CHAPTER I

INTRODUCTION

The discovery, development and clinical use of antibiotics during the 20th century decreased substantially the morbidity and the mortality from microbial infection (1). The emergence of opportunistic pathogens, especially in immunosuppressed hosts, and the widespread use of antibiotics which causes extensive microbial resistance, have resulted in serious problems in treating infectious diseases (2). With the rise of antibiotic drug resistance and unusual microbial threats, the need for more effective antimicrobial agents is increasing (3).

By 1983 about 64,000 fungal species were known, and since then about 1,500 species have been described and named as new discovery each year. However, it is usual that about 60% of such discoveries are fungi that have already been found and described under different names. Hence, the number of fungi known is probably increasing at about 600 species a year, suggesting that about 70,000 species will be known by 1994 (4). Currently, approximately 72,000 species of fungi have been described, and Hawksworth conservatively estimated that there are 1.5 million fungal species in this world (5). Filamentous fungi have traditionally been very fertile source of natural products, many of which are useful prototypes for pharmaceutical agent (6). In London, in 1929, Dr. Alexander Fleming observed the inhibition of growth of the bacterium *Staphylococcus aureus* in the area surrounding the colony of a mold that had contaminated a petri dish plate. The mold was identified as *Penicillium notatum*, and the active compound, which was isolated a short time later, was named penicillin. The success of penicillin led to the screening of thousands of soil microorganisms for antibiotic production. Griseofulvin, from *Penicillium griseofulvum*, was isolated in 1939. Fusidic acid, from *Fusidium coccineum*, is a steroid active against Gram-positive bacteria, and is useful against strains that have acquired a high resistance to beta-lactam antibiotics. Fumagillin, from *Aspergillus* sp., is active against protozoa. A novel antifungal antibiotic, sordarin from the Ascomycete *Sordaria araneosa*, is showing promise for development as an antifungal drug (7).

It is believed that fungi are underexploited source of novel compounds. In addition, fungal metabolites are often species specific (8). The study of unusual microorganisms as a source of novel antimetabolites is one tread of several trends, which are largely responsible for the continuing discovery of novel antimicrobial activities, often with unsuspected application potential (5). Endophytic microbes are an intriguing group of organisms associated with various healthy tissues of terrestrial and some aquatic plant (4). Most plants contain microorganisms –typically fungi– that supply their hosts. Practical application of endophytes includes potential biological control agents, sources of novel metabolites for medicine, plant protection and industrial use. There are perhaps as many as 500,000 distinct varieties of higher plants. The varieties of fungi associated with such plants are estimated to be at least as high, suggesting that the extent of this biodiversity is enormous. These microorganisms represent a pharmaceutically valuable but virtually untapped resource (3).

In order to search for an unexploited source of antimicrobial compounds, endophytic fungi were isolated from Thai medicinal plants reported to have antimicrobial activities. Antibacterial and antifungal activities of the fungal endophyte isolates were screened by dual-culture agar diffusion assay. As previously reported that amphotericin B-resistant strains of *Candida albicans* and *Saccharomyces cerevisiae* were used in general *in vitro* preliminary screening of natural products for new antifungal agent (9), this approach was also applied to this study. Microbial strains with low susceptibility to natural antibiotics were selected by gradient plate technique (10). They were used as test microorganisms in screening the active endophytic fungus isolates obtained in this study and preserved in the culture collection. The endophytic fungus isolates exhibiting activities against low susceptible test microorganisms were grown in liquid medium. Minimum inhibitory concentrations of the crude extracts obtained from culture broth were determined by broth microdilution assay. Taxonomy of the active isolates was done by conventional method based on fungal morphology. This study may lead to selection of endophytic fungus isolates that are a potential source of novel antimicrobial agent(s).