

CHAPTER 1

INTRODUCTION

1.1 Background problems to this research

The fungal kingdom comprises a large and diverse group of organisms estimated to number over one million species (Hawksworth, 1991). Fungi are heterotrophic and absorptive organisms with a saprobic, parasitic or mutualistic existence. As parasites, they cause diseases in plants and animal. Within the Ascomycota, the Hypocreales is an order of many genera and species that are important pathogens of plants and animals. The family of Hypocreales, *Hypocreaceae* de Not., *Niessliaceae* Kircht. and *Clavicipitaceae* (Lindau) O. Eriksson, contain the most numerous invertebrate pathogens. The *Clavicipitaceae* contains *ca.* 500 species (Hawksworth *et al.*, 1995). About 75% of these fungi are pathogenic on the invertebrates (most often on insects). The insect hosts include Coleoptera, Homoptera, Lepidoptera, Hymenoptera, Diptera, Isoptera, Heteroptera, Orthoptera and Odonata. They are also pathogenic on Arachnida (spider, mites and ticks). As well as the invertebrate pathogens, the Clavicipitaceae also contains important plant pathogenic genera such as *Balansia* Speg., *Claviceps* Tul., etc. The genus *Hypocrella* contains less than fifty species and is known to only infect the immature stages of the insect order Homoptera and is further limited within that order to only two families of scale insects, Aleyrodidae and Coccidae (Evans and Hywel-Jones, 1990). Rossman (1983) indicated that over 40% of hypocrealean teleomorphs are known to have anamorphs. The genus *Hypocrella* has few species and a limited number of anamorph forms, mainly from the genus *Aschersonia*. *Hypocrella scutata* and *Hypocrella schizostachyi* were studied by Petch (1921). He reported that there are no records of the anamorph of these species. Recent

work by Hywel-Jones and Evans (1998) described the anamorph of *H. schizostachyi* and mentioned that it is not a true *Hypocrella*. No record of an anamorph of *H. scutata* has been published.

Mycological taxonomy is a constantly changing subject. There are insufficient morphological characters that can be used to draw significant taxonomic conclusions in fungi and also, the evolution of most morphological characters are difficult to trace. Moreover, some characters are possibly acquired by parallel or convergent evolution. Morphology alone is no longer sufficient for the classification of fungi. The addition of molecular phylogenetics has revolutionised the fungal classification. The molecular techniques are useful tools for determining the relationship between known and unknown isolates.

Few studies have been undertaken at the molecular level for *Hypocrella*. The evolutionary relationships of *Hypocrella* are unknown. The taxonomic criteria for separating species of *Hypocrella* are based mainly on host and morphology (Petch, 1921; Mains, 1958; Evans and Samson, 1982; Kobayasi, 1982; Ito and Hirano, 1997). Key morphological characters for the determination of species of *Hypocrella* are poorly defined and often conflicting or overlapping.

1.2 Outline of the research

The *H. scutata* and *H. schizostachyi* were collected from various survey sites around Thailand and isolated into pure culture. The culture collections were kept at the Department of Microbiology, Prince of Songkla University, Songkhla and the BIOTEC culture collection, National Center for

Genetic Engineering and Biotechnology. The macroscopic and microscopic structures of the fungi were observed and photographed. The effect of different media and temperatures on the growth of fungi was studied. The fungal DNA was extracted from the culture mycelia and amplified. The PCR products were purified and DNA was sequenced at the BIOTEC Service Unit. The phylogenetic trees were constructed from partial 28S rDNA gene using standard computer software. The phylogenetic relationship between *H. scutata*, *H. schizostachyi* and *H. discoidea* (type species) was determined.

1.3 Benefits of the research

The study on the problematic *Hypocrella* can add the data to the Thai fungal database. The evolutionary relationship in the genus *Hypocrella* will be shown.

The study on effect of media and temperature will provide us the data which will be useful for further studies on searching of biomass production, bioactive compounds and antibiotic screening in the future. The ecological distribution data will provide the information about the possibility of *Hypocrella* occurrence elsewhere in natural habitats.

1.4 Objectives of the research

- 1) To collect *H. scutata* and *H. schizostachyi* from southern and the other areas in Thailand.
- 2) To isolate *H. scutata* and *H. schizostachyi* into pure culture and maintain in the culture collection.
- 3) To study the morphological structures, the effect of media and temperature on the growth

- 4) To study the ecological distribution of *H. scutata* in nature.
- 5) To determine the relationship of *H. scutata* and *H. schizostachyi* with the type species, *H. discoidea* (B. and Br.) Sacc., by phylogenetic analysis.