CHAPTER 2

LITERATURE REVIEW

2.1 Definition, distribution and habitats of *Dracaena lourieri* and *Pandanus* spp.

In this study fungal saprobes and pathogens of *Dracaena lourieri* and three different species of *Pandanus* were investigated. Descriptions of these monocotyledons are given below.

2.1.1 *Dracaena*

Dracaena lourieri is one of 210 species in family Dracaenaceae, concentrated in tropical Africa and Asia. There are 2 *Dracaena* species in northern Thailand forests. *Dracaena lourieri* is a shrub or slender and much-branched tree, rarely more than 5m high. The bark is pale cream, thin, smooth with close ring-like leaf scars. Leaves are (22)50-80 cm long, 3-7 cm wide, simple, alternate, crowded near the tops of stems, linear with long sharp tips, grasping the stem at the base and lack stalks. Mature leaves are thick and rigid, and completely smooth. Flowers are 0.7-1.2cm, cream or greenish-yellow, in large branched clusters at the top of stems. This species is locally common in the high mountains of northern Thailand, especially on exposed limestone outcrops because it is a xerophyte and halophyte (Gardner *et al.*, 2000). The other rare species *D. angustifolia* was not studied.

2.1.2 *Pandanus*

Pandanus (Pandanaceae) comprise 600-875 species worldwide, distributed from Australia throughout the Borneo, Burma, Pacific, Philippines, India, Indonesia, Japan, Madagascar, Malaysia, Mauritius, Papua New Guinea, Seychelles, South China, Taiwan, Thailand, Vietnam and Western Africa (Stone, 1981, 1990). The leaves are usually linear, often more than 2 m long. They may remain attached for a long time following leaf death, and large amounts of dead leaves may accumulate under plants.

The genus has a wide ecological range and probably is one the most domesticated forms of any native Pacific plants (Yen, 1995). *Pandanus* taxonomy is very complex. Their female fruits are drupes, forming finger-shaped syncarps which are further agglomerated into large cylindrical or globose heads (Figure 2.1).

Pandanus amaryllifolius Roxb. (Toei hom) is distributed over Southern India, peninsular South East Asia, Indonesia and Western New Guinea. Male flowers are extremely rare, and there is no scientific description of a female flower for this species. The only examples of flowers come from the Moluccas, and it is plausible that the species evolved there. It is also interesting to note that *P. amaryllifolius* is the only species with fragrant leaves, and lacking of a wild population and the large distribution, imply a long tradition of cultivation (www.ang.kfunigraz.ac.at/~katzer/engl/Pand_ama.html).

Pandanus odoratissimus L. (Toei thalae) is known as Kewra flower that is usually found along the beach. The tree is distinctly dioecious plant. The male plants are termed *P. odoratissimus* while the female plants are *P. tectorius* (screwpine), so the two species are in reality one. The plant is a small tree that grows up to 6m high. It is supported by prop roots. Its leaves are usually 90-150cm long and 5-7cm wide with saw-like edges. It is a perennial tree and needs to grow in warm, damp areas in partial sunlight (Wikipedia, 2005).

Pandanus penetrans (Toei pa) is an evergreen shrub up to 5 m high, with dense clusters of leaves near the top of slender stems, often with aerial roots near the base, rather like enormous pineapple plants. Leaves are up to 3m, simple, spirally-arranged, linear with vicious spines all along the edges. Fruits (syncarp) are in dense ovoid or oblong heads, and often spiny. This *Pandanus* species is usually found in moist areas, often forming large impenetrable colonies (Gardner *et al.*, 2000).

2.2 Importance of *Dracaena lourieri* and *Pandanus* spp.

Many species of *Dracaena* and *Pandanus* are sources of herbal drugs used in Asia and listed in pharmacopedias and other drug compendia (Pongbunnrod, 1979; Sirisa-ard and Tantipathananandh, 2005). When *Dracaena lourieri* becomes old, it has a red core in the stem, the stem then gradually decays until all it core become red,

this core wood is called Chan Daeng. Retrodihydrochalcone has been extracted from this stem wood, and possesses estrogen antagonist activity (Ichikawa *et al.*, 1977). Furthermore, some other compounds have been extracted from *D. lourieri* e.g., (3S)-7, 4'-dihydroxy-3-(4-hydrobenzyl)-chromane, loureirin D and (2S)-pinocembrin show antibacterial activity against *S. aureus* and *B. subtilis* (Meksuriyen and Cordell, 1988; Ichikawa *et al.*, 1977). In Thailand, *D. lourieri* have been used as a component of traditional herbal medicines, for example Ya Hom, Ya kin-kae-pid, Ya lom, Ya sa-tree, Ya kae-lom-pid-dern. It has been used as a folk medicine e.g., antipyretic, anti-inflammatory, pain relief (Pongbunnrod, 1979). The popular dosage forms were those which are easy to take and prepare, such as powders, capsules or pills for mild illness following strokes (Sirisa-ard and Tantipathananandh, 2005).

Pandanus plants are used as a source of food and fibre (Stone, 1990). A number of ornamental species are cultivated in groves, the fruit is eaten and the leaves are used in thatching for roofing. The trunks of the *Pandanus* are suitable for constructing rafts (Whitton, 1999). *Pandanus amaryllifolius* is regularly used as a nourishing green tea. Leaves of this species contain linalyl acetate, geraniol, coumarin and ethyl vanillin (Reangrungsri and Tantiwat, 1991). Moreover, they are sold along with bunches of orchids for use in floral arrangements, but more importantly, they are the source of well-loved flavoring that goes into a wide assortment of desserts and sweet treats. The juice extracted from the fresh leaves provides a natural green food coloring as well (Figure 2.1).

Pandanus odoratissimus or *P. tectorius* have many traditional uses: the leaves are woven into mats, sails and baskets, the tips of the aerial roots are used in traditional medicine, and the flowers are used to make Monoi oil. The fruit of *P. tectorius* has traditionally been used as both food and medicine. Traditional healers use it for alcohol addiction, where it is said to help reduce craving, prevent liver scarring and to be antifibrotic. In traditional Philippine medicine, the fruit is used in the treatment of dysentery. In traditional Samoan medicine, it has been used for inflammation, fever, stomachache, constipation, dysentery, urinary tract complaints and furuncles. The fruit contains tannins, alkaloids, glycosides, and essential oils, which contain acetates, cinnamates and other compounds (NutriMedical online database of nutritional supplements: http://www.nutrimedical.com).

Furthermore, their colorful shrub or styles make them ideal as ornamentals. For example, many species of these both plant genera are cultivated as garden plants or pot plants for their attractiveness (Figure 2.1).

Figure 2.1 General structure and use of *Pandanus*. 1. Leaves. 2. Young fruit. 3. Mature fruit. 4. Prop roots. 5. Seed. 6. *Pandanus* cultivated as garden plants. 7. The juice extracted from the fresh leaves of *Pandanus amaryllifolius*. (Pictures from: www.biologie.uni-hamburg.de/b-online/d53/pandanus.htm, www.hktraveler.com)

2.3 Fungal life strategies (endophytes, parasites and saprobes)

Fungi are eukaryotic and heterotrophic. They are typically filamentous as well as single celled or pseudo-filamentous (e.g. yeasts). The hyphae (sing. hypha) or individual filaments are surrounded by a wall which often, although not always, contains chitin as a major component. The hypha extends by tip growth (apical growth) and multiplies by branching, creating a fine network, or mycelium. Fungi employ exoenzymes, form spores, and lack flagella (Deacon, 1988; Jennings and Lysek, 1996; Kirk *et al.*, 2001). They obtain carbon compounds and nutrients from external sources as diverse as rock faces in Antarctica and dung heaps in the tropics (Fröhlich and Hyde, 2000). Hawksworth *et al.* (1996) list about 30 niches and

microhabitats for fungi in a tropical forest: e.g. living vascular plants (e.g. endophytes, mycorrhiza), dead plants (e.g. saprobes, soil surface), non-vascular plants (e.g. lichen, aquatic algae), other fungi and fungal analogues (e.g. biotrophs, necrotrophs, myxomycetes), plant exudates (e.g. leaf or fruit surface), invertebrates, vertebrates and fungi in water.

The fungi in this study are mostly saprobes and parasites on plant material. A saprobe is an organism using dead organic material as food and commonly causing its decay (Hawksworth *et al.*, 1983), while a pathogen obtains food directly from its host and causes disease (Deacon, 1988). However, saprobes can switch between one mode of nutrition and another during their life cycle (Boddy and Griffith, 1989; Fisher and Petrini, 1992; Sridhar and Raviraja, 1995). Saprobes are capable of utilizing a wide range of carbon sources, including cellulose and lignin (Nilsson, 1973, 1974).

The close association between a fungus and a living plant, which it utilizes as a food source, is an example of symbiosis. This symbiosis may be detrimental to the host, as in the case of parasitism, but this is not always the case. Many parasitic fungi may also live as a saprobe on the host that they have killed. An example is *Armillaria mellea*, the honey fungus, which is a devastating root parasite of forest trees. After the host has died, the fungus lives as a saprobe and utilizes the nutrients (Hudson, 1980).

Endophytic relationships are usually examples of commensalism, that is the fungus benefits and the plant is neither helped nor harmed by the association. In terrestrial ecosystems, endophytes in living plants often become saprobic colonizers of the dead plant material due to their positional advantage (Carroll and Petrini, 1983; Ingold and Hudson, 1993; Guo *et al.*, 1998). By definition an endophyte cannot be considered as causing disease, however, some disease causing species are regularly isolated as endophytes. The distinction between a pathogen and endophyte is not always clear (Sinclair and Cerkauskas, 1996). Jones *et al.* (1993) found that the degree of correlation between the potential fungal pathogens of the *Musa* and the frequently isolated tropical endophyte genera was high, that including the common fungi as *Cladosporium*, *Colletotrichum*, *Curvularia*, *Drechslera*, *Fusarium*, *Guignardia*, *Lasiodiplodia*, *Nigrospora*, *Phoma*, *Phyllosticta* and *Verticillium*. However species known to be pathogens, which have been isolated as endophytes, are not necessarily always pathogenic strains (Brown *et al.*, 1998). *Cladosporium musae*,

Cordana musae and *Periconiella musae*, which are weak pathogens on banana, were found to persist as saprobes on the dead banana tissue (Photita *et al.*, 2003a).

2.4 Fungal classification

Representatives of the fungi *sensu stricto* include four phyla: Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota (McLaughlin *et al.*, 2001: Seifert and Gams, 2001). Chytridiomycota and Zygomycota are described as lower fungi. They are characterized by vegetative mycelium with no septa, complete septa are only found in reproductive structures. Asexual and sexual reproduction are by sporangia and zygospore formation respectively. Ascomycota and Basidiomycota are higher fungi and have a more complex mycelium with elaborate, perforate septa. Members of Ascomycota produce sexual ascospores in sac-shaped cells (asci) while fungi in Basidiomycota produce sexual basidiospores on club-shaped basidia in complex fruit bodies. Anamorphic fungi are anamorphs of Ascomycota and Basidiomycota and usually produce asexual conidia (Nicklin *et al.*, 1999; Kirk *et al.*, 2001). Three morphological groups of anamorphic fungi have been recognized that have in the past been named as classes, agonomycetes, coelomycetes and hyphomycetes. Agonomycetes are mycelial forms which are sterile, but may produce chlamydospores, sclerotia and/or related vegetative structures. Coelomycete forms produce conidia in pycnidial, pycnothyrial, acervular, cupulate or stromatic conidiomata. Hyphomycetes are mycelial forms which bear conidia on separate hyphae or aggregations of hyphae (as synnematous or sporodochial conidiomata) but not inside discrete conidiomata (Kirk *et al.*, 2001). This study focuses on higher fungi, Ascomycota and Basidiomycota and their anamorphs.

2.5 Ecology and distribution of fungi

There are few studies of saprobic fungal recurrence on different plant tissue types, e.g. on bamboo, Douglas fir, palms and *Rhizophora*. The fungal communities on different tissue types have been investigated within a single host species, for example, on trunks, twigs and branches of *Sonnerratia griffithii* or lignicolous, corticolous parts, twigs, large trees and thick twigs of *Salix* spp (Mathiassen, 1993). These studies and some other investigations have shown that saprobic taxa are

recurrent on different tissue types (Hyde *et al.*, 1990; Yanna *et al.*, 2001a; Photita *et al.*, 2003a; Bussaban, 2005) (Table 2.1). Lumyong *et al.* (2000) investigated the endophytes in twigs and leaves of bamboo, and found that the old tissues had more endophytes than the younger tissues. Different types of palm tissue can support a variety of saprobic fungal communities, and the great difference between the leaves and rachides indicates that saprobic fungi may be specific to different frond parts (Yanna *et al.*, 2001b). The tidal influence was also considered to affect the fungal communities on roots of *Rhizophora apiculata*, with or without bark (Hyde *et al.*, 1990). There have been several studies that have indicated that endophytes may exhibit tissue specificity (Bills and Polishook, 1992; Clay, 1992; Rodrigues, 1994; Taylor *et al.*, 1999; Fröhlich *et al.*, 2000; Photita *et al.*, 2001; Photita, 2003; Bussaban *et al.*, 2004).

Several studies have shown an increase in the number of endophytes recovered with increasing age of tissue (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.*, 2001). Factors that may contribute to changes in the endophyte community with tissue age are weathering of tissue, texture, increased exposure to propagules with time, and physical changes of the plant tissue or degradation of the leaf cuticle (Petrini and Carroll, 1981; Stone, 1987; Hata and Futai, 1993).

Fungal occurrence may be seasonal, possibly because the taxa have specific temperature or moisture requirements. For example, *Digitatispora marina* only occurs in marine habitats during the winter (Jones, 2000). Seasonality not only directly influences fungal occurrence, but may also affect the hosts and thus, in turn, again influence the fungi. A particular nutrient within the host tissue could be more available during a certain season of the year and thus affect fungal activity (Rodrigues, 1994). Fungi on leaf surfaces of 20 tree species in forests of Panama were cultured and examined. One-third of the species were present only in the dry season, one-third only in the wet season and the remainder were present in both seasons (Lodge and Cantrell, 1995).

Table 2.1 Saprobic fungi showing fungal recurrence on different tissues.

Plant name	Substrate	Fungi shown to be tissue specific	Place	References
Rhizophora apiculata	Young roots with bark (above mean tide)	*Leptosphaeria* sp. *Massarina* cf. *velatispora* *Rhizophila marina*	Ranong mangrove, Thailand	Hyde *et al.* (1990)
	Young roots without bark (above mean tide)	*Dactylospora haliotrepha* *Halocyphina villosa* *Hydronectria tethys* *Marinosphaera mangrovei* *Phialophorophoma* cf. *litoralis* *Savoryella lignicola*		
	Young roots with bark (below mean tide)	*Lulworthia grandispora* *Phomopsis* sp.		
	Young roots without bark (below mean tide)	*Didymosphaeria enalia* *Leptosphaeria australiensis* *Xylomyces* sp.		
Salix spp.	Twigs	*Cryptodiaporthe salicella* *Enchnoa infernalis* *Rhynchostoma minutum*	Scandinavian peninsula	Mathiassen (1993)
	Linicolous	*Bertia moriformis* *Capronia collapse* *Glyphium grisonense* *Hypoxylon macrosporum* *Hysterographium elongatum* *Lophiotrma boreale* *Lophiotrema nucula* *Melanopsamma pomiformis*		
	Corticolous	*Arthopyrenia lapponina* *Cryptodiaporthe salicella* *Cryptosphaeria subutanea* *Diatryp bullata* *Enchnoa infernalis* *Hypoxylon mammatum* *Rebentischia massalongii*		
	Large trees and thick twigs	*Bertia moriformis* *Diatryp bullata* *Hypoxylon mammatum* *Hysterographium elongatum*		

Table 2.1 (continued)

Plant name	Substrate	Fungi shown to be tissue specific	Place	References
Livistona chinensis	Leaves (2–4 weeks of decay)	*Pseudospiropes arecacensis* *Pseudospiropes* sp.	Lung Fu Shan	Yanna *et al.* (2001a).
	Leaves (>4 weeks)	*Lachnum palmae* *Zygosporium echinosporum*		
	Petiole (0-4 months)	*Appendicospora hongkongensis* *Oxydothis elaeicola*		
	Tip and mid of petioles (>10 months)	*Astrosphaeriella bakariana* *Cocoicola livistonicola* *Verticillium cf. dahliae*		
	Mid and bases of petiole (>10 months)	*Oxydothis obducens*		
Mesaceae	Leaves	*Anthostomella clypeoides* *Cladosporium cladosporioides* *C. musae* *Exserohilum cf. halodes* *Hansfordia ovalispora* *Helminthosporium velutinum* *Mycosphaerella* *Periconia lateralis* *Pseudocercospora*	Suthep Pui National Park, Thailand	Photita *et al.* (2003a)
	Petioles	*Colletotrichum musae* *Hemicorynespora mitrata* *Helicomyces macrofilamentosus*		
	Pseudostems	*Eupenicillium* *Dictyosporium heptasporum*		
Zingiberaceae	Leaves	*Acremonium* spp. *Cladosporium cladosporioides* *Dactylaria* spp. *Dactylella* spp. *Periconia* spp. *Verticillium* sp.	Suthep Pui National Park, Thailand	Bussaban (2005)
	Pseudostems	*Acremonium* spp. *Chloridium* sp. *Dactylaria* spp. *Dactylella* spp. *Nectria* spp. *Periconia* spp.		

Fungi may be morphologically adapted to a vertical distribution on the host (Jones, 2000). For example, ascomycetes that occur above mean tide generally have pigmented or ornamented spores, while those found throughout the tidal range have hyaline or smoothed-walled or sheathed ascospores (Hyde, 1989). Fungi in the intertidal region are influenced by tidal inundation. For example, *Cirrenalia* spp., *Periconia prolifica* and *Xylomyces* sp. were common on bark below mean tide. However, *Calathella* sp. and *Halorosellinia oceanica* were common at higher intertidal regions (Hyde, 1991).

2.6 Fungal succession

Changes of fungal communities throughout succession (especially secondary succession) have been well-documented but a full understanding of fungal succession is needed (Hyde, 1991; Frankland, 1992, 1998). Various studies on fungal succession including the traditionally studied macrofungi and the less well-studied microfungi were compiled in a publication by Hyde and Jones (2002). Based on the nutritional hypothesis, dead plant material is initially thought to be colonized by lower and non-specialized fungi, which utilize the available sugars (e.g. xylose, cellobiose, and sucrose). These fungi are then succeeded by higher and more specialised fungi, which utilize the complex organic components, including celluloses, hemicelluloses and lignin (Fell *et al.*, 1975; Deacon 1988; Hyde *et al.*, 1990). The occurrence of marine fungi can also be affected by dissolved organic nutrients, hydrogen ion concentration, osmotic effects, oxygen availability, pollutants, hydrostatic pressure, temperature and tidal amplitude (Booth and Kenkel, 1986).

In general, fungal communities go through three succession stages comprising the pioneer community, mature community and impoverished community (Dix and Webster, 1985).

Pioneer community, which consist of pioneer species have a low percentage of occurrence (Dix and Webster, 1985). Pioneer species tend to be fast growing, short lived and capable of rapid and wide dispersal (Luckzkovich and Knowles, 2000). Therefore, this type of community has low species diversity and few species have a high percentage of occurrence (Dix and Webster, 1985).

Mature community, the species diversity in this community is high and has peaked. There is a number of species with low percentage occurrence. However several species have a high level of occurrence. The dominant species have extremely high levels of occurrence. During the later stages of the mature community, the number of dominant species declines, but species diversity is still high (Dix and Webster, 1985)

Impoverished community, the species diversity and the number of species in this community decline. The community is dominated by a few species with high levels of occurrence (Dix and Webster, 1985). These dominant species tend to be persistent and longer-lived (Luckzkovich and Knowles, 2000). However, there are still some species with low levels of occurrence (Dix and Webster, 1985).

2.7 Importance of fungi

Fungi are responsible for plant disease and are the major cause (about 70-80%) of economic crop losses. For example, *Helminthosporium oryzae* and *Phytophthora infestans* caused devastating losses of potato and rice crops respectively in Europe during 1840-1943 (Deacon, 1988). Almost all higher plants in nearly all environments have mycorrhiza fungi living in association with their roots. The fungi form several types of mycorrhiza and they greatly increase the efficiency of mineral nutrient uptake from soil, as in some species of ectomycorrhiza mushroom (*Boletus*, *Russula*). Fortunately, fungi cause relatively few diseases of man and other warm-blooded animals when compared with those of plant.

Fungi also cause disease of *Pandanus* species. For example, *Microcyclus pandani* is associated with large, tan-coloured, distinctly zonate leaf spots which can render *Pandanus* leaves unsuitable for use in handcrafts. There is folklore associated with this pathogen on Aitutaki, Cook Islands, young girls normally harvest *Pandanus* for weaving are not allowed going to the plants during menstruation. The plants are said to develop blood-red leaf spots, caused by *M. pandani*, out of sympathy for the girl (McKenzie and Hyde, 1997).

Fungi are also saprobes, and are the major decomposers in almost all natural and man-made environments e.g. wood, wood-like materials and insect cuticles. They

produce the major depolymerizing enzymes involved in cellulose and lignin breakdown and thus ensure recycling of both carbon and mineral nutrients for continued plant growth. In addition, they produce some extremely complex and resistant polymers as a result of their saprobic activity, these polymers being the main component of the humic acid fraction of soil humus, which contributes to soil fertility.

Fungi are also used directly as food (as mushroom crops) and indirectly food and beverage production e.g. in beer-, bread-, and wine-making. In addition fungi are used in several production of industrial chemicals and drugs e.g. *Aspergillus niger*-organic acid and enzymes, *Penicillium chrysogenum*-Penicillins, *Cephalosporium* sp.-Cephalosporins, etc. (Deacon, 1988)

2.8 Fungi on Pandanaceae and fungi worldwide

During 1931-1932, the first comprehensive account of fungi on the Pandanaceae (90 species including 11 new species) were reported by Verona, principally from Samoa (see McKenzie and Hyde, 1997). Presently, the mycota on the family Pandanaceae (*Freycinetia*, *Pandanus* and *Sararanga*) has been recently reviewed (McKenzie and Hyde, 1996; 1997; McKenzie *et al*., 2002). One-hundred and sixty-nine species of fungi described from members of the Pandanaceae are listed alphabetically by McKenzie and Hyde (1996), comprising 35 species from *Freycinetia* (15 ascomycetes, 4 basidiomycetes, and 16 anamorphic fungi), 134 species from *Pandanus* (59 ascomycetes, 15 basidiomycetes, and 60 anamorphic fungi). No fungi have been recorded on *Sararanga*.

McKenzie and Hyde (1997) found that the biodiversity of fungi on the Pandanaceae was high, and leaves of *Freycinetia* and *Pandanus* have proved to be a rewarding substrate for mycological investigations. There are several species known only from the Pandanaceae, e.g. *Chalarodes bisetis*, *Sporidesmium freycinetiae*, *Stachybotrys freycinetiae*, *S. nephrodes* and *Zebrospora bicolor*. Most ascomycetes on *Pandanus* have been recorded from the Philippines and Indonesia, while fewer have been recorded in India and Hawaii. *Sphaeria fur* and *S. profuga* were the first ascomycetes described from *Pandanus* from the Marshall Islands. The anamorphic fungi have been recorded from various parts of the world. Whitton (1999)

investigated microfungi on the Pandanaceae in 11 widespread tropical countries, from 9 species of *Freycinetia*, 23 species of *Pandanus* and 1 species of *Sararanga*. Anamorphic fungi accounted for 78 genera and 149 species, and ascomycetes 27 genera and 76 species.

McKenzie *et al.* (2002) reported that the fungal diversity of *Pandanus* (336 spp.) differs from *Freycinetia* (133 spp.), with 44 overlapping species (Table 2.2). At that time they also reported an additional 21 ascomycetes and 15 anamorphic fungi described from *Freycinetia* and *Pandanus*.

Table 2.2 Number of fungi and myxomycetes published as occurring on the Pandanaceae (McKenzie *et al.*, 2002).

Fungi	*Freycinetia*	*Pandanus*	*Sararanga*	Overlap between *Freycinetia* and *Pandanus*
Ascomycetes	61	118	2	20
Basidiomycetes	6	17	0	0
Mitosporic fungi	65	198	5	23
Oomycetes	1	1	0	1
Myxomycetes	0	2	0	0
Total	133	336	7	44

There are approximately 40 new fungi recently described from *Freycinetia* and *Pandanus* since McKenzie and Hyde (1996) (Table 2.3).

Most of the fungi described on Pandanaceae are saprobes. The family does not form ectomycorrhizas and supports few basidiomycetes. However, *Pandanus* leaves are invariably spotted, due to fungal infections. *Pandanus tectorius*, the coastal species in Hong Kong is constantly covered with large, brown, necrotic spots, with an associated coelomycete. In northern Queensland the rainforest *Pandanus* sp. is consistently covered with large leaf spots caused by *Annellolacinia pandanicola*. Other anamorphic fungi for example *Diplococcium pandani*, *Phyllosticta pandanicola*, and *Volutellaria fuliginea* have also be shown to cause *Pandanus* leaf spots. The ascomycetes *Echidnodes pandani* and four *Meliola* spp. are major causes sooty blotch on *Pandanus* (McKenzie and Hyde, 1997).

Table 2.3 New fungi described from the Pandanaceae since McKenzie and Hyde 1996.

Ascomycetes described from *Freycinetia*	**References**
Anthostomella kapiti – Java	Lu and Hyde (2000)
A. manawatua – Mauritius	Lu and Hyde (2000)
A. okatina – Mauritius	Lu and Hyde (2000)
Hyphomycetes described from *Freycinetia*	
Dictyochaeta renispora – Philippines	Whitton *et al.* (2000a)
Ascomycetes described from *Pandanus*	
Anthostomella minutoides – Java	Lu and Hyde (2000)
A. petrinensis – Mauritius	Dulymamode *et al.* (1998a)
A. theobromina – Mauritius	Dulymamode *et al.* (1998a)
Astrocystis cepiformis – Mauritius	Dulymamode *et al.* (1998b)
A. fimbriata – Mauritius	Dulymamode *et al.* (1998b)
A. rarissima – Mauritius	Dulymamode *et al.* (1998b)
Fasciatispora pandanicola – Java	Hyde (1995)
Linocarpon appendisporum – Irian Jaya	Hyde (1997)
L. breve – Irian Jaya	Hyde (1997)
L. falciformisporum – Irian Jaya	Hyde (1997)
L. fasciatum – Mauritius	Dulymamode *et al.* (1998c)
L. lammiae– Hongkong	Thongkantha *et al.* (2003)
L. pandanicola – Irian Jaya	Hyde (1997)
L. siamensis – Thailand	Thongkantha *et al.* (2003)
L. spathulatum – Mauritius	Dulymamode *et al.* (1998c)
L. sulcatum – Mauritius	Dulymamode *et al.* (1998c)
L. suthepensis – Thailand	Thongkantha *et al.* (2003)
Meliola kapoorii – India	Hosagoudar (1996)
M. pandacearum – India	Hosagoudar and Abraham (1999)
Nipicola pandani – Hongkong	Hyde and Taylor (1996)
Stictis pandani – Australia	Whitton *et al.* (1999a)
Hyphomycetes described from *Pandanus*	
Acrodictys lamma – Hongkong	Whitton *et al.* (2000b)
A. triarmatus – Mauritius	Whitton *et al.* (2000b)
Bahusutrabeeja dubhashii Bhat, (1994) – India	Dulymamode *et al.* (1998e)
Camposporium fusisporum – Brunei Darussalam	Whitton *et al.* (2002)
C. ramosum – Australia (+ Hawaii)	
Cryptophiale pandanicola – Mauritius	Dulymamode *et al.* (1999)
Dictyochaeta fibriaspora – Philippines	Whitton *et al.* (2000a)
D. microcylindrospora – Hongkong	Whitton *et al.* (2000a)
D. multisetula – Australia	Whitton *et al.* (2000a)
D. seychellensa – Seychelles	Whitton *et al.* (2000a)
Fuscophialis suttonii – Mauritius	Dulymamode *et al.* (1998e)
Paraceratocladium triseptata – Mauritius	Dulymamode *et al.* (1998e)
Rubikia splendida – Mauritius	Dulymamode *et al.* (1998d)
Spadicoides mauritiana – Mauritius	Dulymamode *et al.* (1999)
Troposporopsis atroapicis – Hongkong	Whitton *et al.* 1999b
T. rigidospora – Hongkong	Whitton *et al.* 1999b
Zygosporium pacificum – Vanuatu	Whitton *et al.* 2003
Z. pandanicola – Philippines	Whitton *et al.* 2003
Coelomycete described from *Pandanus*	
Rubikia splendida – Mauritius	Dulymamode *et al.* (1998d)

Recently, the biodiversity and ecology of fungi on bamboo and palm fronds have been studied (Hyde *et al.*, 2001; Yanna, 2001; Yanna *et al.*, 2001b). Two-thousand and forty-four collections of saprobic fungi were examined from dead clums of *Bambusa* spp. and *Dendrocalamus* spp. in the Philippines and Hong Kong comprising 24 ascomycetes, 56 anamorphic taxa and 1 basidiomycete. More saprobes were collected in the Philippines (1278) than in Hong Kong (766) and differences in the mycota between the two sites were observed (Hyde *et al.*, 2001). Yanna (2001) investigated the biodiversity of fungi on fronds of the palms *Arenga engleri*, *Livistona chinensis* and *Phoenix hanceana* (Hong Kong), *Arenga undulatifolia*, *Oncosperma horridum* and *Salacca affinis* (Brunei), *Livistona australis* and *Oraniopsis appendiculata* (Australia). A total of 306 taxa, including one new ascomycete and 12 new anamorphic fungi (four new genera) were recorded. The fungal communities on the leaves differed to that on the rachides. The ascomycetes such as *Anthrostomella*, *Diaporthe*, *Linocarpon*, *Oxydothis* and *Stictis* and the hyphomycetes such as *Acrodictys*, *Colletotrichum*, *Gliomastix*, *Periconia*, *Phoma, Phomopsis* and *Sporidesdium* were recorded as the common genera on various monocotyledonous hosts (Table 2.4).

2.9 Fungi in Thailand

In Thailand, knowledge on fungal diversity and reports were very poor and sporadic before 1990, even though this tropical country has a rich flora and fauna (Dreyfuss and Petrini, 1984; Hyde, 2000; Jones and Hyde, 2004). However, research facilities and literature access improved with the number of fungal species records to over 2000 in 2001 from 700 species in 1989 (Hywel-Jones and Boonpratuang, 2001). Hyde *et al.* (1997a) isolated a rare fungus, *Apiosordaria striatispora*, from young seedlings of *Mesua ferrea* and *Prunus arborea,* studied its morphology and examined the species at the SEM level. Lumyong *et al.* (1998) indicated that *Colletotrichum* sp., *Curvularia* sp., *Fusarium* sp., *Phoma* sp., *Phomopsis* sp. and *Seimatosporium* sp. were dominant endophytic species isolated from indigenous plant species in Doi Suthep-Pui area. Sardsud *et al.* (1998) stated that *Lasiodiplodia* sp., *Pestalotiopsis* sp., *Fusarium* sp. and *Curvularia* sp. were the dominant endophytes isolated from shoot, panicles, stem

Table 2.4 The most common fungal genera recorded on various monocotyledonous hosts.

Palms (Taylor, 1998)	Palm (Yanna *et al.*, 2001a)	Bamboo (Hyde *et al.*, 2001)	Pandanaceae (Whitton, 1999)	Grasses (Wong and Hyde, 2001)	Musaceae (Photita *et al.*, 2003a)	Zingiberaceae (Bussaban, 2005)
Ascomycetes						
*Anthostomella**	*Anthostomella*	*Anthostomella*	*Anthostomella*	*Didymosphaeria*	*Anthostomella*	*Anthostomella*
Botryosphaeria	*Astrosphaeriella*	*Apiospora*	*Linocarpon*	*Diaporthe**	*Chaetomium*	*Nectria*
Diaporthe	*Auerswaldia*	*Arecophila*	*Meliola*	*Linocarpon**		*Nectriopsis*
Fasciatiapora	*Lembosia*	*Astrosphaeriella*	*Nectria*	*Macrospora*		*Phaeosphaeria*
Linocarpon	*Linocarpon*	*Chaetomium*	*Niesslia*	*Massarina**		unknown *Ascomycetes*3
Lophiostoma	*Meliola*	*Hypoxylon*	*Oxydothis*	*Niptera*		
Massarina	*Mycosphaerella*	*Massarina*	*Stictis*	*Paraphaeosphaeria*		
Neolinocarpon	*Oxydothis*			*Phaeospharia*		
*Oxydothis**	*Phyllachora*			*Phragmitensis*		
*Stictis**	*Sphaerodothis*			*Pleospora*		
Anamorphic fungi						
Arthrinium	*Cocoicola*	*Acremonium*	*Acrodictys*	*Colletotrichum*	*Canalisporium*	*Acremonium**
Diplodia	*Cylindrocladium*	*Acrodictys**	*Colletotrichum*	*Fusarium*	*Periconia**	*Canalisporium*
Endocalyx	*Verticillium*	*Cladosporium*	*Dictyochaeta*	*Nigrospora*	*Memnoniella*	*Chloridium*
Lasiodiplodia	*Stilbella*	*Corynespora*	*Dictyosporium*	*Petrakia*	*Pseudobotrytis*	*Cladosporium*
Melanographium	*Volutella*	*Curvularia*	*Gliomastix*	*Phaeoisaria**	*Pyriculariopsis*	*Colletotrichum*
Microsphaeropsis	*Zygosporium*	*Ellisembia*	*Periconia*	*Phoma**	*Verticillium*	*Curvularia*
*Periconia**		*Gliomastix**	*Pestalotia*	*Phomopsis**	*Zygosporium*	*Dactylaria*
Pestalotiopsis		*Phaeoisaria*	*Sporidesmium*	*Septoria*		*Dactylella*
Phoma		*Podosporium*	*Stachybotrys*	*Sporidesmium**		*Fusarium*
Phomopsis		*Trichocladium*	*Zygosporium*	*Tetraploa*		*Phomopsis**

* Common genera on 2-3 hosts.

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, found that mycelia sterilia, *Fusarium* spp., *Phoma* sp. and xylariaceous species were the dominant species. Recently, wild banana (*Musa acuminata*) has been investigated for endophytes and saprobes, 61 and 80 taxa respectively have been isolated. Fewer endophytic isolates were recovered from younger than older samples. Xylariaceous taxa and *Guignardia cocoicola* were the most frequently isolated endophytes from leaves, *Dactylaria* sp. and *Pyricularia parasitica* were most common in the pseudostems, while *Colletotrichum* sp. was most common in the midribs and petioles (Photita *et al.*, 2001; Photita, 2003). Eighty-nine fungal species were reported from submerged wood of *Dipterocarpus alatus* and *Xylia dolabrifomis* in Khao Yai National Park streams by Sivichai *et al.* (2000). Two years later they found 73 species of freshwater fungi from a stream at Tad Ta Phu, Khao Yai National Park (Sivichai *et al.*, 2002). Somrithipol *et al.* (2002) recorded 70 fungi during a study of fungal succession on pods of *Delonix regia* from Khao Yai National Park. Finally, 163 species of saprobes were listed from decaying leaves and pseudostem tissues of zingiberaceous plants by Bussaban (2005).

A good start has been made to study saprobes and pathogens on plants in Thailand. Most plants studied have been found to support a rich diversity of fungi, and several new species have been described (Table2.5). However, further investigations in Thailand on fungal diversity and ecology are necessary to clarify the numerous saprobes, endophytes and parasites present.

Table 2.5 New selected fungi described from various hosts/habitats in Thailand since 1996.

New fungi	Host/Habitat	References
Chaetomium floriforme	fallen leaves	Gené and Guarro (1996)
Apiosordaria striatispora	*Mesua ferrea* and *Pranus arborea*	Hyde *et al.* (1997a)
Brachydesmiella verrucosa	wood in freshwater	Sivichai *et al.* (1998)
Biflagellospora papillata	wood in freshwater	Sivichai and Hywel-Jones (1999)
B. gracilis		
B. siamensis		
Melanochaeta garethjonesii		
Micropeltopsis quinquecladiopsis		

Table 2.5 (continued)

New fungi	Host/Habitat	References
Sigmoidea contorta	wood in freshwater	Marvanová and Hywel-Jones (2000)
Aliquandostipite khaoyaiensis	wood	Inderbitzin *et al.* (2001);
Lollipopaia minuta		Inderbitzin and Berbee (2001)
Berkleasmium nigroapicale	dead pseudostems of	Bussaban *et al.* (2001a)
B. sutheppuiense	*Amomum siamense*	
Leiosphaerella amomi		
Gaeumannomyces amomi	*Alpinia malaccensis*	Bussaban *et al.* (2001c)
Pyricularia longispora	Zingiberaceous	Bussaban *et al.* (2003)
P. kookicola	endophytes	
P. variabilis		
Chalara siamense	Submerged *Eleiodoxa conferta*	McKenzie *et al.* (2002)
Dictyosporium musae	*Musa acuminata*	Photita *et al.* (2002);
Stachybotrys suthepensis		Photita *et al.* (2003b)
Jahnula appendiculata	palms in peat swamp	Pinruan *et al.* (2002)
Dokmaia monthadongii	senescent leaves of *Manglietia garrettii*	Promputtha *et al.* (2003)
Anthostomella monthadoia	*Magnolia liliifera*	Promputtha *et al.* (2004a, 2005a, b)
Cheiromyces magnoliae		
Pseudohalonectria suthepensis		
Amanita simensis	Associate with plants in Doi Suthep Pui National Park	Sanmee *et al.* (2003)
Custingophora undulatistipes	palm	Pinnoi *et al.* (2003a, b, 2004)
Dactylaria uliginicola		
D. flammulicornuta		
D. palmae		
Submersisphaeria palmae		
Unisetosphaeria penguinoides		
Vanakripa minutiellipsoidea		
Berkleasmium typhae	*Typha angustifolia*	Somrithipol and Jones
Digitoramispora lageniformis	*Arundinaria pusilla*	(2003a, b, c)
Pseudoacrodictys dimorphospora	(pygmy bamboo)	
Linocarpon siamensis	dead laves of	Thongkantha *et al.* (2003)
L. suthepensis	*Pandanus penetrans*	
Craspedodidymum licualae	palm	Pinruan *et al.* (2004a, b, c)
C. microsporum		
C. siamense		
Phruensis brunniespora		

2.10 Phylogenetic relationships of fungi

Taxonomists are not only interested in characterizing and describing species, but also in demonstrating relationships between them. Understanding relationships between species will not only assist in developing classification schemes but also to understand morphological, physiological, biochemical, and ecological relationships. All of these types of data can and should be used to classify fungi (Shearer, 1986). Pleomorphism (having more than one form) is one of the great challenges of systematic mycology. Fungi may have sexual or asexual forms or rarely both forms will develop together. Holomorphic fungi that are known to produce both sexual and asexual forms have caused difficulties for taxonomists attempting to place them within classification systems (Weresub and Pirozynski, 1979). Because the sexual and asexual forms are different and are usually not in association with each other, the same species has been given more than one name. Therefore integrating classification and naming systems for anamorphs and teleomorphs has become more and more important.

The application of polymerase chain reaction (PCR) in mycology was firstly described by White *et al.* (1990) and concerned the amplification and direct sequencing of ribosomal RNA gene (rDNA) to establish the taxonomic and phylogenetic relationship of fungi. There are three major steps in a PCR including denaturation, annealing and extention, which are repeated for 30 or 40 cycles (Figure 2.2). This is done on an automated cycler, which can heat and cool the tubes with the reaction mixture in a very short time. During PCR processes, both strands are copied, there is an exponential increase of the number of copies of the gene (Figure 2.3) and the PCR product could be verified by using gel electrophoresis (Figure 2.4) (http://users.ugent.be/~avierstr/index.html).

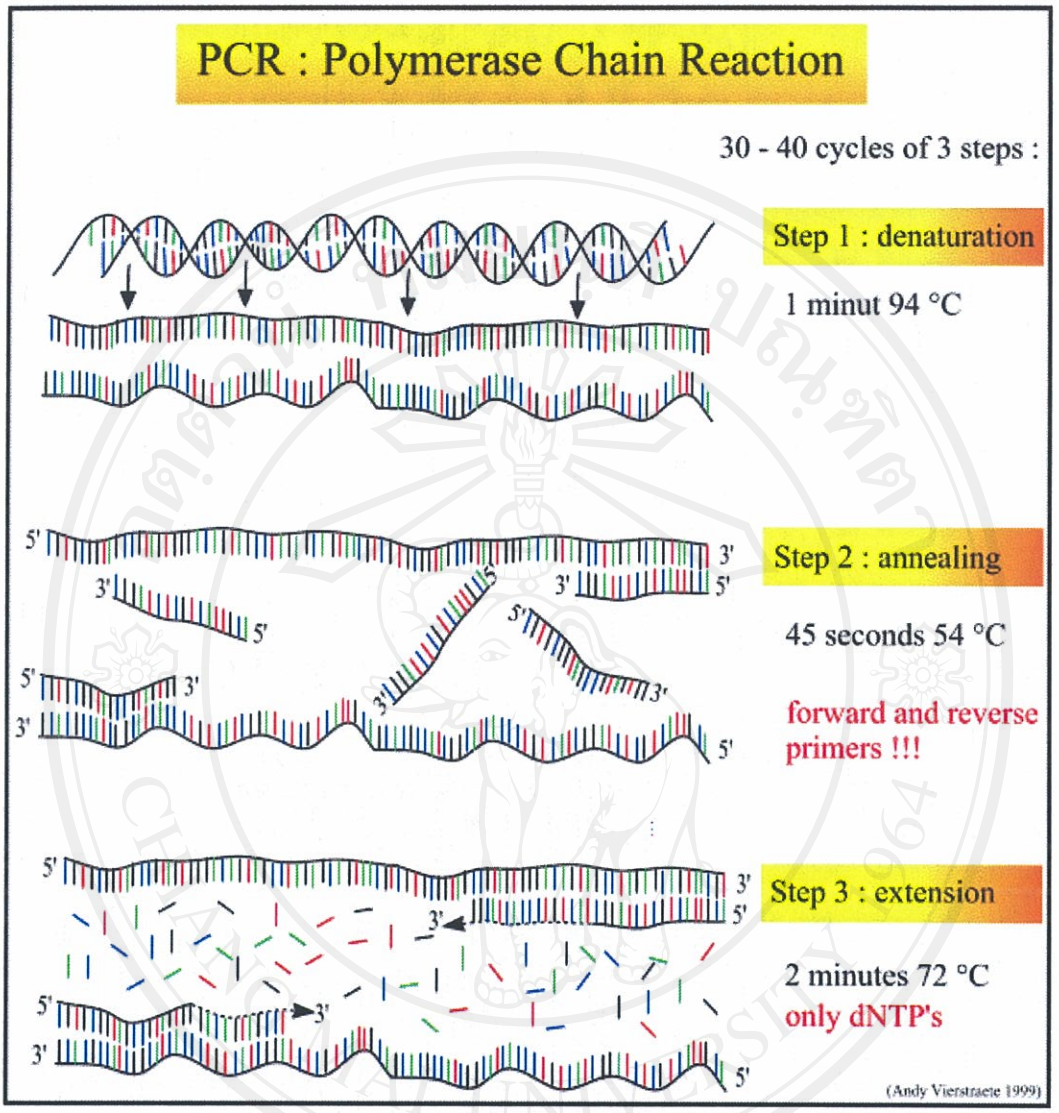

Figure 2.2 The different steps in PCR.

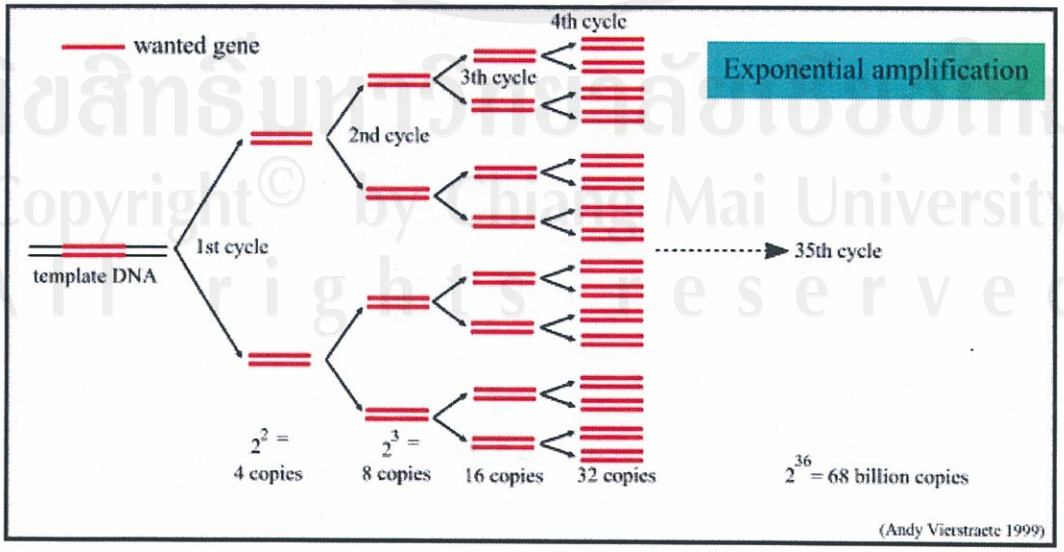

Figure 2.3 The exponential amplification of the gene in PCR.

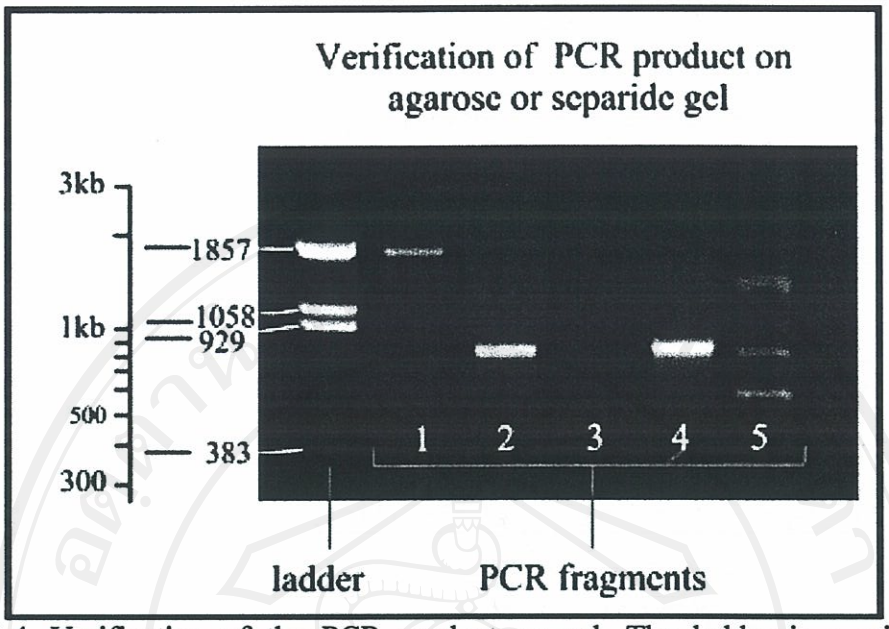

Figure 2.4 Verification of the PCR product on gel. The ladder is a mixture of fragments with known size to compare with the PCR fragments. Notice that the distance between the different fragments of the ladder is logarithmic. Lane 1: PCR fragment is approximately 1850 bases long. Lane 2 and 4: the fragments are approximately 800 bases long. Lane 3: no product is formed, so the PCR failed. Lane 5: multiple bands are formed because one of the primers fits on different places.

With the advent of PCR and its associated methodologies e.g. random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and DNA sequencing have frequently been used for the construction of phylogenetic relationships of fungi at different classification levels. RAPD is a simple and rapid method for detecting genetic diversity as well as it is a powerful tool used to differentiate morphologically similar microorganisms (Welsh and McClelland, 1990). AFLP is a modified technique of simple PCR. There is amplification of fragments from restriction enzyme digestion of genomic DNA. AFLP is similar to RAPD in that it analyses the whole genome but is different in that it uses stringent PCR conditions and produces more reproducible results. AFLP has been applied to the detection of inter- and intraspecific genetic variation in fungi and has more advantages than restriction fragment length polymorphism analysis (RFLP) (Majer *et al.*, 1996). The maximum degree of polymorphism can be detected by sequencing appropriate region of DNA and identifying which of the four possible nucleotides (ATGC) occurs at each position (Talbot, 2001). The simply in sequencing of DNA and computerization of analytic methods together with the rapid increasing

amount of sequences in database (GenBank, EMBL and DDBJ), therefore sequenced data are usually used to infer phylogenies of fungi.

Ribosomal RNA genes (rDNA) of fungi comprise two major regions; the highly conserved region and variable region which are slowly and rapidly evolving respectively (Figure 2.5). These genes are often used for taxonomic and phylogenetic relationships of fungi because they are found universally in living cells in which they have important functions; thus, their evolution might reflect the evolution of the whole genome. This multiple copy gene of fungi can be easily extracted from the fungal genome and amplified using primers for these genes (White *et al.*, 1990), then DNA can be easily sequenced either directly from PCR product or from cloned fragments. In addition, many sequences of the same studied genes can be easily downloaded from the molecular genetic databases for data comparison. Molecular tools use sequence analyses of the variable and short genes, such as internal transcribed spacer (ITS) rDNA and some other genes e.g. β-tubulin and histone genes are now established to prove relationship of closely related taxa (Risède and Simoneau, 2001; Roux *et al.*, 2001; Zhou and Stanosz, 2001; Bussaban *et al.*, 2005). The conserved gene (large subunit 28S rDNA) have often been useful in finding relationships at the genus level (Inderbitzin and Berbee, 2001; Rossman *et al.*, 2001a; Jeewon *et al.*, 2002; Jeewon *et al.*, 2004; Sakayaroj *et al.* 2005), while the most conserved regions, namely the small subunit rDNA (18S) have been used to examine the relationships between distantly related taxa or families (Chen *et al.*, 1999; Kong *et al.*, 2001; Wandelei-Silva *et al.*, 2003; Hansen *et al.*, 2005).

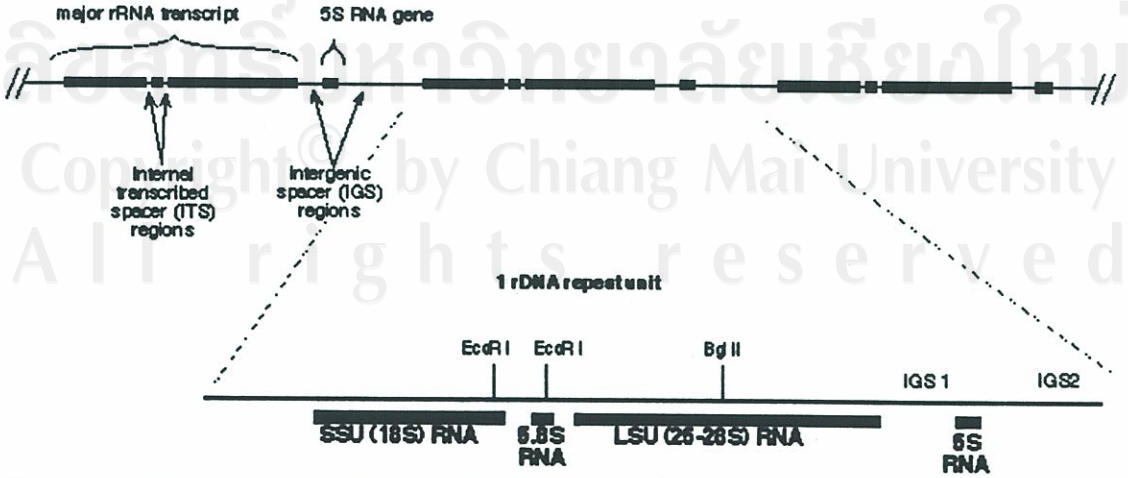

Figure 2.5 Ribosomal RNA gene (rDNA) diagrams.