CHAPTER 1

GENERAL INTRODUCTION

The Zingiberaceae is a monocotyledonous plant family, members of which are used as a source of spices, dyes, perfumes, and medicines, while a number of its ornamental species are cultivated for their showy flowers. The family is widely distributed in tropical regions and comprises 53 genera with about 1,300 species (Mabberley, 1987; Griffiths, 1992; Kress *et al*., 2002). In Thailand, there are 21 genera of Zingiberaceae, with about 200 species, occurring throughout the country (Larsen, 1996).

Numerous studies have reported that infections of terrestrial and aquatic plants by endophytes are ubiquitous, having been found throughout a broad range of host orders, families, and genera worldwide. However, the endophytes, saprobes and pathogens of Zingiberaceae have been poorly studied. Fungal endophytes become established within living plant tissue without causing overt symptoms or apparent injury to the host (Fisher and Petrini, 1990; Petrini, 1991; Hirsch and Braun, 1992). These symbioses are diverse in nature, and can be mutualistic, neutral or antagonistic, even though the endophyte may cause disease under appropriate ecological and physical conditions or develop as saprobes, once a plant senesces or dies (Latch, 1993; Brown *et al*., 1998; Photita *et al*., 2004). Some endophytes are thought to benefit host plants by protecting them against insect pests and plant pathogens, conferring drought tolerance, or enhancing absorption of soil nutrients (Webber 1981;

Funk *et al*., 1983; Carroll, 1986, 1988; Thomson *et al*., 1986; Clay, 1989; Breen 1993, 1994; Stone *et al*., 2000; Arnold *et al*., 2003; Newman *et al*., 2003; Barrow, 2004). Studies on this group of fungi on zingiberaceous plants are, therefore, important in determining relationships among endophytes, saprobes and pathogens.

The investigation of fungi occurring on the Zingiberaceae is important in terms of providing answers to questions on global fungal numbers, diversity of fungi in Thailand and establishing whether the fungi on the Zingiberaceae are unique or the same as those found on other families. Moreover, there is a great potential for use of data obtained from this study in terms of answering questions on the endophytic mode of life. By establishing which enzymes that the endophytes, saprobes and pathogens produce, it should be possible to provide data on the mode of life of the endophytes. In addition, the isolates obtained from this study could be used in the development of Thai biotechnology. Possible outcomes could be new fungi for enzyme production, fungi for biological control of zingiberaceous pathogens, fungi that produce new antibiotic/anti-fungal compounds, and novel compounds from fungi for the agricultural or pharmaceutical industries.

This study was, therefore, initiated in order to establish the ecology and diversity of endophytic and saprobic fungi on Zingiberaceae in northern Thailand. Six species in the Zingiberaceae (Figure 1.1), including four wild and two cultivated species, were selected for the present study. The wild species were *Alpinia malaccensis* (Brum.) Rosc., *Amomum siamense* Craib., *Etlingera elatior* (Jack) Smith, *Etlingera littoralis* (Kon.) Gisc., while the cultivated species were *Alpinia galanga* Willd. and *Zingiber officinale* Rosc. The four wild species and *Alpinia galanga* are

Figure 1.1 Wild and cultivated zingiberaceous plants selected for the present study: a. *Alpinia malaccensis*, b. *Amomum siamense*, c. *Etlingera elatior*, d. *Etlingera littoralis*, e. *Alpinia galanga*, f. *Zingiber officinale*.

evergreen species having fibrous rhizomes. *Zingiber officinale* is herbaceous species having dormancy period and fleshy rhizomes. The plant species were examined to establish whether the pathogens, endophytes and saprobes differ between zingiberaceous hosts especially between the wild and cultivated species. Representative endophytes, saprobes and pathogens were examined for pathogenicity test and antagonistic test in order to answer questions on the mode of life of

endophytes. Thailand is in the tropics and the fungi 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, selected isolates obtained from this study were provided to screen the zingiberaceous fungi producing antimicrobial compounds and enzyme cellulase, mannanase and protease.

This thesis (Figure 1.2) reviews knowledge of Zingiberaceae and fungi (Chapter 2), explores the biodiversity of endophytic and saprobic fungi (Chapters 3, 4, respectively) on wild and cultivated Zingiberaceae in northern Thailand. It also addresses zingiberaceous diseases and their pathogenicity, including the pathogenicity of endophytic and saprobic fungi which are though to be latent pathogens and their capability to be antagonistic agents (Chapter 5). *Myrothecium* and *Pyricularia* strains were identified and isolated as part of this study. The distinction between *Myrothecium* and some synnematous fungi, e.g., *Solheimia*, and between *Pyricularia* and some *Dactylaria* species is not always clear. The morphological characterization of *Myrothecium* and *Pyricularia* are discussed and phylogenetic relationships among isolates of these fungi and their related genera were determined by analyzing complete sequences of the ITS regions (including 5.8S rRNA gene), to determine whether the morphological characters used to distinguish these fungi are supported by molecular data (Chapter 6). Fungi isolated from Zingiberaceae were tested to establish their potential to produce antimicrobial compounds and enzyme cellulase, mannanase and protease (Chapter 7). A general discussion of the results of this study and their implications is also presented (Chapter 8).

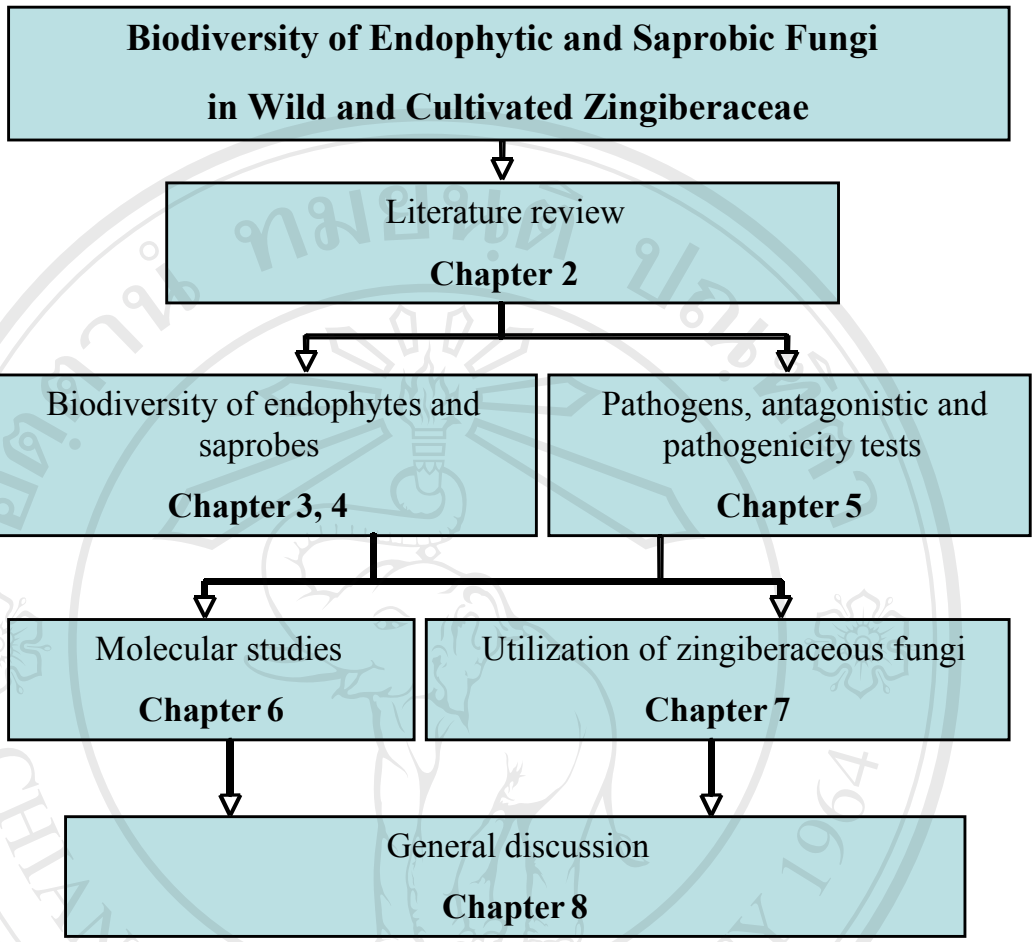

Figure 1.2 Schematic presentation of the relationships between chapters of the thesis.