]CHAPTER ONE

INTRODUCTION

Screening of microorganisms for the production of novel antibiotics has been intensively practiced for many years by scientists. Of all the known microbes, actinomycetes represent a major source of biologically active metabolites such as antibiotics, agrochemicals, enzymes, immunosuppressants, antiparasitics and anticancer agents (Phipps *et al.*, 2004). In the screening of actinomycetes which are remarkably prolific sources of structurally diverse secondary metabolites, include many isolates that possess pharmaceutically relevant biological activities (Oskay *et al.*, 2004: Jensen *et al.*, 2006).

However, as the frequency of novel bioactive compounds discovered from terrestrial actinomycetes reduces with time, industrial programs are increasing the screening of actinomycetes from various environments for novel metabolites. Furthermore in recent years, the production of new compounds from common soil-derived actinomycetes has decreased significantly (Fenical, 2007), thus providing incentive to broaden the search for new metabolites to include actinomycetes that occur in the sea.

The importance of marine sources for the discovery of novel natural products with a pharmaceutical potential has been shown during the last decade and was highlightened in various excellent review articles (Fiedler *et al.*, 2005; Boonlarppradab *et al.*, 2008; Ye *et al.* 2009; Ogunmwonyi *et al.*, 2010). In fact, according to Boonlarppradab *et al.* (2008), marine actinomycetes have become the major contributors for novel natural products.

The recognition of increased diversity and the success of marine natural products have promoted the marine environment as a source of novel chemical diversity for drug discovery (Fiedler *et al.*, 2005: Hong *et al.*, 2009). Novel metabolites, such as carsinostatic substances, aminoglycoside antibiotics, cytotoxic substances and other bioactive compounds were isolated from marine actinomycetes (Imada *et al*., 2010). Thus, marine environment is expected to be a new screening target for finding novel bioactive compounds. Kurtboke (1996) explained that the screening for novel metabolites and evaluation of new bacterial taxa is necessary for development of improved compounds for chemical modification.

Among the actinomycetes, *Streptomyces* spp. has been the most abundant source of all types of antibiotics and are producers of a number of useful bioactive compounds (Hayakawa, 2008). Some studies had been done to search for antifungal activity from marine-derived *Streptomyces* spp. Woo *et al.* (2002) reported a discovery of a novel antifungal protein from a marine culture supernatant of *Streptomyces* strain AP77. It is specific for *Pythium porphyrae*, a red rot disease causative agent in *Porphyra* spp. Toxicity tests for both in vitro and in vivo of this protein demonstrated that this novel antifungal protein only showed toxicity against *Pythium porphyrae*. This discovery indicated that *Streptomyces* strain AP77 might be implemented as source of gene for safe transgenic *Porphyra* breeding for tolerance to infection of *Pythium*.

There were also research which reported interesting secondary metabolites compound discovery from marine-derived *Streptomyces* spp. Phipps *et al*. (2004) reported that four new anthracycline derivatives were isolated from a New Zealand marine-derived streptomycete which were cytotoxic against P33 murine leukaemia cell line. Piperazimycin which is a cytotoxic hexadepsipeptides (Miller *et al*., 2006) and salinamides an anti-inflammatory agent (Moore *et al*., 1999) were also discovered from marine-derived *Streptomyces* spp.

At the University of Malaya, researchers have turned to the seas to tap their vast and unexploited microbial diversity and in particular the actinomycetes which are prolific producers of bioactive agents. Recent research have revealed that Malaysian mangrove mud, rhizosphere of mangrove plants, near shore sediments, soft coral and sponge samples contain diverse actinomycete population (Vikineswary *et al*., 2003; Ismet *et al*., 2004).

Research has shown that true marine actinomycetes do exists (Weyland, 1969; Lam, 2006). Further seaweeds, corals, nudibranchs and sponges were among the macroorganisms investigated for their actinomycete inhabitants (Tan *et al.*, 2004: Vikineswary, 2005). In Malaysia, the marine actinomycetes niche remains virtually unexplored and is a promising resource for the biotechnological applications including drug discovery (Vikineswary, 2005). Kavithambigai *et al.* (2001) and Tan *et al*. (2001) reported on the isolation of indigenous actinomycetes from marine organisms. Lam (2006) also stated that isolation of novel actinomycetes from samples collected at different marine environments and habitats had been in progress. There are various new secondary metabolites produced by these marine actinomycetes, which possess biological activities and are potential to be developed as therapeutic agents. However, marine actinomycetes are still underexploited source for the discovery of novel secondary metabolites (Lam, 2006). From the view of many reports on actinomycetes isolates from marine environment producing various bioactive metabolites, a study was carried out to examine the marine *Streptomyces* spp. isolated from Tioman Island, Malaysia.

In order to explore the potential of discovering novel strains and/or bioactive compounds, bioactivity of the isolates against *Candida albicans* and *Schizosaccharomyces pombe* were examined. These fungi could be indicators for antifungal (Jayaraman *et al.*, 2008) and antitumor agent (Cassone *et al.*, 1982),

especially *S. pombe* as it is used as a model organism in molecular and cell biology. The sequence of *S. pombe* genome was published in 2002, becoming the sixth model eukaryotic organism whose genome has been fully sequenced. This fungi has become a gold medium in research for DNA damage and the process of DNA replication as identification of various genes homologous to human disease genes is being done.

Houghton & Raman (1998) explained that it is obviously important to know the amount of a particular active substance or substances present in an organism in order to assure the degree of efficacy and safety. But this can only be implemented by identification of the active ingredient/s. Charlotte *et al.* (2002) screened volatile production capacity of twenty six *Streptomyces* spp. on yeast starch agar. Characterization by gas chromatography –mass spectrometry analysis was done and they identified fifty three compounds as terpenoids, among which eighteen could be identified. Among the volatile compounds were alkanes, alkenes, alcohols, asters, ketons, sulfur compounds and isoprenoid compounds. Ethyl acetate extract of ten most potent antimicrobial *Streptomyces* spp. bioactive compounds were characterized partially by IR spectra analysis (Ogunmwonyi *et al.,* 2010). This study revealed the possible presence of terpenoids, long chain fatty acids and secondary amine derivatives in the extracts.

OBJECTIVES

The objectives of this study were to:

i) study the antifungal potential of selected marine and marine-derived *Streptomyces* spp.

ii) characterize the potential strains using morphological, biochemical and molecular methods.

iii) identify the active ingredients of the potential strains.