root was determined by using the paralogous gene couple, translation elongation factors EFTu and EFG (Iwabe *et al*., 1989; Woese, 1994).

Table 2.2 Differences between the three domains (Campbell *et al*., 2003).

Characteristic	**Bacteria (Prokaryote)**	**Archaea (Prokaryote)**	**Eukarya (Eukaryote)**
Nuclear envelop	no	no	yes
Membrane-enclosed organelles	no	no	yes
Peptidoglycan in cell wall	yes	no	no
Membrane lipids	unbranched hydrocarbons	some branched hydrocarbons	unbranchced hydrocarbons
RNA polymerase	one gene	several genes	several genes
Start amino acid	formyl-Met	Met	Met
Introns	no	some species	yes
Sensitivity to antibiotics streptomycin and chloramphenicol	yes	no	no

Representative of the fungi *sensu stricto* include four phyla: Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota (McLaughlin *et al*., 2001; Seifert and Gams, 2001). The important differences between the taxonomic groups are summarized in Table 2.3. Chytridiomycota and Zygomycota are described as lower fungi, in which the vegetative mycelium is non-septate, and complete septa are only

found in reproductive structures. Asexual reproduction is by the formation of sporangia, and sexual reproduction by the formation of zygospores. Ascomycota and Basidiomycota are described as higher fungi and have a more complex mycelium with elaborate, perforate septa. Members of Ascomycota produce sexual ascospores in sac-shaped cells called asci, while fungi from Basidiomycota produce their sexual spores from club-shaped basidia in complex fruit bodies. Anamorphic fungi are anamorphs of Ascomycota or Basidiomycota and produce asexual conidia (Nicklin *et al*., 1999; Kirk *et al*., 2001). This study focuses on higher fungi, Ascomycota and Basidiomycota and their anamorphs.

Table 2.3 Most significant morphological and biochemical features of the main groups of fungi (modified from Jennings and Lysek, 1996; Nicklin *et al*., 1999).

Features	**Lower fungi**		**Higher fungi**	
	Chytridiomycota	**Zygomycota**	**Ascomycota**	**Basidiomycota**
Perforate septa	Absent	Absent	Present	Present
Asexual sporulation	Motile zoospores	Non-motile sporangiospores	Conidiospores	Conidiospores
Sexual sporulation	Oospores	Zygospores	Ascospores	Basidiospores
Mannitol as a major polyol	+	-	+	+
Glutamate dehydrogenase				
NADP-linked	-	-	+	+
NAD-linked	+	+	-	-

NADP, nicotinamide adenine dinucleotide phosphate; NAD, nicotinamide adenine dinucleotide

About 70,000 species of fungi have been described (Hammond, 1992; Wilson, 1992; Hawksworth *et al*., 1995). However, Hawksworth (1991) estimated a global number of 1.5 million species. This figure was based on a ratio of approximately six species of fungi for every vascular plant. This ratio was derived from the ratio of plant and fungal species in the United Kingdom, an area believed to be better studied than any other in the world in respect to plant and fungal communities. The extrapolation

of this ratio on a worldwide scale gave an estimate of 1.5 million fungal species. Most authors accept that the estimate of Hawksworth (1991) is reasonably accurate, although probably conservative (Rossman, 1994; Cannon, 1997; Wildman, 1997; Frö hlich and Hyde, 1999). Rossman (1994) noted that the number of fungal species associated with insects has been estimated at between 500,000 and 1.5 million, and that large numbers of fungi are also found associated with other non-plant substrates. Furthermore, Hawksworth *et al*. (1996) listed about 30 niches and microhabitats to examine for fungi in a tropical forest (Table 2.4). May (1991, 1994), however, argued that the figure of 1.5 million is too high, and that the diversity of fungi could be as low as a few hundred thousand species. Hyde and Hawksworth (1997) believed these low estimates to be due to a lack of familiarity with fungal distributions and host specificity. Analysis of 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 fungal species is 1:26 (Hyde *et al*., 1997) and appears to be higher than the generally accepted ratio of 1:6 for other plants (Hawksworth, 1991). Fröhlich and Hyde (1999) provided data on the numbers of fungi occurring on the above-ground tissues of six individual *Licuala* species in Australia and Brunei Darussalam (Borneo) and indicated that 33:1 would be a more accurate estimate of the ratio of host specific fungal to palm species in the tropics. Photita *et al*. (2001a, b) provided data on number of endophytic and saprobic fungi occurring on *Musa* spp. in Thailand and Hong Kong, and listed six fungal species that are probably unique to *Musa* species. Together with a list of fungi on *Musa* spp. (Farr *et al*., 1989; Brown *et al*., 1998), Photita *et al*. (2001b) noted that the ratio of 1:6 appeared to hold for *Musa* species.

Table 2.4 Principal niches and microhabitats for fungi in a tropical forest (Hawksworth *et al*., 1996).

Living vascular plant-associated fungi
Biotrophs and necrotrophs of leaves, stems, fruits, seeds, etc.
Endophytes of leaves, stems, bark and roots
Secondary colonizers of dead attached tissue
Arbuscular mycorrhizal and ectomycorrhizal species
Fungi associated with plant exudates
Fungi on leaf and fruit surfaces
Yeasts and other fungi associated with nectar, resin, etc.
Dead plant-associated fungi
Saprobes of wood, bark and litter
Fungi on soil surfaces
Fungi isolated from soil core
Fungi associated with burnt plant tissue
Fungi of submerged and inundated vegetation
Chytrids associated with pollen in water samples
Fungi associated with non-vascular plants
Lichenized fungi on leaves, bark, rock and soil surfaces
Algal parasites on leaves and trunks, in water, etc.
Fungi associated with bryophytes
Fungi associated with aquatic algae
Fungi associated with other fungi and fungal analogues
Biotrophs, necrotrophs and saprobes of other fungi
Lichenicolous species
Myxomyceticolous species
Fungi associated with vertebrates
Mammal and bird skins, feathers, hair, bone, etc.
Fungi from dung, pellets, etc.
Fungi associated with nests, lairs, etc.
Ruminant guts
Fungi on fish scales, in fish guts, etc.
Fungi associated with invertebrates
Biotrophs and necrotrophs of insects
Fungi from invertebrate guts (arthropods, annelids, etc.)
Fungi associated with insect nests (bees, termites, etc.)
Fungi on arthropod scales
Nematode-trapping fungi, fungi associated with rotifers, etc.
Fungi in water
Water and foam isolations from streams, permanent and temporary ponds etc.
Water retained in plants (e.g., bromeliads)

2.5 Fungi in Thailand

Thailand has a rich, diverse flora and fauna, but lagged behind with respect to research on the biodiversity of its fungi (Jones and Hyde, 2004). Before 1990, reports on fungal diversity in Thailand were sporadic, and knowledge of Thailand's fungal diversity was very poor. This may be the result of a lack of funding, lack of research facilities and, in particular, a lack of access to the literature on fungi. However, there has been greater interest on Thai fungi since 1990. The number of fungal records for Thailand has increased from 700 species (in 1989) to over 2,000 species in 2005. There were 13,696 fungal collections, with 2,200 species in approximately 800 genera in the database complied by BIOTEC (Hywel-Jones and Boonpratuang, 2001), and a number of new taxa have recently been described from Thailand (e.g., Photita *et al*., 2002, 2003a; Pinruan *et al*., 2002, 2004a, b, c; Promputtha *et al*., 2003, 2004a, 2005a, b; Bussaban *et al*., 2003a, b; Pinnoi *et al*., 2003a, b, 2004; Thongkantha *et al*., 2003). Thai fungi described since 2000 are from various microhabitats including on living or dead plants tissues (leaves, seeds or wood), on animals (insects, crustraceans, fish), fungi from soil, endophytes, lichens, and mycorrhizas in forests or national parks.

There have been several studies focusing on fungal communities on wood (Sivichai and Hywel-Jones, 1999; Sivichai *et al*., 2000, 2002a; Kodsueb *et al*., 2004). These studies have addressed both freshwater and terrestrial fungi. Sivichai *et al*. (2000) studied fungal colonization on wood of *Dipterocarpus alatus* and *Xylia dolabrifomis* from a stream in Khao Yai National Park and 89 fungal species were reported. Sivichai *et al*. (2002a) collected samples from a stream at Tad Ta Phu, Khao Yai National Park and recorded 73 fungal taxa. Seven new species of freshwater fungi were described from Thailand, including three new species of *Biflagellospora*

(Sivichai and Hywel-Jones, 1999) and one new species of *Brachydesmiella* (Sivichai *et al*., 1998), *Micropeltopsis* (Jones *et al*., 1999) *Melanochaeta* (Sivichai and Hywel-Jones, 1999) and *Sigmoidea* (Marvanová and Hywel-Jones, 2000). Kodsueb *et al*. (2004) studying the fungi on terrestrial wood samples of *Magnolia lillifera* at Doi Suthep-Pui National Park, have identified more than 60 fungal taxa.

Microfungi have been studied on dead plant tissues of several plant species. Promputtha *et al*. (2002) studied fungal succession on senescent leaves of *Magnolia liliifera* at Doi Suthep-Pui National Park and 22 fungal taxa were identified. Promputtha *et al*. (2004b) also studied fungal saprobes on randomly collected dead leaves of *Magnolia liliifera* in the same location, and 37 fungal taxa were identified, including two new species of ascomycetes, *Anthostomella* (Promputtha *et al*., 2005b), and *Pseudohalonectria* (Promputtha *et al*., 2004a), and two new species of anamorphic fungi, *Cheiromyces magnoliae* (Promputtha *et al*., 2005a), and *Dogmaia monthadangii* (Promputtha *et al*., 2003). In the same location, Photita *et al*. (2003b) studied fungi on *Musa acuminata* and identified 80 fungal taxa, including two new species, *Dictyosporium musae* (Photita *et al*., 2002), and *Stachybotrys suthepensis* (Photia *et al*., 2003a). Somrithipol *et al*. (2002) recorded 70 fungi during a study of fungal succession on pods of *Delonix regia* from Khao Yai National Park. Studies of microfungi on palms (*Eleiodoxa conferta*, *Licuala longecalycata*) in Sirindhorn Peat Swamp Forest, Narathiwat has resulted in a number of new species, including new species of *Craspedodidymum*, *Jahnula*, *Phruensis*, and *Stachybotrys* (Pinruan *et al*., 2002, 2004a, b, c), *Custingophora*, *Dactylaria*, *Submersisphaeria*, *Unisetosphaeria* and *Vanakripa* (Pinnoi *et al*., 2003a, b, 2004). There have been a few studies on some macrofungi in Thailand. Sanmee (2004) found 21 taxa of *Amanita* in the northern

provinces (Chiang Mai, Chaing Rai and Payaou) of Thailand. Fifteen new records of *Amanita* and one new species, *A*. *siamensis*, were listed (Sanmee *et al*., 2003).

Endophytic fungi have also been investigated in some indigenous plant species in Doi Suthep-Pui area of Thailand (Lumyong *et al*., 1998). This study indicated that *Colletotrichum, Curvularia, Fusarium, Phoma, Phomopsis* and *Seimatosporium* were dominant genera isolated from most plants. The morphology of *Apiosordaria striatispora*, which is an endophyte of *Mesua ferrea* and *Prunus arborea* has been examined at the SEM level (Hyde *et al*., 1997). Sardsud *et al*. (1998) stated that *Lasiodiplodia*, *Pestalotiopsis*, *Fusarium* and *Curvularia* were the dominant endophytes isolated from shoot, panicle, stem end and seeds of longan (*Dimocarpus longana*) and these fungi are recognized as causing fruit rot in longan after harvest. Lumyong *et al*. (2000) investigated the endophytes in twigs and leaves of bamboo and showed the effects of factors such as tissue type and tissue age on endophyte assemblages and colonization. The result was consistent with other studies in that the old tissues had far more endophytes than the younger tissues. Mycelia sterilia, *Fusarium* spp., *Phoma* sp. and xylariaceous species were the dominant endophytes. Screening for carbohydrase enzyme production by endophytic fungi, indicated that the fungi have the potential to produce mannanase rather than other enzymes tested (Lumyong *et al*., 2000). Sangthong (2002) stated that xylariaceous taxa and *Fusarium* spp. were the dominant endophytes isolated from root of Orchidaceae and some of these fungal isolates significantly increased growth and survival percentage in infected *Dendrobium scabrilingue* and its hybrids. A study of endophytes in wild banana (*Musa acuminata*) resulted in the isolation of 61 taxa (Photita *et al*., 2001b). This study showed the effects of factors such as tissue age,

tissue type and plant growing site on endophyte assemblages and colonization. Fewer isolates were recovered from younger than older samples. Xylariaceous taxa and *Guignardia cocoicola* were the most frequently isolated endophytes from leaves, *Dactylaria* sp. and *Pyriculariopsis parasitica* were most common in the pseudostems, while *Colletotrichum* sp. was most common in the midribs and petioles (Photita *et al*., 2001b). A wide range of the endophytic fungi isolated from wild banana showed the potential to produce bioactive compounds, e.g., *Guignardia cocoicola* and *Fusarium* sp. produced the greatest growth inhibition of banana pathogens, *Colletotrichum musae* and *Fusarium* sp., and of a yeast, *Saccharomyces cerevisiae* (Photita, 2003). Further investigations are necessary to clarify endophyte ecology and species diversity on various plants. Thailand is in the tropics and the endophytes are therefore believed to be diverse and may provide an excellent source of isolates for screening and the potential discovery of biological active novel compounds (Dreyfuss and Petrini, 1984; Hyde, 2001; Strobel and Daisy, 2003; Strobel *et al*., 2004).

2.6 Fungi described from Zingiberaceae

The Zingiberaceae comprises 53 genera with about 1,300 species (Mabberley, 1987; Griffiths, 1992), and 147 fungal species have been described from 16 genera (about 70 species) of Zingiberaceae, with most records from *Zingiber*, *Amomum* and *Alpinia*. Most described taxa are ascomycetes or their anamorphs with basidiomycetes being poorly represented on these plants (Table 2.5). The fungi on Zingiberaceae have been mainly described from India (34 species), and China (16). In Thailand, 11 fungi (including 5 species isolated as endophytes) have been described from wild gingers, comprising 1 basidiomycete, 2 ascomycetes and 8 anamorphic fungi (Table 2.6).

Table 2.5 Number of fungi described from the Zingiberaceae worldwide (Braun, 2001; Chen *et al*., 2002; Bussaban *et al*., 2004).

Host genus	Basidiomycetes	Ascomycetes	Anamorphic fungi	Total
Aframomum	6	1		7
Alpinia	2	8	17	27*
Amomum	4	10	12	26
Catymbrium			1	1*
Curcuma	2	1	15	18
Elettaria	2	3	5	10
Geostachys			1	1
Globba	2		1	3
Hedychium	2	3	5	10
Hitchenia			1	1
Kaempferia	1			1
Nicolaia			2	2
Renealmia		4	1	5
Roscoea	1			1
Stahlianthus			1	1
Zingiber	1	8	18	27
Unknown Zingiberaceae	2	4	1	7
Total	**25**	**42**	**81**	**147**

* *Pyricularia distorta* was described on both host genera

The first fungus to be described from a zingiberaceous plant was the basidiomycete, *Crepidotus alpiniae* Berk. (Hooker, 1856) on *Alpinia aromatica* from Brazil. There are most records of basidiomycetes on *Aframomum* (6) and *Amomum* (4). The best-represented basidiomycete genus is *Uredo*, with 7 species described from Zingiberaceae.

Most ascomycete species on Zingiberaceae are described from Brazil (5), China (5), India (4), Malaysia (4) and Dominican Republic (4). Currently the best-represented ascomycete genus is *Mycosphaerella*, with 5 species described from Zingiberaceae. There are most records of ascomycetes on *Amomum* (10).

Table 2.6 Index of fungi described from the Zingiberaceae (Braun, 2001; Bussaban *et al*., 2002, 2003a, b; Chen *et al*., 2002).

Aframomum

Basidiomycetes

Desmellopsis aframomicola J.M. Yen, *Rev. Mycol. (Paris)* 34: 21 (1969).
Gabon, Kanga, on living leaves of *Aframomum citratum*, 21 Jul. 1969, G. Gilles.
Puccinia aframomi Hansf., in Wakefield & Hansford, *Proc. Linn. Soc. London* 161: 176 (1949).
Uganda, Masakka Rd, on *Aframomum citratum*, Nov. 1937, C.G. Hansford (2250).
Puccinia aframomi-gigantei J.M. Yen & Gilles, *Cah. Maboké* 8: 38 (1970).
Gabon, on leaves of *Aframomum giganteum*.
Tubulicium vermiferum* ssp. *raphidosporum Boidin & Gilles, *Bull. Soc. Mycol. France* 102: 283 (1986).
Gabon, on *Aframomum* sp., 8 May 1976, LY7771.
Uredo aframomi Har. & Pat., *Bull. Mus. Hist. Nat. (Paris)* 5: 365 (1911).
Congo, Mandgi, on leaves of *Aframomum* sp.
Uredo longozyi Bouriquet & Bassino, *Rev. Mycol (Paris)* 31: 326 (1966).
Madagascar, on *Aframomum daniellii*.

Ascomycetes

Aulographum aframomi Hansf., *Proc. Linn. Soc. London* 156: 112 (1944).
Uganda, Entebbe Rd, on leaves of *Aframomum* sp., C.G. Hansford (3163).

Alpinia

Basidiomycetes

Crepidotus alpiniae Berk., in Hooker, *J. Bot. (Hooker)* 8: 133 (1856).
Brazil, Rio Negro, on dead stems of *Alpinia aromatica*, R. Spruce (114).
Marasmiellus pacificus Desjardin, in Desjardin *et al*., *Canad. J. Bot.* 70: 533 (1992).
USA, Hawaii, Kauai, Lawai, National Tropical Botanical Garden, on dead leaves of *Alpinia purpurata*, 25 Nov. 1990, G. Wong (BISH 889).

Ascomycetes

Leptosphaeria alpiniae Maubl., *Bull. Soc. Mycol. France* 21: 89 (1905).
Brazil, Sao Paulo, on leaf spot of *Alpinia nutans*, Puttemans.
Linocarpon alpiniae K.D. Hyde, *Bot. J. Linn. Soc.* 123: 113 (1997).
Malaysia, Taman Ulu Bendol, in rain forest by side of stream, on basal stem of *Alpinia* sp., Nov. 1992, K.D. Hyde (HKU(M) 1632).
Meliola monopla Cif., *Mycopathol. Mycol. Appl.* 7: 159 (1954).
[Dominican Republic] Santo Domingo, Peninsula de Samaná, Sánchez, on leaves of *Alpinia aromatica*, 30 Apr. 1930, E.L. Ekman (4192).
Mycosphaerella alpiniae S.Q. Chen & P.K. Chi, in Chi, *Fung. Dis. Cultivated Medicinal Plants Guangdong*: 159 (1994).
China, Guangdong, on leaves of *Alpinia katsumadai*, Chen (114).
Mycosphaerella alpiniicola S.Q. Chen & P.K. Chi, in Chi, *Fung. Dis. Cultivated Medicinal Plants Guangdong*: 35 (1994).
China, Guangdong, on leaves of *Alpinia oxyphylla*, Chen (109).
Pestalosphaeria alpiniae P.K. Chi & S.Q. Chen, in Chi, *Fung. Dis. Cultivated Medicinal Plants Guangdong*: 35 (1994).
China, Guangdong, on leaves of *Alpinia oxyphylla*, Chen (91).
****Phyllachora alpiniae*** Sacc. & Berl., *Atti Ist. Veneto Sci. Lett. Arti* 6: 715 (1885).
Australia, Queensland, Tallebud, on dead leaves of *Alpinia coerulea*, 1883, B. Scortechini.
Phyllachora alpiniae Cooke & Massee, in Cooke, *Grevillea* 17: 56 (1889).
Australia, Brisbane, on fading leaves of *Alpinia coerulea*, Bailey (623).

Anamorphic fungi
Aposphaeria alpiniae Massee, *Bull. Misc. Inform.*: 182 (1899).
Solomon Islands, New Georgia, on inflorescences of *Alpinia* sp., 1894, Officers of HMS Penguin.
Cercospora alpiniae Syd. & P. Syd., *Ann. Mycol.* 12: 202 (1914).
Philippines, Los Banos, Laguna, Mt Maquiling, on leaves of *Alpinia* sp., 18 Dec. 1913, C.F. Baker (2221).
Cercospora alpiniae-katsumadae S.Q. Chen & P.K. Chi, *J. South China Agric. Univ.* 11: 58 (1990).
China, Guangdong, on leaves of *Alpinia katsumadai*, Chen (112).
Cercospora alpiniae-katsumadaicola S.Q. Chen & P.K. Chi, *J. South China Agric. Univ.* 11: 58 (1990).
China, Guangdong, on leaves of *Alpinia katsumadai*, Chen (111).
Cercospora alpiniicola S.Q. Chen & P.K. Chi, *J. South China Agric. Univ.* 11: 57 (1990).
China, Guangdong, on leaves of *Alpinia oxyphylla*, Chen (102).
Coniothyrium alpiniicola Tassi, *Bull. Lab. Orto Bot. Reale Univ. Siena* 2: 152 (1899).
Italy, Senensi Botanical Gardens, on leaf sheaths of *Alpinia nutans*.
****Dactylium alpiniae*** Sawada, *Rep. Gov. Res. Inst. Formosa* 35: 102 (1928).
Taiwan, on leaves of *Alpinia speciosa*.
****Monosporium alpiniae*** Sawada, *Special Publ. Coll. Agric. Natl. Taiwan Univ*. 8: 185 (1959).
Taiwan, Taipei, on fruit of *Alpinia speciosa*, 27 & 28 Aug. 1946, C.C. Chen.
Pestalotiopsis alpiniae Y.X. Chen & G. Wei, in Chen, Wei & Chen, *Mycosystema* 21: 317 (2002).
China, Guangxi, on living leaves of *Alpinia galanga*, 9 Dec. 1975, Y.X. Chen & Y.Q. He (DPPGU Hsp93 II-5100).
Phomopsis alpiniae Sousa da Câmara, *Agron. Lusit*. 11: 57 (1949).
Portugal, Lisboa, Hortum Botanicum Facultatis Scientiarum, on living leaves of *Alpinia nutans*, 1948, T. Vasconcelos.
Phomopsis conspicua Syd. & P. Syd., *Ann. Mycol.* 18: 102 (1920).
Philippines, Mindanao, Davao, Pantucan, on living leaves of *Alpinia* sp., 24 Apr. 1918, O.A. Reinking (6918).
Phyllosticta alpiniae Bat., in Batista & Vital, *Biol. Soc. Agric. Pernambuco* 19: 5 (1952).
Brazil, on *Alpinia speciosa.*
****Phyllosticta alpiniae-kelungensis*** Sawada, *Rep. Gov. Res. Inst. Formosa* 85: 59 (1943).
Taiwan, on *Alpinia kelungensis*.
Pseudocercospora alpiniae S.Q. Chen & P.K. Chi, *J. South China Agric. Univ.* 11: 48 (1990).
China, Guangdong, on leaves of *Alpinia offcinarum*, Chen (105).
Pseudocercospora alpiniicola S.Q. Chen & P.K. Chi, *J. South China Agric. Univ.* 11: 48 (1990).
China, Guangdong, on leaves of *Alpinia officinarum*, Chen (106).
Pyricularia distorta Hashioka, *Trans. Mycol. Soc. Japan* 12: 133 (1971).
Thailand, Mt Khaoyai, on living leaves of *Alpinia* sp. and *Catymbrium* sp., 9 Sep. 1968, Y. Hashioka.
Stenella alpiniae (Syd. & P. Syd.) U. Braun, *Fungal Diversity* 8: 68 (2001).
Philippines, Laguna, Los Banos, Mt Maquiling, on *Alpinia* sp., Dec. 1914, C.F. Baker (PC).

Amomum

Basidiomycetes
Arrhenia minuta Petch, *Trans. Brit. Mycol. Soc*. 27: 137 (1944).
Sri Lanka, Hakgala, on dead leaves of *Amomum* sp., May 1912, T. Petch (4547).
Marasmius amomi Petch, *Trans. Brit. Mycol. Soc.* 31: 43 (1947).
Sri Lanka, Hakgala, on dead stems of *Amomum* sp., Apr. 1915, T. Petch.
Mycena aculeifera Petch, *Ann. Roy. Bot. Gard. (Peradeniya)* 10: 132 (1926).
Sri Lanka, Peradeniya, on dead rhizomes of *Amomum* sp., 17 Dec. 1914.
Uredo amomi Petch, *Ann. Roy. Bot. Gard. (Peradeniya)* 5: 252 (1912).
Sri Lanka, Hakgala, on *Amomum involucratum*.

Ascomycetes
Amphisphaeria amomi Henn. & E. Nyman, in Hennings, *Monsunia* 1: 166 (1899).
Indonesia, Java, Salak, on decaying stems of *Amomum* sp., E. Nyman.
Bertia tessellata Petch, *Ann. Roy. Bot. Gard. (Peradeniya)* 7: 304 (1922).
Sri Lanka, Hakgala, on dead rhizomes of *Amomum* sp.

Gaeumannomyces amomi Bussaban *et al*., *Nova Hedwigia* 73: 488 (2001).
Thailand, Chiang Mai, Doi Suthep-Pui National Park, on healthy leaves of *Amomum siamense*, Feb. 2000, B. Bussaban (BCC4066).
Gnomonia scitaminearum Höhn., *Mitt. Bot. Lab. TH Wien* 4: 7 (1932).
Indonesia, Java, on leaves of *Amomum* sp.
Guignardia amomi S.M. Lin & P.K. Chi, in Chi, *Fung. Dis. Cultivated Medicinal Plants Guangdong*: 43 (1994).
China, Guangdong, on leaves of *Amomum krervanh*, Lin (156).
Leiosphaerella amomi Bussaban *et al*., *Nova Hedwigia* 73: 490 (2001).
Thailand, Chiang Mai, Doi Suthep-Pui National Park, on healthy leaves of *Amomum siamense*, Aug. 1999, B. Bussaban (BCC4065).
Meliola amomicola F. Stevens, *Illinois Biol. Monogr.* 2: 40 (1916).
Puerto Rico, Mayaguez, Mesa, on leaves of *Amomum caryophyllata*, 15 Jun. 1915.
Microthyrium subulatum P.C. Gupta, *Mycopathol. Mycol. Appl.* 54: 129 (1974).
India, U.P., Varanasi, on living leaves of *Amomum subulatum*, 17 Oct. 1964, P.C. Gupta (PCG-38).
Mycosphaerella amomi P.K. Chi, *Fung. Dis. Cultivated Medicinal Plants Guangdong*: 44 (1994).
China, Guangdong, on leaves of *Amomum compactum*, Lin (261).
Phaeochaetia amomicola **var. *minispora*** Bat. & Peres, in Batista *et al*., *Brotéria Ci. Nat.* 31: 115 (1962).
Brazil, Pernambuco, on leaves of *Amomum magnificum*, 16 Sep. 1960.

Anamorphic fungi
Berkleasmium nigroapicale Bussaban *et al*., *Fung. Divers.* 8: 80 (2001).
Thailand, Chiang Mai, Doi Suthep-Pui National Park, on dead pseudostems of *Amomum siamense*, 15 Oct. 2000, B. Bussaban (PDD 74415).
Berkleasmium sutheppuiense Bussaban *et al*., *Fung. Divers.* 8: 82 (2001).
Thailand, Chiang Mai, Doi Suthep-Pui National Park, on dead pseudostems of *Amomum siamense*, 15 Oct. 2000, B. Bussaban (PDD 74416).
Cercospora amomi A.K. Kar & M. Mandal, *Trans. Brit. Mycol. Soc*. 53: 358 (1969).
India, West Bengal, Darjeeling, Tung, on *Amomum dealbatum*, 1768 m, 12 May 1967 (IMI 135184).
Gonatopyricularia amomi Z.D. Jiang & P.K. Chi, *J. South China Agric. Univ.* 10: 11 (1989).
China, Guangdong, Yang-Chun, on living leaves of *Amomum villosum*, Feb. 1985, Z.D. Jiang & P.K. Chi (001).
Phoma amomi P.K. Chi, *Fung. Dis. Cultivated Medicinal Plants Guangdong*: 45 (1994).
China, Guangdong, on leaves of *Amomum compactum*, Lin (77).
Pyricularia kookicola Bussaban, *Mycologia* 95: 520 (2003).
Thailand, Chiang Mai, Doi Suthep-Pui National Park, on healthy leaves of *Amomum siamense*, Feb. 2000, B. Bussaban (CMUZE0501).
Pyricularia longispora Bussaban, *Mycologia* 95: 522 (2003).
Thailand, Chiang Mai, Doi Suthep-Pui National Park, on healthy leaves of *Amomum siamense*, Feb. 2000, B. Bussaban (BCC11377).
Pyricularia variabilis Bussaban, *Mycologia* 95: 522 (2003).
Thailand, Chiang Mai, Doi Suthep-Pui National Park, on healthy leaves of *Amomum siamense*, Feb. 2000, B. Bussaban (BCC8210).
Ramichloridium amomi P.K. Chi & S.Q. Chen, in Chi, *Fung. Dis. Cultivated Medicinal Plants Guangdong*: 46 (1994).
China, Guangdong, on leaves of *Amomum krervanh*, Chen (115).
Rhombostilbella crus-pavonis Cif., Bat. & Nascim., *Atti Ist. Bot. Lab. Crittog. Univ. Pavia* 14: 13 (1957).
Brazil, Pernambuco, on *Amomum magnificum*, 22 Mar. 1956.
Septoria amomi Z.D. Jiang & P.K. Chi, in Chi, *Fung. Dis. Cultivated Medicinal Plants Guangdong*: 29 (1994).
China, Guangdong, on leaves of *Amomum villosum*, Jiang (56).
Xenosporium amomi Bussaban, *Fung. Divers.* 14: 62 (2003).
Thailand, Chiang Mai, Doi Suthep-Pui National Park, on dead pseudostems of *Amomum siamense*, 15 October 2000, B. Bussaban (PDD 77014).

Catymbrium

Anamorphic fungi

Pyricularia distorta Hashioka, *Trans. Mycol. Soc. Japan* 12: 133 (1971).
Thailand, Mt Khaoyai, on living leaves of *Alpinia* sp. and *Catymbrium* sp., 9 Sep. 1968, Y. Hashioka.

Curcuma

Basidiomycetes

Klastopsora curcumae Höhn., *Sitzungsber. Kaiserl. Akad. Wiss., Matt.-Naturwiss. Cl., Abt. 1* 121: 339 (1912).
Indonesia, Java, Buitenzorg Botanical Garden, on leaves of *Curcuma longa*, 1907, von Höhnel.
Puccinia curcumae T.S. Ramakr. & Sundaram, *Proc. Indian Acad. Sci. B* 38: 192 (1953).
India, Madras, Mundage, on living leaves of *Curcuma* sp., 27 Aug. 1952, T.S. Ramakrishnan.

Ascomycetes

****Pyrenochaetina curcumae*** Sawada, *Special Publ. Coll. Agric. Natl. Taiwan Univ.* 8:145 (1959).
Taiwan, Tainan, Wanli, on leaves of *Curcuma aromatica*, 4 Nov. 1909, K. Sawada.

Anamorphic fungi

Cercospora curcumae Govindu & Thirum., *Sydowia* 10: 275 (1956).
India, Bangalore, Hebbal, on leaves of *Curcuma longa*, 12 Apr. 1954, H.C. Govindu (IMI 125195).
Cercospora curcumae-longae Pavgi & R. Upadhyay, *Sydowia* 21: 102 (1967).
India, U.P., Varanasi, on leaves of *Curcuma longa*, 3 Jan. 1964, R. Upadhyay (MSP 341).
Cercospora curcumina R.K. Srivast., N. Srivast. & A.K. Srivast., *Proc. Natl. Acad. Sci. India, Sect. B, Biol. Sci.* 64: 107 (1994).
India, Madhya Pradesh, University of Gorakhpur, on leaves of *Curcuma angustifolia* (GPU 1341).
****Dactylaria curcumae*** Sawada, *Special Publ. Coll. Agric. Natl. Taiwan Univ.* 8: 188 (1959).
Taiwan, Nantou, Fenshuiliao, on leaves of *Curcuma aromatica*, 8 Oct. 1910, K. Sawada.
Neottiospora curcumae K. Ramakr. & Sundaram, *Proc. Indian Acad. Sci. B* 45: 150 (1957).
India, Madras, Chingleput, on leaves of *Curcuma amada*, 9 Feb. 1956, N.V. Sundaram.
Passalora curcumae Purkay. & Mallik, *Beih. Nova Hedwigia* 63: 132 (1979).
India, West Bengal, on leaves of *Curcuma reclinata*.
Phaeodactylium curvularioides Matsush., *Matsushima Mycol. Mem.* 1: 56 (1980).
Taiwan, Kuantzuling, on dead leaves of *Curcuma aromatica*, 6 Jun. 1978 (9082).
Phaeorobillarda curcumae Pavgi & R. Upadhyay, *Sydowia* 21: 100 (1967).
India, U.P., Varanasi, on leaves of *Curcuma longa*, 12 Nov. 1963, R. Upadhyay (MSP 340).
****Phyllosticta curcumae*** Sawada, *Special Publ. Coll. Agric. Natl. Taiwan Univ*. 8: 135 (1959).
Taiwan, Taipei, on leaves of *Curcuma longa*, 2 Dec. 1914, Y. Fujikuro.
Pyricularia curcumae Rathaiah, *Pl. Dis.* 64: 104 (1980).
India, Assam, Diphu, on leaf of *Curcuma longa*, 9 Aug. 1977, Y. Rathaiah (IMI 216922).
Sphaceloma curcumae Thirum., *Trans. Brit. Mycol. Soc*. 31: 6 (1947).
India, Kemmangundi, Mysore, on leaves of *Curcuma* sp., 9 Oct. 1945, M.J. Thirumalachar (HCIO).
Sporidesmina malabarica Subram. & Bhat, *Kavaka* 15: 69 (1987).
India, Kerala, Palghat, Silent Valley, on dead petiole of *Curcuma* sp., 11 Jul. 1980, D.J. Bhat (FFSI 4328).
Sporidesmiopsis malabarica Subram. & Bhat, *Kavaka* 15: 71 (1987).
India, Kerala, Palghat, Silent Valley, on dead petiole of *Curcuma* sp., 23 Apr. 1980, D.J. Bhat (FFSI 3637).
Thirumalacharia curcumae Rathaiah, *Mycologia* 72: 1211 (1980).
India, Assam, Shillong, on leaves of *Curcuma longa*, Y. Rathaiah, 9 Nov. 1978 (IMI 233681).
Vermicularia curcumae Syd., *Ann. Mycol.* 11: 329 (1913).
India, Madras, Kistna, Angalur, on dying leaves of *Curcuma longa*, 24 Oct. 1912, W. McRae (24).

Elettaria

Basidiomycetes

Schroeteriaster elettariae Racib., *Parasit. Algen Pilze Javas* II: 28 (1900).
Indonesia, Java, on leaves of *Elettaria* sp.

Uredo elettariae Thirum., *Curr. Sci.* 12: 232 (1943).
India, Mysore, Balehonnur, on *Elettaria cardamomum*.

Ascomycetes

Catacauma elettaria T.S. Ramakr. & K. Ramakr., *Proc. Indian Acad. Sci. B* 32: 99 (1950).
India, Madras, Papanasam, on living leaves of *Elettaria cardamomum*, 15 May 1949, K. Sundaram.

Ceriospora elettariae Ponnappa & C.G. Shaw, *Mycologia* 70: 861 (1978).
India, Cherambane, Coorg, Karnataka, on leaf of *Elettaria cardamomum*, K.M. Ponnappa, 1 Nov. 1975 (IMI 198915).

Placostroma elettariae Berk. & Broome, in Theissen & Sydow, *Ann. Mycol.* 13: 408 (1915).
Sri Lanka, Habgalla, on leaves of *Elettaria floribunda*.

Anamorphic fungi

Phaeodactylium venkatesanum Agnihothr., *Proc. Indian Acad. Sci. B* 68: 208 (1968).
India, Kerala, High Ranges, Mat. Sathurangapara estate, on living leaves of *Elettaria cardamomum*, 10 Oct. 1967, V. Agnihothrudu (IMI 129936).

Phyllosticta elettariae S.R. Chowdhury, *Lloydia* 21: 153 (1958).
India, Assam, Nongpoh, on leaves *of Elettaria cardamomum*, 4 Nov. 1957, S.R. Chowdhury (HCIO 25592).

Rhabdospora elettariae Penz. & Sacc., *Malpigia* 15 : 235 (1901).
Indonesia, Java, Tjibodas, on stems of *Elettaria* sp.

Sphaceloma cardamomi Muthappa, *Sydowia* 19: 145 (1966).
India, Mysore, Coorg, on leaves of *Elettaria cardamomum*, 5 Jan. 1965, B.N. Muthappa (MACS 250).

Xenosporium intermedium Vittal, *Trans. Brit. Mycol. Soc.* 76: 513 (1981).
India, Karnataka, Nemmar, on dead rachis of *Elettaria cardamomum*, 12 Nov. 1974, B.P.R. Vittal (MUBL 2544).

Geostachys

Anamorphic fungi

Chalara rostrata Nag Raj & W.B. Kendr., *Monogr. Chalara & Allied Genera*: 132 (1975).
Malaysia, Cameron Highlands, on *Geostachys rupestris*, 6 Sep. 1953, W.J. Cherewick (IMI 54897).

Globba

Basidiomycetes

Chrysocelis globbae Syd., in Sydow & Petrak, *Ann. Mycol.* 29: 165 (1931).
Philippines, Manila, on living leaves of *Globba marantina*, 9 Oct. 1923 (2984).

Maravaria pseudosuprastomatalis Y. Ono & Kakish., in Ono *et al*., *Trans. Brit. Mycol. Soc.* 91: 471 (1988).
Thailand, Phetchabun, Nam Nao National Park, on leaves of *Globba* sp., 22 Dec. 1985 (TSH-R7174).

Anamorphic fungi

****Pyricularia globbae*** Siwasin & Giatgong, *Newslett. Int. Rice Commiss.* 20: 16 (1971).
Thailand, on *Globba* sp.

Hedychium

Basidiomycetes

Lecanocybe lateralis Desjardin & E. Horak, *Sydowia* 51: 21 (1999).
Indonesia, Java, Cibodas Botanical Garden, on senescent leaves of *Hedychium flavescens*, 11 Jan. 1998, E. Horak & D.E. Desjardin (SFSU 6752).
Typhula thindii Khurana, *Mycologia* 72: 714 (1980).
India, U.P., Nainital, Tiffońs Top, on stem and leaves of *Hedychium acuminatum*, 6 Aug. 1973, I.P.S. Khurana (PAN 4881).

Ascomycetes

Microthyriella azorica Dennis & Spooner, in Dennis *et al*., *Kew Bull*. 32: 131 (1977).
Azores, Vila Nova, Terceira, on dead leaves of *Hedychium gardnerianum*, 25 Mar. 1975 (K).
Mycosphaerella hedychii F. Stevens & P.A. Young, in Stevens, *Bernice P. Bishop Mus. Bull.* 19: 103 (1925).
USA, Hawaii, on leaves of *Hedychium coronarium*.
Patinellaria hedychii K.S. Thind & Saini, *Proc. Indian Acad. Sci. B* 67: 143 (1968).
India, U.P., Mussoorie, Jabber Khet Khud, on decaying stems and leaf sheaths of *Hedychium acuminatum*, 27 Aug. 1960, K.S. Thind (448).

Anamorphic fungi

Cercospora hedychii Boedijn, *Nova Hedwigia* 3: 432 (1961).
Indonesia, Java, on leaves *of Hedychium coccineum*, Jun. 1950, K.B. Boedijn.
Macrophoma hedychii Mariani, *Atti Soc. Ital. Sci. Nat.* 50: 169 (1911).
Portugal, Botanical Garden, on petioles of *Hedychium coronarium*.
Phomopsioides natalinae A.C. Santos, *Bol. Soc. Brot.* 40: 42 (1966).
Azores, Ilha de S. Miguel, Mata da Granja, on rhizome of *Hedychium gardnerianum*, Jun. 1960.
Phyllosticta hedychii Petr., *Ann. Mycol.* 14: 168 (1916).
[Austria] Moraviae, Weisskirchen, on dead leaves of *Hedychium* sp., Oct. 1914, J. Petrak.
Pseudocercospora hedychii (Boedijn) U. Braun, *Nova Hedwigia* 73: 424 (2001).
Indonesia, Java, on leaves *of Hedychium coccineum*, Jun. 1950, K.B. Boedijn.

Hitchenia

Anamorphic fungi

Cercospora hitcheniae Chidd., *Indian Phytopathol.* 12: 112 (1959).
India, Bombay, Mahabaleshwar, on leaves of *Hitchenia caulina*, 19 Jan. 1955, P.P. Chiddarwar (14).

Kaempferia

Basidiomycetes

Uredo kaempferiae Syd. & P. Syd., *Ann. Mycol.* 12: 263 (1914).
Angola, Quelimane, on leaves of *Kaempferia ethela*, 8 Sep. 1913, I.B. Pole-Evans (7387).

Nicolaia

Anamorphic fungi

Cercospora nicolaiae Boedijn, *Nova Hedwigia* 3: 432 (1961).
Indonesia, Java, on leaves of *Nicolaia* sp., Feb. 1949, K.B. Boedijn.
Pseudocercospora nicolaiae (Boedijn) U. Braun, *Nova Hedwigia* 73: 427 (2001).
Indonesia, Java, on leaves of *Nicolaia* sp., Feb. 1949, K.B. Boedijn.

Renealmia

Ascomycetes

Dictyopeltis domingensis Petr. & Cif., *Ann. Mycol.* 30: 179 (1932).
[Dominican Republic] Santo Domingo, Peninsula de Samaná, ca. 300 m, on living leaves of *Renealmia aromatica*, 30 Apr. 1930, E.L. Ekman (3595).

Dothidella renealmiae Rehm, *Hedwigia* 36: 377 (1897).
Brazil, Sierra Org., on leaves of *Renealmia* sp., E. Ule.

Micropeltis ekmanii Petr. & Cif., *Ann. Mycol.* 30: 205 (1932).
[Dominican Republic] Santo Domingo, Cordillera Central, Villa Altagracia, on living leaves of *Renealmia aromatica*, 7 Jan. 1930, E.L. Ekman (3811).

Phyllachora renealmiae Rehm, *Hedwigia* 36: 373 (1897).
Brazil, Catharina, on leaves of *Renealmia* sp., E. Ule.

Anamorphic fungi

Septoria renealmiae Tassi, *Bull. Lab. Orto Bot. Reale Univ. Siena* 2: 159 (1899).
Italy, Senensi Botanical Gardens, on dying leaves of *Renealmia cinnamomum*.

Roscoea

Basidiomycetes

Puccinia roscoeae Barclay, *Descr. List Uredineae Simla II*: 237 (1889).
India, Simla, on leaves of *Roscoea alpina*.

Stahlianthus

Anamorphic fungi

Cercospora stahlianthi Z.D. Jiang & P.K. Chi, in Chi, *Fung. Dis. Cultivated Medicinal Plants Guangdong*: 162 (1994).
China, Guangdong, on leaves of *Stahlianthus involucrata*, Jiang (303).

Zingiber

Basidiomycetes

Puccinia zingiberis T.S. Ramakri., *Proc. Indian Acad. Sci. B* 44: 117 (1956).
India, Thodupuzha, on living leaves of *Zingiber officinale*, 26 Sep. 1955, T.S. Ramakrishnan.

Ascomycetes

Dimeriella dendrocalami Sawada & Yamamoto, in Sawada, *Special Publ. Coll. Agric. Natl. Taiwan Univ*. 8: 37 (1959).
Taiwan, Taipei, on leaves of *Dendrocalamus latiflorus*, *Litchi sinensis* and *Zingiber mioga*, 14 Sep. 1929, C.C. Chen.

Hypocrella zingiberis Massee, *Bull. Misc. Inform.* 1899: 174 (1899).
Malaysia, Perak, on petioles of *Zingiber* sp., Ridley (10).

Mycosphaerella zingiberis Shirai & Hara, *Bot. Mag. (Tokyo)* 25: 70 (1911).
Japan, Shimotsuke, on leaves of *Zingiber miogae*.

Nectria egans Corner, *Gard. Bull. Straits Settlem.* 8: 135 (1935).
Malaysia, on leaves of *Zingiber crescentia*.

Nectriella zingiberis F. Stevens & Atienza, *Philipp. Agric.* 20: 176 (1931).
Philippines, Laguna, Agricultural College, on rhizomes of *Zingiber officinale*, 18 Nov. 1930, F. Stevens (1126).

Nectriella zingiberis* var. *pallida F. Stevens & Atienza, *Philipp. Agric*. 20: 176 (1931).
Philippines, Laguna, Agricultural College, on rhizomes of *Zingiber officinale*, 18 Nov. 1930, F. Stevens (1128).

****Phaeosphaeria zingiberis*** Sawada, *Special Publ. Coll. Agric. Natl. Taiwan Univ*. 8: 67 (1959).
Taiwan, Ilan, Mt Chentou, on leaves of *Zingiber officinale*, 18 Jul. 1907, R. Suzuki.

Rosellinia zingiberis F. Stevens & Atienza, *Philipp. Agric*. 20: 174 (1931).
Philippines, Laguna, Agricultural College, on rhizomes of *Zingiber officinale*, 5 Oct. 1930, F. Stevens (878).

Anamorphic fungi
Aschersonia philippinensis Petch, *Ann. Mycol.* 30: 119 (1932).
Philippines, Cagayan, on leaves of *Zingiber* sp., Jan. 1924 (2827).
****Ascochyta zingiberi*** Sawada, *Special Publ. Coll. Agric. Natl. Taiwan Univ*. 8: 152 (1959).
Taiwan, Taipei, on leaves of *Zingiber mioga*, 16 Aug. 1929, K. Sawada.
Ascochyta zingibericola Punith., *Mycol. Pap.* 159: 156 (1988).
Ethiopia, Bako, on living leaves of *Zingiber officinale*, 17 Dec. 1980 (IMI 255877a).
Cercoseptoria zingiberis Rathaiah, *Mycologia* 73: 774 (1981).
India, Assam, Haflong, on leaves of *Zingiber officinale*, 21 Jul. 1978, Y. Rathaiah (IMI 231501).
Cercospora zingiberi Togashi & Katsuki, *Bot. Mag. (Tokyo)* 65: 25 (1952).
Japan, Fukuoka, Takawa, Soeda, on *Zingiber mioga*, 13 Sep. 1949, S. Katsuki.
Cercospora zingibericola A.K. Kar & M. Mandal, *Trans. Brit. Mycol. Soc.* 53: 359 (1969).
India, West Bengal, Murshidabad, Khargram, on *Zingiber officinale*, 1 Feb. 1967 (IMI 135186).
Coniothyrium zingiberis F. Stevens & Atienza, *Philipp. Agric*. 20: 174 (1931).
Philippines, Laguna, Agricultural College, on leaves of *Zingiber officinale*, 24 Oct. 1930. F. Stevens (856).
Fusarium oxysporum* f.sp. *zingiberi E.E. Trujillo, *Phytopathology* 53: 1371 (1963).
USA, Hawaii, on leaves and rhizomes of *Zingiber officinale*.
Geotrichum zingiberis-saccharati Overeem, *Bull. Jard. Bot. Buitenzorg* 5: 283 (1923).
Indonesia, Java, Buitenzorg, on *Zingiber saccharati*.
****Hendersonia zingiberi*** Sawada, *Special Publ. Coll. Agric. Natl. Taiwan Univ*. 8: 156 (1959).
Taiwan, Ilan, Mt Chentou, on leaves of *Zingiber officinale*, 18 Jul. 1907, R. Suzuki.
Memnoniella zingiberis V.G. Rao, *Sydowia* 16: 43 (1962).
India, Poona, on old rhizomes of *Zingiber officinale*, Jan. 1961, V.G. Rao (MACS 95).
Phomopsis zingberis M.S. Ali & Saikia, *Indian Phytopathol.* 46: 228 (1993).
India, Assam, on leaves of *Zingiber officinale* (HCIO 40716).
Phyllosticta zingiberis F. Stevens & Ryan, in Stevens, *Bernice P. Bishop Mus. Bull.* 19: 133 (1925).
USA, Hawaii, on living leaves of *Zingiber zerumbet*.
Phyllosticta zingiberis T.S. Ramakr., *Proc. Indian Acad. Sci. B* 15: 170 (1942).
India, Godavari and Malabar, on living leaves of *Zingiber officinale*.
****Pyricularia zingiberis*** Nishik., *Ber. Ohara Inst. Landwirt. Forsch.* 1: 216 (1917).
Japan, Kuraschiki, on living leaves of *Zingiber mioga* and *Zingiber officinale*.
Pyriculariopsis miogae Matsush., *Icon. Microfungorum Matsushima Lectorum*: 121 (1975).
Japan, Chiba, Kiyozumi Exp. Forest, Univ. Tokyo, on a dead leaf of *Zingiber mioga*, Oct. 1967 (2175).
Septoria zingiberis Sundaram, *Indian Phytopathol.* 14: 208 (1961).
India, Wynaad, Kerala, on living leaves of *Zingiber officinale*, 25 Sep. 1955, N.V. Sundaram (G.M. Herb. 2862).
****Vermicularia zingiberae*** Sundaram, *Yearb. Dept. Agric. Madras* 1926: 10 (1927).
India, on living leaves of *Zingiber officinale*.

Zingiberaceae

Basidiomycetes
Helicogloea indica Boedijn, *Bull. Jard. Bot. Buitenzorg* 14: 188 (1937).
Indonesia, Java, Tjibodas, on dead stems of Zingiberaceae, Apr. 1930, K.B. Boedijn (604).
Mycena digitata Maas Geest. & E. Horak, *Biblioth. Mycol.* 159: 193 (1995).
Papua New Guinea, Morobe, Bulolo, Manki Ridge, Rd 5, on rotten rhizomes of wild ginger, 8 Nov. 1971, E. Horak (ZT 71/256).

Ascomycetes
Leptophyma grandispora M.L. Farr, *Mycologia* 79: 113 (1987).
Brazil, Terr. Roraima, Boa Vista- Sta. Elena Venezuela Rd, on living leaves of Zingiberaceae, 2 Dec. 1977, Dumont *et al*. (BR-873, NY).
Linocarpon zingiberacicola K.D. Hyde, *Bot. J. Linn. Soc.* 123: 129 (1997).
Malaysia, Taman Ulu Bendul, in rain forest by side of stream, on basal stem of unidentified Zingiberaceae, Nov. 1992, K.D. Hyde (ML 10, HKU(M) 1920).

Nectria sesquiphialis Samuels, *Mem. New York Bot. Gard.* 49: 276 (1989).
Venezuela, Edo. Bolivar, Km 110-111 S of E1 Dorado on road between E1 Dorado and Sta Elena, on leaf of Zingiberaceae, 6 Aug. 1972, Dumont (VE 7184) (Teleomorph of *Sesquicillium asymmetricum*).
Protocreopsis zingibericola Yoshim. Doi, *Kew Bull*. 31: 552 (1977).
Papua New Guinea, New Britain, Rabaul, on decayed stems and leaves of Zingiberaceae, 1 Jan. 1970, Doi (TNS, F-192961).

Anamorphic fungi
Sesquicillium asymmetricum Samuels, *Mem. New York Bot. Gard.* 49: 276 (1989).
Venezuela, Edo. Bolivar, Km 110-111 S of E1 Dorado on road between E1 Dorado and Sta Elena, on leaf of Zingiberaceae, 6 Aug. 1972, Dumont (VE 7184) (Anamorph of *Nectria sesquiphialis*).

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*Fungus pulished without a Latin diagnosis, as required by Art. 36 of the International Code of Botanical Nomeclature (Greuter, 2000)

Aposphaeria alpiniae on *Alpinia* sp. (Massee, 1899), *Coniothyrium alpinicola* on *Alpinia nutans*, and *Septoria renealmiae* on *Renealmia cinnamomum* (Tassi, 1899) were the first anamorphic fungi described. The best represented genus is *Cercospora*, with 4 species described on *Alpinia*, 3 species on *Curcuma*, 2 species on *Zingiber*, and 1 species on *Amomum*, *Hedychium*, *Hitchenia*, *Nicolaia*, and *Stahlianthus*.

2.7 Life strategies of fungi

Fungi are a diverse assemblage of eukaryotic organisms united primarily by their absorptive mode of nutrition. They secrete digestive enzymes onto living or dead organic material and then absorb small organic molecules of the predigested food through their cell walls and membranes (Cooks and Rayner, 1984). Because fungi are opportunists that produce a vast range of enzymes (e.g., cellulases, chitinases, proteases and multicomponent lignin-degrading enzymes), they can degrade many kinds of organic as well as some inorganic substrates. Some fungi can detoxify polyphenols, degrade plastics (Edwards, 1988) or break down complex polymer such

as cellulose, chitin or lignin (Nicklin *et al*., 1999). Thus they can grow in a wide range of environments.

In this study, fungi are divided into three categories according to their mode of life. A saprobe is an organism that is a decomposer, utilizing non-living organic material as food and commonly causing its decay (Kirk *et al*., 2001). A pathogen is a parasite living in or on another living organism, obtaining nutrients from the host and harmful to the host (Raven *et al*., 1992). An endophyte is an organism living in healthy plant tissues but causing symptomless infection (Petrini, 1991). Endophytic fungi, however, represent a divesity of nutritional modes from biotrophic parasites to interim or facultative saprotrophs, and association with their hosts span the continuum from biotrophic mutualists and benign commensals to nectotrophic, antagonistic pathogens (Bertoni and Cabral, 1988; Johnson and Whitney, 1992; Bettucci and Saravay, 1993; Fisher *et al*., 1993; Schulz *et al*., 1993; Hata and Futai, 1995; Rodrigues and Petrini, 1997; Brown *et al*., 1998; Okane *et al*., 1998; Taylor *et al*., 1999; Umali *et al*., 1999; Fröhlich *et al*., 2000; Shamoun and Sieber, 2000; Swart *et al*., 2000; Photita *et al*., 2004).

2.8 Endophytic fungi

2.8.1 Definition of endophytes

The term endophyte was introduced by de Barry (1887) and was initially applied to any organism found within a plant (Wilson, 1995). The meaning of the term endophyte has been refined over time with the addition of new information (Carroll, 1986; Petrini, 1986; Siegel *et al*., 1991). Petrini (1991) considered the term endophyte to be “endophytes colonize symptomlessly the living, internal tissues of

their host, even though the endophyte may, after an incubation or latency period, cause disease." This definition is broad to include virtually any microbe that colonizes the internal tissues of plants. This definition also includes virtually the entire spectrum of symbiotic interactions in which fungi and plants participate, parasitism, commensalism, and mutualism (Carroll, 1988; Wilson, 1995; Bills, 1996).

The term endophyte has been used in a variety of ways, and Hawksworth *et al*. (1995) suggested that the term should be clearly defined when used. Since this study focusing on fungi, the definition proposed for the term endophytic fungi is "fungi colonizing living plant tissue without causing any immediate, overt negative effects" (Hirsch and Braun, 1992).

2.8.2 Biological role of endophytes

Fungal endophytes have been isolated from a broad range of dicotyledonous, monocotyledonous and coniferous plants worldwide (Table 2.7). These fungi can live for a certain period as neutral endophytes and produce symptoms only after appropriate ecological and physical conditions occur (Chapela and Boddy, 1989). It is often difficult to differentiate between endophyte and pathogen as many plant pathogens also undergo an extensive phase of asymptomatic latent infection before the appearance of disease symptoms. Additionally, the mutation in a single genetic locus can change a pathogen to non-pathogenic endophyte with no effect on its host specificity (Freeman and Rodriguez, 1993). Fungal endophytes can also have mutualistic relations with their host, e.g. study of grass endophytes by Hammond and Faeth (1992), showed that there is a greater probability of mutualism in the fugal

Table 2.7 Endophytic fungi reported for various hosts worldwide.

Host	Tissue/organs	No. of species	Comments	Location	References
Abies alba	branch bases	44	17 common	Germany, Poland	Kowalski and Kehr, 1992
	needles	4	2 common	Switzerland	Carroll *et al*., 1974
	roots	4	-	Switzerland	Ahlick and Sieber, 1996
	twigs	50	2 endemic	Switzerland	Sieber, 1989
Abies amabilis	needles	4	-	Oregon,Washington	Carroll and Carroll, 1978
Abies balsamea	needles	19	4 common	Canada	Johnson and Whitney, 1989
Abies concolor	needles	5	2 endemic	Oregon, Washington	Carroll and Carroll, 1978
Abies grandis	needles	6	1 endemic	Oregon, Washington	Carroll and Carroll, 1978
Abies lasiocarpa	needles	4	3 endemic	Oregon, Washington	Carroll and Carroll, 1978
Acer macrophyllum	leaves, twigs	9	-	British Columbia	Sieber and Dorworth, 1994
Acer pseudoplatanus	branch bases	28	15 common	Germany, Poland	Kowalski and Kehr, 1992
Alnus rubra	leaves	25	2 endemic	Canada	Sieber *et al*., 1991
	twigs	27	-	Canada	Sieber *et al*., 1991
Amaranthus hybridus	leaves	11	-	South Africa	Blodgett *et al*., 2000
	petioles	13	-	South Africa	Blodgett *et al*., 2000
	roots	17	-	South Africa	Blodgett *et al*., 2000
Araceae	leaves	29	*Anthostomella aracearum* sp. nov., *Chaetosphaeria endophytica* sp. nov.	Guyana	Dreyfuss and Petrini, 1984
Arctostaphylos uvaursi	leaves	13	-	Oregon	Petrini *et al*., 1982
	leaves	176	23 common	Switzerland	Widler and Müller, 1984
	roots	14	8 common	Switzerland	Widler and Müller, 1984
	twigs	35	29 common	Switzerland	Widler and Müller, 1984
Bambusa tuldoides	leaves	37	-	Kong Kong	Umali *et al*., 1999
Betula nana	stems	1	*Myrothecium gronlandicum* sp. nov.	Greenland	Bohn, 1993
Betula papyrifera	roots	5	Aquatic hyphomycetes	Nova Scotia	Sridhar and Barlocher, 1992a, b
Betula pendula	branch bases	23	14 common, 3 endemic	Germany, Poland	Kowalski and Kehr, 1992
Bromiliaceae	foliage	16	*Chaetosphaeria endophytica* sp. nov.	Guyana	Dreyfuss and Petrini, 1984
Bruguiera gymnorrhiza	leaves	14	*Surculiseries rugispora* sp. nov.	Japan	Okane *et al*., 2001
Calocedrus decurrens	foliage	15	-	Oregon	Petrini and Carroll, 1981
Carpinus betulus	branch bases	29	17 common, 3 endemic	Germany, Poland	Kowalski and Kehr, 1992
Carpinus caroliniana	bark	155	11–12 species per tree, 5 basidiomycetes	New Jersey	Bills and Polishook, 1991a
Castanea sativa	stems	14	9 common	Switzerland	Bissegger and Sieber, 1994

Table 2.7 (Continued).

Host	Tissue/organs	No. of species	Comments	Location	References
Chamaecyparis lawsoniana	foliage	18	1 basidiomycete, *Luellia* sp.	Oregon	Petrini and Carroll, 1981
Chamaecyparis thyoides	leaves, twigs	88	8–12 species per tree	New Jersy, West Virginia	Bills and Polishook 1992
Cordemoya integrifolia	leaves	27	15 common	Mauritius	Toofanee and Dulymamode, 2002
Cuscuta reflexa	stems	40	-	India	Suryanarayanan *et al*., 2000
Dendrobium scabrilingue	roots	20	14 common	Thailand	Sangthong, 2002
Dimocarpus longana	shoots, panicles, fruits	18	-	Thailand	Sardsud *et al*., 1998
Dryas octopetala	leaves	23	-	Switzerland	Fisher *et al*., 1995
Ericaceae	leaves, twigs	23	-	UK	Petrini, 1984
Ericaceae	leaves	18	3 common	Japan	Okane *et al*., 1998
Eucalyptus globulus	stems	41	9 basidiomycetes	Uruguay	Bettuci and Saravay, 1993
Eucalyptus nitens	bark, leaves, xylem	61	2 common	Australia	Fisher *et al*., 1993
	bark, leaves	49	2 common	UK	Fisher *et al*., 1993
Eucalyptus viminalis	leaves	23	-	Argentina	Cabral, 1985
Euphoria longana	shoots, fruits	14	-	Thailand	Sardsud *et al*., 1998
Euterpe oleracea	leaves	57	21 common, *Idriella amazonica* sp. nov., *I. asaicola* sp. nov., *I. euterpes* sp. nov.	Brazil	Rodrigues, 1994
Fagus sylvatica	branch bases	37	23 common, 3 endemic	Germany, Poland	Kowalski and Kehr, 1992
	branches	18	-	UK	Chapela and Boddy, 1988
	leaves	64	1 basidiomycete	Switzerland	Sieber and Hugentobler, 1987
	roots	12	*Cryptosporiopsis radicicola*, *Phialocephala fortinii*	Germany, Switzerland	Alick and Sieber, 1996
	stems	18	-	UK	Petrini and Fisher, 1988
	twigs	21	-	Switzerland	Sieber and Hugentobler, 1987
Ficus benghalensis	leaves, petioles, roots	28	-	India	Suryanarayanan and Vijaykrishna, 2001
Fraxinus excelsor	branch bases	36	18 common, 1 endemic	Germany, Poland	Kowalski and Kehr, 1992
Gaultheria shallon	leaves	13	-	Oregon	Petrini *et al*., 1982
Gynoxis oleifolia	leaves, stems, roots	42	-	Ecuador	Fisher *et al*., 1995

Table 2.7 (Continued).

Host	Tissue/organs	No. of species	Comments	Location	References
Herbaceous and shrub plants living on gypsum soil	leaves, stems, twigs	152	-	Spain	Peláez *et al*., 1998
Hordeum vulgare	leaves	14	*Didymella phleina*	New Zealand	Riesen and Close, 1987
Ipomoea pes-caprae	roots	21	5 common	India	Beena *et al*., 2000
Juncus acutus	culms, leaves	8	-	Argentina	Menendez *et al*., 1995
Juncus bufonius	leaves	14	-	Oregon	Cabral *et al*., 1993
	culms, leaves	27	-	Argentina	Menendez *et al*., 1995
Juncus imbricatus	culms, leaves	9	-	Argentina	Menendez *et al*., 1995
Juncus spp.	culms, leaves	6	-	Oregon	Cabral, *et al*., 1993
Juniperus communis	leaves	114	-	Switzerland	Petrini and Müller, 1979
Juniperus occidentalis	leaves	6	-	Oregon	Petrini and Carroll, 1981
Larix decidua	branch bases	27	17 common, 1 endemic	Germany, Poland	Kowalski and Kehr, 1992
	roots	51	-	Germany	Kehr, 1995
Launaea sarmentosa	roots	16	5 common	India	Beena *et al*., 2000
Leucadendron salignum × *Leucadendron laureolum*	leaves, petioles	22	*Botryosphaeria proteae*	South Africa	Swart *et al*., 2000
Leucospermum cordifolium	leaves, petioles	26	*Botryosphaeria proteae*	South Africa	Swart *et al*., 2000
Licuala ramasayi	leaves	11	1 new species, *Idriella licualae* sp. nov.	Australia	Rodrigues and Samuels, 1990
	leaves, petioles	36	16 common	Australia	Fröhlich *et al*., 2000
Licuala sp.	leaves, petioles	54	15 common	Brunei	Fröhlich *et al*., 2000
Livistona chinensis	leaves, petioles	16	4 species identified using rDNA-ITS sequences	Hong Kong	Guo *et al*., 2000
Lolium spp.	leaves	2	2 species identified using *tub2* and rDNA-ITS sequences and microsatellite DNA profiles	New Zealand	Moon *et al*., 2000
Mahonia aquifolia	leaves	9	-	Oregon	Petrini *et al*., 1982
Mahonia nervosa	leaves	6	-	Oregon	Petrini *et al*., 1982
Manikara bidentata	leaves	23	-	Puerto Rico	Lodge *et al*., 1996

Table 2.7 (Continued).

Host	Tissue/organs	No. of species	Comments	Location	References
Musa acuminata	leaves, pseudostems	16	10 common	Australia	Brown *et al*., 1998
	leaves, pseudostems	61	39 common	Thailand	Photita *et al*., 2001b
Musa sp.	leaves, pseudostems	12	8 common	Hong Kong	Brown *et al*., 1998
Orchidaceae	leaves	21	-	Guyana	Dreyfuss and Petrini, 1984
	roots	67	-	Costa Rica	Richardson and Currah, 1995
Oryza sativa	leaves, roots	30	-	Italy	Fisher, 1992
Parthenium hysterophorus	leaves	21	1 endemic	Mexico	Romero *et al*., 2001
Pasania edulis	leaves	21	16 common	Japan	Hata *et al*., 2002
Picea abies	branch bases	30	21 common, 1 endemic, *Phialocephala compacta* sp. nov., *P. scopiformis* sp. nov.	Germany, Poland	Kowalski and Kehr, 1992
	branches	85	*Tryblidiopsis pinasti*	Sweden	Barklund and Kowalski, 1996
	roots	20	*Phialocephala fortinii*	Germany, Switzerland	Ahlick and Sieber, 1996
	roots	120	25 common	Germany	Holdenrieder and Sieber, 1992
	twigs	58	-	Switzerland	Sieber, 1989
Picea breweriana	needles	2	2 endemic	Oregon, Washington	Carroll and Carroll, 1978
Picea engelmannii	needles	6	6 endemic	Oregon, Washington	Carroll and Carroll, 1978
Picea excelsa	needles	29	8 common	Switzerland	Carroll *et al*., 1974
Picea glauca	roots	9	Aquatic hyphomycetes	Nova Scotia	Sridhar and Bärlocher, 1992
Picea mariana	needles	10	*Phaeocoocus catenatus* sp. nov.	New Brunswick	Johnson and Whitney, 1992
	roots	97	-	Ontario	Summerbell, 1989
Picea rubens	needles	19	4 common	Canada	Johnson and Whitney, 1989
Pinus attenuata	needles	3	1 endemic	Oregon, Washington	Carroll and Carroll, 1978
Pinus banksiana	needles	6	-	Quebec	Legault *et al*., 1989
Pinus contorta	needles	4	1 endemic	Oregon, Washington	Carroll and Carroll, 1978
Pinus densiflora	needles	9	-	Japan	Hata and Futai, 1995
Pinus lambertiana	needles	3	1 endemic	Oregon, Washington	Carroll and Carroll, 1978
Pinus monticola	needles	1	-	Oregon, Washington	Carroll and Carroll, 1978

Table 2.7 (Continued).

Host	Tissue/organs	No. of species	Comments	Location	References
Pinus nigra	needles	15	4 common	France	Carroll *et al*., 1974
Pinus ponderosa	needles	7	3 endemic	Oregon, Washington	Carroll and Carroll, 1978
Pinus resinosa	needles	8	-	Quebec	Legault *et al*., 1989
Pinus sitchensis	needles	10	1 endemic	Oregon, Washington	Carroll and Carroll, 1978
Pinus sylvestris	branch bases	28	18 common, 2 endemic	Germany, Poland	Kowalski and Kehr, 1992
	roots	15	*Phialocephala fortinii*	Findland, Germany, Switzerland	Ahlick and Sieber, 1996
	stems	16	*Pezizella pulvinata* var. *lignicola*	UK	Petrini and Fisher, 1988
Plumeria rubra	leaves	46	21 common	India	Suryanarayanan and Thennarasan, 2004
Polycarpaea corymbosa	roots	15	5 common	India	Beena *et al*., 2000
Protea cynaroides	leaves, petioles	30	*Botryosphaeria proteae*	South Africa	Swart *et al*., 2000
Pseudotsuga menziesii	needles	9	2 endemic	Oregon, Washington	Carroll and Carroll, 1978
	needles	20	-	Switzerland	Carroll *et al*., 1974
Pteridium aquilinum	leaf vein, pinnules, rachis, rhizome	61	6 common, *Stagonospora pteridicola* sp. nov.	UK	Petrini *et al*., 1992
Quercus ilex	leaves	50	27 common	UK, Switzerland, Spain	Fisher *et al*., 1994
	twigs	7	-	UK, Switzerland, Spain	Fisher *et al*., 1994
Quercus petraea	leaves	49	13 common	Austria	Halmschlager *et al*., 1993
	twigs	20	8 common	Austria	Halmschlager *et al*., 1993
Quercus robur	branch bases	31	23 common, 2 endemic	Germany, Poland	Kowalski and Kehr, 1992
	twigs	36	12 common	UK	Petrini and Fisher, 1990
Rhizophora apiculata	leaves	25	18 common	India	Kumaresan and Suryanarayanan, 2002
Rubus parviflorus	leaves, twigs	15	-	Canada	Shamoun and Sieber, 2000
Rubus spectabilis	leaves, twigs	13	-	Canada	Shamoun and Sieber, 2000
Salicornia perennis	stems	31	-	UK	Petrini and Fisher, 1986
Salix fagilis	twigs	33	9 common	UK	Petrini and Fisher, 1986

Table 2.7 (Continued).

Host	Tissue/organs	No. of species	Comments	Location	References
Sequoia sempervirens	needles	3	-	California	Carroll and Carroll, 1978
	needles	26	-	California	Espinosa-Garcia and Langenheim, 1990
	needles	12	-	France	Carroll *et al*., 1974
Stylosanthes guianensis	leaves	13	3 common	Brazil	Periera *et al*., 1993
Suaeda fruticosa	leaves	7	-	UK	Fisher and Petrini, 1987
Taxus baccata	stems	9	-	UK	Fisher and Petrini, 1987
Taxus brevifolia	needles	6	1 common	Switzerland	Carroll *et al*., 1974
Thuja brevifolia	foliage	5	4 endemic	Oregon, Washington	Carroll and Carroll, 1978
Tilia cordata	leaves	17	1 basidiomycete, new genus, *Taxomyces andreanae* gen. nov. *et* sp. nov.	Oregon, Montana	Strobel *et al*., 1993
Trachycarpus fortunei	leaves, petioles	29	12 common	Australia	Taylor *et al*., 1999
	leaves, petioles	49	16 common	China	Taylor *et al*., 1999
	leaves, petioles	32	13 common	Switzerland	Taylor *et al*., 1999
Triticum aestivum	culms, leaves, glumes, roots, seeds	163	*Phaeosphaeria nodorum*	Switzerland	Riesen and Sieber, 1985
Triticum spp.	seeds	2	*Aremonium* sp., *Neotyphodium* sp.	Turkey	Marshall *et al*., 1999
Tsuga heterophylla	needles	10	9 endemic	Oregon, Washington	Carroll and Carroll, 1978
Tsuga mertensiana	needles	8	3 endemic	Oregon, Washington	Carroll and Carroll, 1978
Ulex europeaus	stems	22	-	UK	Fisher *et al*., 1986
Ulex galii	stems	21	-	UK	Fisher *et al*., 1986
Umbellularia californica	leaves	5	-	Oregon	Petrini *et al*., 1982
Zea mays	leaves, stems	23	-	UK	Fisher *et al*., 1992
Zea mays	leaves, stems, roots	1	*Fusarium moniliforme*	USA	Bacon and Hinton, 1996

species that are transmitted through seeds, as transmission will increase directly as a result of host survival. Arnold *et al*. (2003) showed that inoculation of endophyte-free leaves of *Theobroma cacao* with endophytes isolated frequently from the naturally infected, asymptomatic host significantly decreased both leaf necrosis and leaf mortality when *T. cacao* seedlings were challenged with the pathogenic, *Phytophthora* sp. These results suggested that the associations between *T. cacao* and diverse, horizontally transmitted foliar endophytes could enhance or supplement host defense.

The endophytes associated with grasses have received much attention, and many of these have been found to produce alkaloids and other mycotoxins that appear to be toxic to mammals and/or play a role in host plant defence, often increasing resistance to mammalian and insect herbivores (Cheplick and Clay, 1988; Siegel and Schardl, 1991; Clay, 1992; Breen, 1994; Bultman *et al*., 1997). Endophytes also increased growth and drought tolerance in infected plants (Bacon, 1993; Latch, 1993). Endophytes have been also reported to promote the onset of senescence (Petrini, 1991; Petrini *et al.*, 1992; Wilson, 1993).

Several reviews discuss secondary metabolite production by endophytic fungi in graminicolous and non-graminicolous hosts (Petrini *et al*., 1992; Bultman *et al*., 1997; Bush *et al*., 1997; Strobel and Daisy, 2003; Strobel *et al*., 2004). Endophytes have been found to play a crucial role in the production of extremely beneficial chemical compounds. Endophytes, *in vitro*, can produce biologically active compounds including several alkaloids, paxillines, lolitrems, tetraenone steroids (Dahlman *et al*., 1991; Brunner and Petrini, 1992) and antibiotics. For example, *Acremonium* sp. from European yew produced leucinnostatin (Strobel *et al*., 1997),

Monochaetia sp. and *Pestalotiopsis* sp. isolated from rain forest plants produced ambuic acid (Li *et al*., 2001), *Cryptosporiopsis* sp. isolated from *Vaccinium myrtillus* produced a compound with fungicidal activity against *Candida albicans* and *Trichophyton mentagrophytes* (Fisher *et al*., 1984b), *Hormonema dematiooides* isolated frequently from *Chamaecyparis thyoides* produced Preussomerin D (Polishook *et al*., 1993). Additionally, *Taxomyces andreanae*, *Pestalotiopsis* sp., *Pestalotiopsis microspora*, and *Periconia* sp. isolated from *Taxus brevifolia*, *T. wallachiama* and *Torreya grandifolia* are taxol producers (Stierle *et al*., 1993; Strobel *et al*., 1996; Pulici *et al*., 1997; Li *et al*., 1998).

2.8.3 Ecology of endophytes

The colonization of plant tissues by endophytes, plant pathogens and mycorrhizae comprise a sequence of steps involving host recognition by the fungi, spore germination, penetration of the epidermis, and tissue colonization (Petrini, 1991, 1996). The source of fungal inoculum involved in infection and colonization is widely considered to be mainly in the form of air borne spores, seed transmission, or by the transmission of propagules by insect vectors (Petrini, 1991). Evidence for air borne inoculation has been shown using fine-scale sampling techniques (Bertoni and Cabral, 1988) which revealed discontinuous patterns of endophyte infection within a leaf. Umali *et al*. (1999) found less endophyte from the tissue of bamboo leaves including the primary veins than those with secondary veins. The occurrence of more endophytes in tissue with secondary veins may indicate that the source of inoculum is from the air rather than being systemically distributed though the veins. A high degree of genetic diversity of endophyte isolates suggested that infection loci arise from

different strains of fungi derived from constant new inoculum (Hammerli *et al*., 1992; Rodrigues *et al*., 1993). Endophytes have been found to be virtually absent from rolled up leaves enclosed in bud scales suggesting that in non-graminicolous endophytes, systemic infection does not occur and air borne inoculum is the method of infection (Johnson and Whitney, 1992; Toti *et al*., 1993).

In terms of the mechanical and enzymatic elements of penetration by endophytic fungi, it can be assumed that endophytes adopt the same strategy for penetration of host tissues as pathogens (Petrini *et al*., 1992). Infection studies have shown that fungi can invade plant tissues by direct cuticular penetration (Carroll, 1988; Stone, 1988; Cabral *et al*., 1993; Viret *et al.*, 1993; Viret and Petrini, 1994) and via appressoria formed on the cuticle after which penetration occurs through the cuticle and epidermal cell walls (Carroll, 1988; Stone, 1988) or via stomata (O'Donnell and Dickinson, 1980; Kulik, 1988; Cabral *et al.*, 1993). Infection can occur intra- or intercellularly (Suske and Acker, 1987; Bacon and Battista, 1991; Cabral, *et al*., 1993; Youssef and Dugan, 2000) and may be limited to one cell (Stone, 1987), or to a limited area around the point of penetration (Verhoeff, 1974; O'Donnell and Dickinson, 1980; Suske and Acker, 1987). Endophytes have been also reported producing intercellular networks of hyphae (Johnson and Whitney, 1989).

several studies have indicated that endophytes may exhibit tissue specificity (Bills and Polishook, 1992; Clay, 1992; Rodrigues, 1994; Fisher *et al*., 1994, 1995; Taylor *et al*., 1999; Fröhlich *et al*., 2000; Photita *et al*, 2001b). Differences in endophyte assemblages in different tissue types might be a reflection of tissue preferences of individual dominating taxa (Taylor *et al*., 1999) and might reflect their capacity for utilizing or surviving within a specific substrate (Rodrigues, 1994). The

factors that may be important in this respect include the weathering of leaf cuticle, tissue texture and changes in the tissue physiology and chemistry (Petrini and Carroll, 1981; Stone, 1987; Arnold and Herre, 2003).

Several studies showing an increase in the number of endophytes recovered with increasing age of tissue have been reported (Bertoni and Cabral, 1988; Hata and Futai, 1993; Rodrigues, 1994; Brown *et al*., 1998; Taylor *et al*., 1999; Umali *et al*., 1999; Photita *et al.*, 2001b; Kumaresan and Suryanarayanan, 2002; Toofanee and Dulymamode, 2002). Factors that may contribute to a change in the endophyte community with tissue age are weathering of tissue texture, increased exposure to propagules with time, and chemical and physical changes of the plant tissue or degradation of the leaf cuticle (Petrini and Carroll, 1981; Stone, 1987; Hata and Futai, 1993, Arnold and Herre, 2003).

2.9 Relationships among endophytic, saprobic and pathogenic fungi

The fungal endophytes isolated from several plants are primarily common genera found to be either necrotrophic or saprotrophic (Kulik, 1984; Petrini and Fisher, 1988; Rodrigues and Samuels, 1990; Sieber *et al*., 1991; Fisher and Petrini, 1992; Fisher *et al*., 1994; Whalley, 1996; Brown *et al*., 1998; Okane *et al*., 1998; Sahashi *et al*., 1999; Photita *et al*., 2001b; Hata *et al*., 2002; Kumaresan and Suryanarayanan, 2002; Bussaban *et al*., 2003b; Suryanarayanan and Thennarasan, 2004). Xylariaceae are known to biodegrade cellulose and lignin and their ecological role is primarily in decomposing senescing plants material (Petrini and Petrini, 1985; Whalley, 1993, 1996). *Xylaria* species have been found to be common in all tropical hosts surveyed (Rodrigues and Samuels, 1990; Rodrigues, 1994; Perreira *et al*., 1993,

Photita *et al*., 2001b). This fungal family is particularly well adapted to an endophytic existence (Whalley, 1997). *Colletotrichum*, *Fusarium*, *Phomopsis*, *Phyllosticta*, and *Pyricularia* species have been often isolated as endophytes (Kulik, 1984; Petrini and Fisher, 1988; Rodrigues and Samuels, 1990; Sieber *et al*., 1991; Fisher and Petrini, 1992; Fisher *et al*., 1994; Brown *et al*., 1998; Okane *et al*., 1998, 2003; Sahashi *et al*., 1999; Photita *et al*., 2001b; Bussaban *et al*., 2003b; Rodgrigues *et al*., 2004). Finding such a large number of potential plant pathogenic genera as endophytes supports the theory that endophytes can act as latent pathogens (Carroll, 1988; Stone, 1990; Petrini, 1991). Previous studies (Bernstein and Carroll, 1977; Sieber *et al*., 1988, 1989, Photita *et al*., 2004) indicated that some pathogenic fungi have a latency period during which they live endophytically in apparently healthy plant tissues. Latency can last several days to many years depending on fungus virulence, host, climate and ecological condition. Thus, these pathogenic fungi could potentially be detected as endophytes in healthy tissues long before symptoms develop (Latch, 1993; Photita *et al*., 2004).

2.10 Importance and biotechnological potential of fungi

Fungi are important in an enormous variety of ways. As decomposers they break down organic matter and release nutrients back to the environment in a form in which it can be reused (Lynch and Poole, 1979; Killham, 1994; Boddy and Watkinson, 1995). Many fungi form mutualistic relationships with other organisms. For example, mycorrhizae, the fungi associated with roots, are essential for the growth of over 90% of all vascular plants as both endomycorrhizae in crops and ectomycorrhizae developing in many woody plants (Allen, 1993). Marx *et al*. (1993)

described the remarkable beneficial effect of inoculating tree seedlings with selected ectomycorrhizal fungi. Mycorrhizae can reduce the use of chemical fertilizers a step towards sustainable agricultural and forest systems (Wood, 1992; Jong and Birmingham, 1993).

Fungi are also important directly as food for humans. Many mushrooms are edible and different species are cultivated for sale worldwide. Global production of top-ten edible cultivated mushrooms is in the region of eight million tonnes with about 60% grown in China (Moore and Chiu, 2001). In Thailand mushroom production in 1998 was 141,700 tons with a value of 4,615 million Thai bath (Thaithatgoon *et al*., 2004). In addition to a gourmet food item, *Fusarium graminearum* serves as a low-cost protein source sold as Quorn, with a market value of 25 million British pounds (Trinci, 1992; Moore and Chiu, 2001). While this is a small proportion of the food that we eat, fungi are also widely used in the production of many foods and drinks including cheeses, beer, wine, bread, cakes and some soya bean products (Moore and Chiu, 2001).

Higher fungi have been used as a source of medicinal products, especially in China. Ying *et al*. (1987) reported more than 270 species known to have medicinal value while Ooi (2001) reported about 100 fungi newly discovered with medicinal properties. The most important and widespread species (e.g., *Ganoderma lucidum*, *Lentinula edodes*, *Poria cocos*, *Polyporus umbellatus*) are used for a variety of medicinal conditions including chronic hepatitis, kidney disease, and coronary heart disease (Ooi, 2001), with an effect in enhancing the immune system (Halpern and Miller, 2002). Penicillin, perhaps the most famous of all antibiotic drugs, is derived from the common fungus, *Penicillium chysogenum.* Many other fungi also produce

antibiotic substances, which are now widely used to control diseases in human and animal populations. The discovery of antibiotics revolutionized health care worldwide (Wildman, 1997).

Many fungi can be used as biocontrol agents for insects, pathogenic fungi and weed management. Such biocontrol is generally cheaper and less damaging to the environment than using chemical pesticides (Templeton, 1992; Hajek *et al*., 2001; Tang *et al*., 2001). A successful biological control programme against mist flower (*Ageratina riparia*) in Hawaii was established for mist flower in New Zealand by introducing the fungus, *Entyloma ageratinae* followed by the gall fly, *Procecidochares alani* (Morin *et al*., 1997). *Phoma clematidina* and *Phragmidium violaceum* have been used to control exotic weeds, *Clematis vitalba* and *Rubus fruticosus* in New Zealand and Australia, respectively (Evans *et al*., 2001). Commercial *Colletotrichum* strains used as mycoherbicides have been listed e.g., Collego, BioMal, Lubao (Templeton, 1992). Many species of *Trichoderma* are targeted against soil-borne plant pathogens, such as *Botrytis*, *Fusarium*, *Rhizoctonia* (Elad and Kapat, 1999; Tang *et al*., 2001). Greatest commercial success has been achieved with well-known species of entomopathogenic *Beauveria*, *Metarhizium*, *Paecilomyces* and *Verticillium*. Worldwide, more than 30 products based on those pathogens have been registered or are being developed, primarily for control of a broad range of stem-boring and foliage- and root-feeding pests of agriculture and forestry (Bateman *et al*., 1993; Higuchi *et al*., 1997; Wraight *et al*., 2000; Hajek *et al*., 2001).

Fungal parasites may be useful in biocontrol, but they can also have enormous negative consequences for crop production (Farr *et al*., 1989; Bailey and Jeger, 1992)

e.g., *Magnapothe grisea* caused rice blast disease, *Colletotrichum* species, cause anthracnose, leaf spot, leaf blight, or rot of various legumes, perennial and other cash crops (Dillard, 1992; Lenné, 1992; Nicholson, 1992; Waller, 1992). Fungi can also cause animal diseases and food spoilage (Chaiprasert, 2004). Many mushrooms contain toxins that are extremely poisonous to animals that ingest them, resulting in severe disease symptoms or death. Aflatoxins, produced by some *Aspergillus* species growing on grain products, are very toxic carcinogens and cause disease in poultry and other populations that consume the toxin-containing food products (Edds, 1979; Atlas and Bartha, 1998).

Fungi are a source of commercially important enzymes and natural products ranging from abscisic acid to zymosterol that results in a billion dollar industry (Edwards *et al*., 1988; Lambert, 1983). They are increasingly used to ferment solid organic waste substrates into useable products such a methane and fertilizers (Fox, 1993), and are invaluable as substitutes for chemicals in the pulp and paper industry (Kirk *et al*., 1993, 2002). Fungal species screened for secondary metabolites using modern techniques are less than 1% of those that may exist (Nisbet and Fox, 1991). Thus, the potential is enormous for the discovery of valuable natural products resulting from a directed search and screening of fungi from unexplored habitats (Concepcion *et al*., 2001; Strobel *et al*., 2004).

There are few fungal species utilized in biotechnological processes or in the production of novel compounds, and a huge potential is seen in the pharmaceutical and health-care industries (Nisbett and Fox, 1991; Fox, 1993; Rossman, 1994; Wildman, 1997). Potentially, fungi have great biotechnological importance as a source of new pharmaceutical compounds, secondary metabolites, and other useful

compounds and as agents of biological control especially by further exploration of tropical fungi (Wildman, 1997; Azevedo *et al*., 2000). It is expected that new drugs of biotechnological importance will be discovered with increased focus on tropical endophytic fungi. Such bioactive chemicals may be useful to the agricultural, biotechnological, forestry, pharmaceutical or food industries (Hyde, 2001; Strobel and Daisy, 2003; Strobel *et al*., 2004).

2.11 Measurement of fungal biodiversity

Biodiversity is the range of organisms present in a given ecological community or system. Biodiversity is the result of evolutionary process (Figure 2.5) and can be divided into three hierarchical categories, i) genetic diversity refers to the variation of genes within species covering distinct populations of the same species or genetic variation within a population, ii) species diversity refers to the variety of living organisms, iii) ecological diversity refers to the variability of habitat and biotic communities including the variety of ecological processes within ecosystems (Smitinand, 1995; Templeton, 1995; Baimai, 2002).

Although current measures select different levels of the biosystem for emphasis, the species, population, ecosystem or landscape levels, the species are the primary unit of measure in discussing fungal biodiversity (Gajaseni and Boonpragob, 1995; Rossman, 1997). Among the fungi, microfungi present the biggest challenge as they are the most abundant mycota and their inventory would be unmanageable in large plots. For these fungi it may be necessary to select smaller plots or individual host plants and sample within these elements (Hyde, 1995).

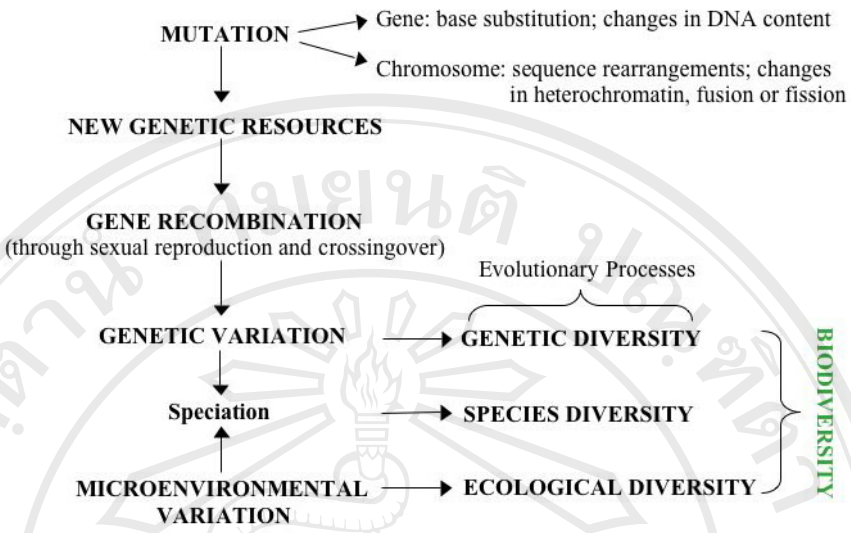

Figure 2.5 Major factors involved in biodiversity.

In investigating fungi on specific substrates such as soil, leaf litter, air or the interior of living plants, one must isolate from the substrate in order to determine species presence. Techniques for the isolation of particular groups of microfungi or from specific substrates vary considerably. The handling of the substrates and the medium on which the fungi are isolated is important. Additional factors may influence which fungal species grow out from the substrate including incubation temperatures, light and humidity regimes, as well as colony density (Bills and Polishook, 1994a, b; Hyde, 1995). With judicious selection of techniques, the result may be a realistic account of the fungal species diversity in that substrate at one point in time (Hyde, 1995).

Standard procedures of fungal endophyte isolation from plant tissue have been integrated from microbiology, plant pathology, and plant tissue culture. A typical

isolation procedure would involve some or all of the following steps: plant collection, transport of collected material to the laboratory, water wash of large segments of tissue, surface sterilization, dissection of tissue into 1–2 cm segments using aseptic techniques, a second surface sterilization, water rinse, and plating the plant tissue on a fungal growth medium. Any hyphae growing from the tissue segments are presumed to have originated from within the plant segment and the fungus designated as endophytic (Rodrigues and Samuels 1990; Fisher *et al*., 1993, 1994, 1995; Bills, 1996; Taylor *et al*., 1999; Fröhlich *et al*., 2000; Guo *et al*., 2000; Photita *et al*., 2001b; Hata *et al*., 2002; Suryanarayanan and Thennarasan, 2004). This type of data is a necessary first step for identifying an endophytic fungal colonization and permits taxonomic classification of the fungus involved.

Microscopic methods, the use of light and electron microscopy reveals the precise internal location of endophytic fungi and can shed some light on the plant's response to the infection. Generally, assessments are made of whether the fungal infection is intercellular or intracellular, and of the plant cellular anatomy. Cabral *et al.* (1993) examined the patterns of fungal endophytes infection and colonization of various *Juncus* species. Two infection types were described, intracellular (colonization limited to a single host epidermal cell, colonization beyond one cell) and intercellular (with in substomatal chambers, within air chamber). The infections of larch and barley roots by either endophytes *Cryptosporiopsis* sp., *Fusarium* sp. or pathogen *Heterobasidium annosum*, *Drechslera* sp. showed that both endophytes and pathogens extensively colonized roots of hosts, both inter- and intracellularly. Infections by the two endophytes, however, resulted in neither growth inhibition nor disease symptoms, whereas infections with the pathogens led to both disease

symptoms and diminished growth of seedling (Schulz *et al*., 1999). Ultrastructural characteristics of endophytic colonization of kernels and vegetative tissues were examined by scanning and transmission electron microscopy. The studies confirmed that endophytic colonization of maize is present in both reproductive and vegetative tissues of the plant (Bacon *et al*., 1992; Bacon and Hinton, 1996). Scanning electron microscopy also demonstrated fungal hyphae located within the pedicel or tip cap of asymptomatic maize (Bacon *et al*., 1992).

In endophytic study, isolation techniques typically involve plating healthy, surface-sterilized plant tissues on agar media and observing the outgrowth of fungi (Rodrigues and Samuels 1990; Bills, 1996; Taylor *et al.*, 1999). However, the identification of endophytic fungi has proved to be extremely difficult, because of the lack of information on the cultural characters of species already described. The task of identification of some endophytic fungi to species level from their culture characteristics is very difficult as they rarely produce morphologically diagnostic structures and teleomorphs are seldom formed (Brunner and Petrini, 1992; Rodrigues *et al*., 1993; Guo *et al*., 2000). Variable proportions of sterile mycelia have been reported in endophyte communities, e.g., 10% of isolates from wild banana, *Musa acuminata* in Thailand (Photita *et al*., 2001b), 11% of isolates from the palm, *Trachycarpus fortunei* in China (Taylor *et al*., 1999), 13% of endophytes from *Licuala* spp. in Brunei and Australia (Fröhlich *et al*., 2000), 16.5% of endophytes from *Livistona chinensis* in Hong Kong (Guo *et al*., 2000), and 54% of isolates from *Quercus ilex* in Switzerland (Fisher *et al*., 1994). Cultural, biochemical and numerical techniques have been applied to solve taxonomic problems of endophytic fungi (Suske and Acker, 1987, 1989; Sieber-Canavesi *et al*., 1991; Guo *et al.*, 1998). Guo *et*

al. (1998) inoculated the mycelia that they obtained from natural palm petioles onto a palm petiole in a flask and obtained better sporulation. They were able to identify two species that were saprobes of *Livistona chinensis* in this way. The evidence of Guo *et al*. (1998) suggests that some of the mycelia sterilia isolated in endophyte studies may in fact be specific to that host or host family. Molecular biology techniques have been widely used to solve taxonomic problems within fungal taxa (Leuchtmann and Clay, 1990; Bonde *et al*., 1991; Oudemans and Coffey, 1991, Leuchtmann *et al*., 1992; Leuchtmann, 1994; Kato *et al*., 2000; Couch and Kohn, 2002; Okane *et al*., 2003; Pandey *et al*., 2003; Tomita, 2003; Menkis *et al*., 2004; Rodrigues *et al*., 2004) and to determine taxonomic relationships of endophytic, saprobic or pathogenic fungi (Brunner and Petrini, 1992; Rodrigues *et al*., 1993).

2.12 Molecular characterization of fungi

Polymerase chain reaction (PCR) has become the standard tool in molecular biology (White, 1996; Piercey-Normore and Egger, 2001). It is an *in vitro* method for amplifying specific DNA sequences that may be present in only trace amounts in a DNA sample from any source. The typical components of a PCR reaction are deoxynucleotide triphosphates (dNTP), oligonucleotide primers, magnesium salts and buffers, *Taq* DNA polymerase, and the template DNA. All PCR reaction components are mixed and the procedure consists of a succession of three steps, which are determined by temperature conditions: template denaturation, primer annealing and extension (Figure 2.6) (Mullis and Faloona, 1987).

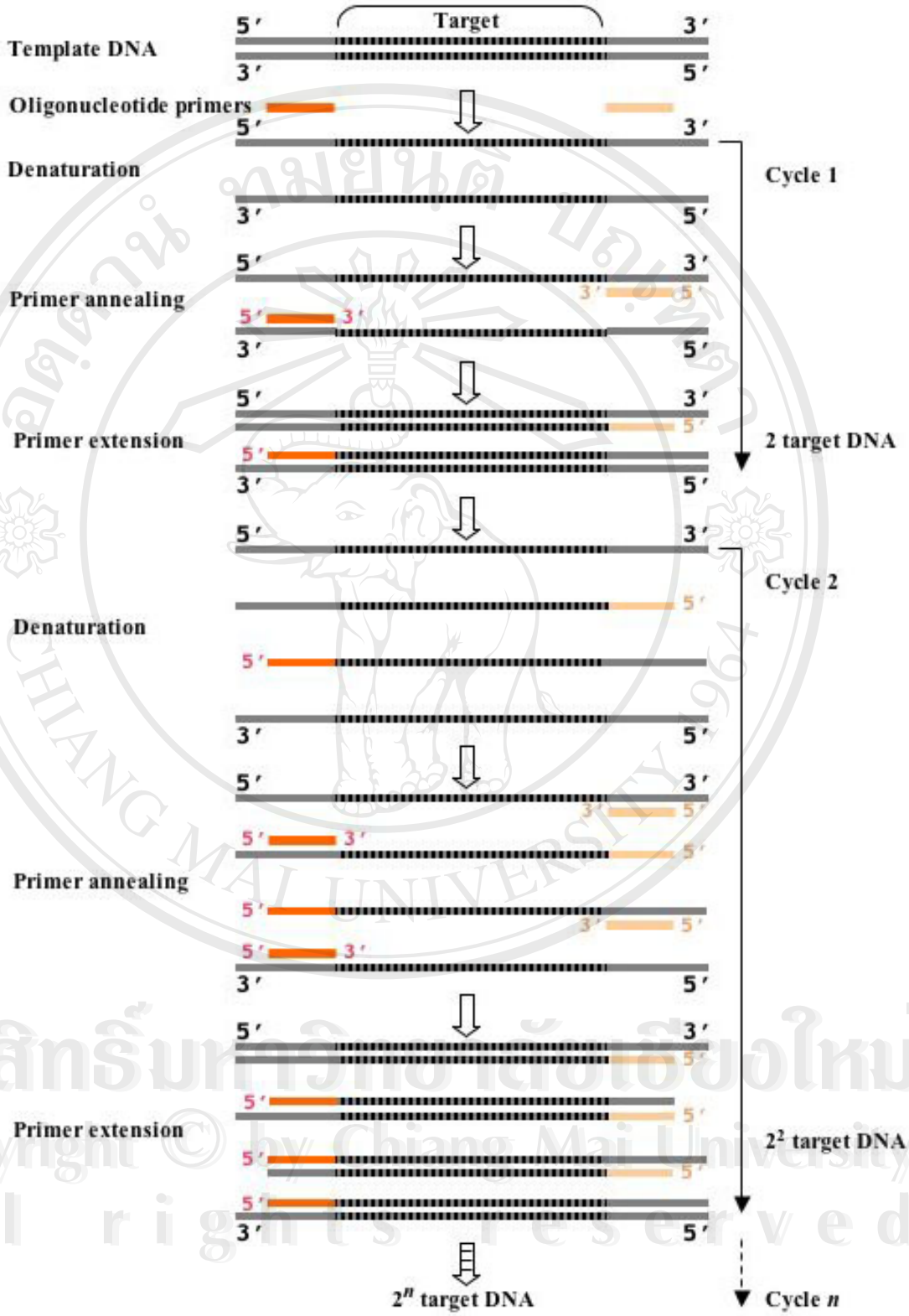

Figure 2.6 Principle of PCR amplification.

The ribosomal RNA genes (rDNA) of fungi exist, as a multiple-copy gene family comprised of highly similar DNA sequences that provide a large number of characters, typically from 8–12 kb. The mode of sequence variation among fungal species at different taxonomic level can be further investigated since the ribosomal DNA consists of regions of highly conserved sequences and regions which are rapidly evolving, which contain highly variable sequences. The more conserved regions, namely the small subunit (SSU) and large subunit (LSU) rDNA, have been useful in finding relationships between distantly related taxa or family. The internal transcribed spacers (ITSs) have been used to examine relationships between closely related taxa (Gardes and Bruns, 1991; Baura *et al*., 1992; Lee and Taylor, 1992; McCullough *et al*., 1998; Callac and Guinberteau, 2005; Didukh *et al*., 2005). Many sequences from fungal taxa are already in the molecular genetic databases (GenBank, EMBL and DDBJ), and can be easily downloaded for data comparison. Sequences of the universal primers for these genes are also available (White *et al*. 1990), and DNA can be easily sequenced either directly from PCR product or from cloned fragments. Thus DNA sequence analysis has been widely used in fungal phylogeny and has been used to infer phylogenetic relationships between fungi at all taxonomic levels (Bruns *et al*., 1991; Hibbett, 1992; Lee and Taylor, 1992; Li, 1997; Okane *et al*., 2003; Pandey *et al*., 2003; Tomita, 2003; Menkis *et al*., 2004; Rodrigues *et al*., 2004).

By the advances in automation of DNA sequencing and computerization of analytic methods, sequenced data are usually used to infer phylogenies in fungi (Sreenivasaprasad and Mills, 1998; Takamatsu, 1998). Molecular characters have been essential for phylogenetic analysis in cases when morphological characters are convergent, reduced, or missing among the taxa considered. This is especially true of

species that never reproduce sexually, because characters of sexual reproduction traditionally have been the basis for classification of fungi. Guo *et al.* (2000) identified 19 morphospecies of endophytes from *Livistona chinensis* by using 5.8S rDNA sequencing. Use of molecular characters allows asexual fungi to be placed among their closest relatives. Carbone and Kohn (1993) demonstrated the confirmations of anamorph-teleomorph connection by comparative sequence analysis of amplified products of *Sclerotinia* and *Sclerotium*, which showed 98% sequence homology in the ITS region of rDNA. Kuhls *et al.* (1997) established the connection between *Trichoderma* anamorphs and *Hypocrea* teleomorphs where five *Trichoderma-Hypocrea* connections were supported by 100% identity in ITS1 and ITS2 sequences. Egger and Sigler (1993) investigated the extype strains of the anamorph *Scytalidium vaccinii* and the ascomycete *Hymenoscyphus ericae*. They found 1.2–3.5% divergence in the ITS1 and ITS2 regions, and concluded from these data, and morphological observations, that *S. vaccinii* and *H. ericae* are anamorph and teleomorph of a single taxon. Likewise, Couch and Kohn (2002) extracted DNA directly from freeze-dried perithecia of *Magnaporthe* and from mycelia of anamorphic *Pyricularia* isolates and the result, based on three genes (actin, beta-tubulin and calmodulin), supported the anamorph-teleomorph connection demonstrated by Hebert (1971) and Yaegashi (1977).

2.13 Antimicrobial agents

Fungal secondary metabolites, which are usually produced during the stationary phase of growth or the idiophase, have a great diversity of molecular structure and frequently show taxonomic specificity in their production (Bu'lock, 1980). Secondary metabolites are known to have medicinal, industrial, or agricultural impact such as antibiotics, antimycotics, anticancer drugs, dyes, growth promoters, hallucinogens, immunosuppressants (Stierle *et al*., 1993; Strobel *et al*., 1996a, b, 2004; Tyler, 1996; Pulici *et al*., 1997; Wildman, 1997; Li *et al*., 1998; Nicklin *et al*., 1999). The discovery of penicillin, the first effective antimicrobial drug, from *Penicillium chysogenum* led to the search for other fungi, Actinomycetes and bacteria that produced antibiotics and other medicinal and bioactive compounds. Most screening programs have concentrated on fungi isolated from soil and it is only recently that mycologists and pharmaceutical industries have begun to consider the vast array of fungi or Actinomycetes from other habitats (Stierle *et al*., 1993; Strobel *et al*., 1996a, b, 2004; Wildman, 1997).

There are many means of classifying antimicrobials based upon their effects on target cells, range of activity, chemical structure, or mechanisms of action. In the early days, the mechanism of action of many antimicrobials was not fully understood. Today, however, the modes of action of many newly described antimicrobials are studied soon after discovery, and there is a large pool of knowledge available for most of the antimicrobials of commercial importance. Antimicrobial drugs work in a variety of ways: i) inhibition of cell wall synthesis, ii) disruption of cell membrane permeability, iii) inhibition of protein synthesis, iv) prevention of the formation of DNA or RNA, or v) inhibition of metabolic pathways (antimetabolites) (Gale *et al*.,

1981; Murray *et al*., 1999; Madigan *et al*., 2003; Franklin and Snow, 2005). Classification by mechanisms of action, with examples of antimicrobials for each group, are outlined in Table 2.8. In addition to being classified by their mechanisms of action, antimicrobial drugs are further classified as bacteriocidal or bacteriostatic.

Table 2.8 Classification of antibiotics by mechanisms of action.

Mechanism of action	Drug	Type of activity[a]
Weaken bacterial cell wall and cause cell death		
♦ Inhibit cross-linking of peptidoglycan	Penicillins	Bactericidal
	Cephalosporins	Bactericidal
♦ Inhibit forming of linear polysaccharide polymer	Vancomycin	Bactericidal
	Bacitracin	Bactericidal
Increase cell membrane permeability		
♦ Cause leakage of intracellular constituents	Polymyxin	Bactericidal
Inhibit protein synthesis		
♦ Bind to 50S ribisome subunit	Chloramphenicol	Bacteriostatic
	Lincosamides	Bacteriostatic
	Macrolines	Bacteriostatic
	Streptogramins	Bactericidal
♦ Bind to 30S ribosome subunit	Aminoglycosides	Bactericidal
	Spectinomycin	Bacteriostatic
	Tetracyclines	Bacteriostatic
♦ Interfere with elongation factors	Fusidic acid	Bacteriostatic
Inhibit nucleic acid synthesis		
♦ Inhibit nucleotide synthesis[b]	Sulfonamides	Bacteriostatic
	Trimethoprim	Bacteriostatic
♦ Inhibit DNA-dependent RNA polymerase	Rifampin	Bactericidal
♦ Inhibit DNA supercoiling and DNA gyrase	Novobiocin	Bactericidal
	Quinolones	Bactericidal

[a]Bacteriostatic drugs inhibit microorganisms, but their effect is reversible if the drug is removed, unless the host defense mechanisms have eradicated the organism. Bactericidal drugs actually kill microorganisms**.**

[b]Antimetabolites (inhibit folic acid synthesis)

Clinical resistance to an antimicrobial agent occurs when the minimum inhibitory concentration (MIC) of the drug for a particular strain of bacteria exceeds, that capable of being achieved with safety *in vivo*. Antimicrobial resistance is the result of complex interactions among antimicrobial agents, microorganisms, and the environment in which they are brought together (Quintiliani *et al*., 1999). The

problem of antibiotic resistance is increasing as more and more strains of pathogenic microorganisms become untreatable with commonly used antimicrobials. This problem can be attributed to a variety of factors, including overuse of antibiotics in agriculture and medicine and misuse of antibiotics by consumers. In addition, antibiotic resistance is often plasmid-borne, the resistance can be readily transferred from one organism to another. The mechanisms for antibiotic resistance include altered receptors for the drug, decreased entry into the cell, and destruction or inactivation of the drug (Quintiliani *et al*., 1999; Tenover and Rasheed, 1999).

Although a variety of antimicrobial agents are available for the treatment of mycobacterial disease, not all agents are suitable for treating all types of infections. Several factors (identification of the pathogen, drug susceptibility, drug spectrum, drug dose, time to affect the pathogen, site of infection and patient assessment) must be considered when choosing the drug of choice or an alternative (Murray *et al*., 1999). To select the right drugs for the infection, an antimicrobial susceptibility test is optimal (Turnidge and Jorgensen, 1999). Sensitivity testing can be done by a disk diffusion test (Figure 2.7) or broth dilution procedure (Figure 2.8).

Disk diffusion test is the most commonly performed test to determine drug susceptibility. In this method, dried paper disks containing a standardized amount of an antimicrobial agent are placed on an agar plate inoculated with the infecting organism. After incubation, a growth inhibition zone will appear around each antibiotic that affects the microbe. The diameter of the visible growth inhibition zone correlates with the MIC, which is the lowest concentration of an antibiotic that prevents visible growth of a microbe. Organisms are rated as sensitive (S) to a

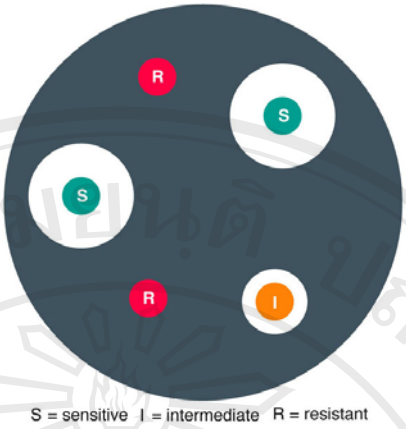

Figure 2.7 Disk diffusion test. In this test, disks impregnated with antibiotics are placed on a plate of the infecting organism. The organism is 'sensitive' to the antibiotic if there is a bacterial free zone around the antibiotic disk and 'resistant' if the bacteria remain around the antibiotic disk.

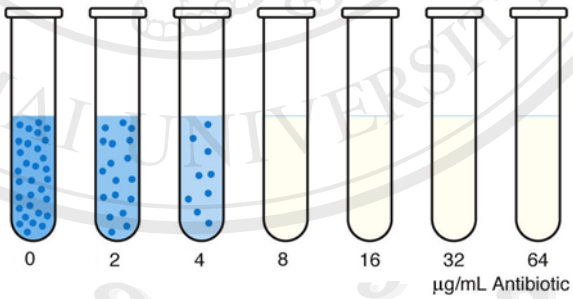

Figure 2.8 Broth dilution procedure. Bacteria are inoculated into a liquid medium containing graduated concentrations of the test antimicrobial. A clear test tube indicates that the concentration of the antimicrobial is sufficient to eradicate the microbe.

particular antibiotic if they are affected by it and resistant (R) if they are not (Jorgensen *et al*., 1999). With the broth dilution procedure, bacteria are inoculated into a liquid medium containing graduated concentrations of the test antimicrobial. This method directly determines the MIC and determines the minimum bactericidal concentration (MBC), the lowest concentration that will kill more than 99.9% of the original inoculum of the microbe. The broth dilution procedure is particularly helpful in managing difficult infections because it demonstrates both MIC and MBC (Jorgensen *et al*., 1999).

Antimicrobial resistance is an important driving force for the continued search for new antimicrobial agents. Many intelligent screening methods have been applied in the search for novel molecules. One approach has involved the investigation of the microbes associated with medicinal plants. From such an approach the antitumor drug taxol was discovered, produced by *Taxomyces andreana*, an endophytic fungus isolated from Pacific yew, *Taxus brevifolia* (Stierle *et al*., 1993). *Cryptosporiopsis quercina* (anamorph of *Pezicula cinnamomea*) isolated as an endophyte from *Tripterigenum wilfordii* (a native medicinal plant of Eurasia) produced cryptocandin, which has antifungal activity against some important human fungal pathogens including *Candida albicans* and *Trichophyton* spp. Cryptocandin is also active against a number of plant pathogenic fungi including *Sclerotinia sclerotiorum* and *Botrytis cinerea*. *Cryptosporiopsis quercina* also produce cryptocin, an unusual tetramic acid possessing potent activity against *Pyricularia oryzae* and a number of other plant pathogenic fungi (Strobel *et al*., 1999; Li *et al*., 2000). The compound was generally ineffective against an array of human pathogenic fungi. With a minimum inhibitory

concentration agaist *P. oryzae* of 0.39 µg/ml, this compound is being examined as a natural chemical control agent for rice blast and is being used as a model to synthesize other antifungal compounds (Strobel *et al*., 2004). Over the last 20 years, filamentous fungi have been a consistent source of biochemical innovations in natural product screening programs (Monagham and Tkacz, 1990). Endophytic fungi have been shown to be a significant source of novel compounds, which have proved effective against cancers and infectious diseases including malaria and viruses (e.g., herpes simplex virus type 1) (Wiyakrutta *et al*., 2003; Strobel and Daisy, 2003; Strobel *et al*., 2004).