

CHAPTER 1

INTRODUCTION

Microbiologists have long realized that communities of microorganisms that inhabit plant leaf surfaces and interiors are very diverse and abundant. One group of microorganisms that has recently attracted the attention of ecologists and evolutionary biologists are the endophytic fungi because of their demonstrable role in mediating interactions of herbivores with some grass host plants (Petrini, 1991). In some grasses, especially clavicipitaceous grasses, fungi are known to produce alkaloidal mycotoxins that, reduce growth and fecundity, or kill invertebrate and vertebrate herbivores and thus are thought to be plant mutualists. In addition, the presence of endophytic fungi may also increase drought and flood resistance, germination success and plant competitive abilities of host grasses (Clay, 1988, 1989). Symptomless endophyte infections of plants other than grasses have been known for more than seventy years (Petrini, 1996). Most endophyte research has been carried out using plants from temperate regions and there are few reports of endophytes from plant material collected in tropical regions.

Petrini and Dreyfuss isolated fungal endophytes of Araceae, Bromeliaceae and Orchidaceae from French Guyana, and from Piperaceae and Crassulaceae collected in Brazil and Colombia (1981 cited in Rodrigues and Petrini, 1997). Rodrigues and Samuels (1990) reported on the endophytes of the fan palm *Licuala ramsayi* (Muell.) Domin. from a tropical rain forest in Australia. More recent studies provided some preliminary information on the endophytes assemblages of an important pasture legume in Brazil, *Stylosanthes guianensis* Swart. (Pereira, Azevedo and Petrini, 1993), of the Amazonian palm *Euterpe oleracea* (Rodrigues, 1994), of teak (*Tectona grandis* L.) (Mekkamol *et al.*, 1996), and terrestrial orchid in Thailand (Manoch and Busarakam, 2001), of tropical fruit trees e.g. banana (*Musa acuminata*) (Azevedo *et al.*, 2000 ; Photita *et al.*, 2001), *Citrus* sp., cashew (*Anacardium occidentale*) and mango (*Mangifera indica*) (Azevedo *et al.*, 2000), of Ericaceae (*Bruguiera gymnorrhiza*) from

Japan (Okane, Nakagiri and Ito, 2001) and some halophytes from mangrove forest in India (Suryanarayanan and Kumaresan, 2000).

The data from tropical regions is scarce but preliminary results have shown that tropical plant hosts contain a great diversity of endophytic microorganisms, many of them not yet classified and possibly belonging to new genera and species. Potentially they are of biotechnological importance. There is the possibility that new pharmaceutical compounds, agents for biological control of insect-pests and plant disease besides other useful characteristics and secondary metabolites could be found, by further exploration of tropical endophytic communities (Azevedo *et al.*, 2000).

In Thailand, there are few reports of endophytic fungi. In a recent investigation endophytic fungi were isolated from leaves of *Tectona grandis* L. in plantations as well as in natural stands from northern Thailand (Mekkamol *et al.*, 1996; Mekkamol, 1998). Teak is one of the most valuable timber resources in the tropics, it is also one of the most widely studied tropical plants in terms of ecology and silviculture. Its distribution is discontinuous, covering the Indian Peninsula and Myanmar as well as northern and western Thailand. This species has been planted in various areas outside of its natural distribution area (Tanaka, Hamazaki and Vacharangkura, 1998).

Studies by Fisher *et al.* (1994) have shown that the endophyte assemblages of trees planted outside their original range are depauperate and consist of species different from those in native habitats. In this investigation the endophytic assemblage of the leaves of teak leaves were taken from campus of Chulalongkorn University. This investigation aims to isolation and identification of endophytic fungi found within healthy leaves, comparison of the endophytes assemblages of young and mature leaves and the investigation seasonal effects.

In addition, this research represents the first survey of foliar endophytes from *Samanea saman* Merr. (Rain tree), a large leguminous tropical tree from the tropics of America, belonging to the family Leguminosae the leaves of which fold together in

cloudy weather and in darkness. *S. saman* has a wide range of useful products, the pod in particular producing an edible pulp. When ripe, the pulp is sweet and sugary. It can also be dried and ground into a meal for animal feed. The timber is strong and hard, with a rich, dark colour, and makes furniture. It is also valuable as a shade tree in pastures, stimulating grass growth (Allen and Allen, 1981; Skerman, Cameron and Riveros, 1998). The purpose of the investigation is to compare the endophytic fungi between rain tree leaves and teak leaves in Chulalongkorn University.

The demand for novel compounds with antibiotic properties produced by microorganisms has increased considerably in recent years and the expectation to find organisms that produce pharmacologically useful metabolites can legitimately rely upon the great fungal diversity present in the tropic (Rodrigues and Petrini, 1997). To evaluate the potential of this valuable fungal source for industrial screening programs, cultures representing different species, or morphologically different strains, were tested for the production of antimicrobial activities against a panel of target microorganisms, including Gram-positive, Gram-negative bacteria and one species of fungus.

Objectives

1. Isolation and identification of the endophytic fungi were found within healthy teak and rain tree leaves.
2. Comparison of the endophytes assemblages between young and mature of teak and rain tree leaves and the investigation seasonal effects
3. To test for the production of antimicrobial substances from endophytic fungi.