## CHAPTER I

## INTRODUCTION

Antibiotics are secondary metabolites. Most of them are produced from microorganisms in the group of actinomycetes. They have been extensively studied and have been used in agriculture, food and nutrition, and especially medical science. Actinomycetes have been described as the greatest source of antibiotics since Waksman introduced *Streptomycetes* into his systematic screening program for new antibiotics in early 1940s. They have provided about two-thirds (more than 4000) of the naturally occurring antibiotics discovered, including many of those important in medicine, such as aminoglycosides, anthracyclines, macrolides and tetracyclines (Goodfellow, 1988).

The most useful in the chemical discoveries and biological activities reported from the actinomycetes are terrestrial actinomycetes. Actinomycetes are numerous and widely distributed not only in soil but also in a variety of other habitats including composts, river mud, and lake bottoms. Organisms belonging to the group of actinomycetes are *Streptomyces, Micromonospora, Microbispora, Actinomadura, Actinomyces, Actinoplanes* and *Streptoverticillium* and others. They have been searched for antibiotic production. The best-known genus of actinomycetes is *Streptomyces*, which is one of the bacteria most commonly isolated from soil (Tortora *et al., 1995).* Since the frequency of novel bioactive compounds discovered from terrestrial actinomycete decreases with times, academic and industrial programs are increasingly screening actinomycetes from diverse environments for their ability to generate new metabolites such as shore environment.

Streptomyces is in the family Streptomycetaceae of the order Actinomycetales. Strains in this genus have substrate mycelium and aerial mycelium, vegetative hyphae averaging 0.5-2.0  $\mu$ m in diameter. Substrate mycelium color and soluble pigment are

blue, dark, green, red-orange and violet. Spore surface have been characterized by the terms smooth, hairy, spiny, warty or rugose. Spore chain morphology is spirales, rectiflexibiles, retinaculiaperti. Spore color is blue, gray, green, red, violet, white and yellow. Walls contain L-diaminopimelic acid. Glucose, glycerol, mannitol, fructose and sucrose are present in cell hydrolysates. Colonies on agar media are granula, powder, velvety or floccuse. Optimal pH averaging 6.5-8.0. Growth occurs normally between 25°C and 30°C but not above 50°C (Goodfellow, 1988).

The strains of *Streptomyces* are valuable because they produce most of commercial antibiotics. Examples include chloramphenicol produced by *Streptomyces venezuelae*, cycloheximide produced by *Streptomyces griseus*, cycloserine produced by *Streptomyces orchidaceus*, kanamycin produced by *Streptomyces kanamyceticus*, lincomycin produce by *Streptomyces lincolnensis*, neomycin produced by *Streptomyces fradiae*, nystatin produced by *Streptomyces nourisei*, streptomyces nouced by *Streptomyces griseus*, tetracyclin produced by *Streptomyces rimosus* (Oki, 1994), luisol A and B produced by *Streptomyces* sp. CNH-370 and arenaric acid produced by *Streptomyces* sp. CNH-248 (Cheng, Jensen and Fenical, 1999)

The misuse of antibiotics in the past decade has led to increasing number of resistant strains. Thus there is an immediate need for researchers to study and develop new antimicrobial substances to replace the current antibiotics which effectiveness is reducing. Despite enormous effort in searching natural substance produced from *Streptomyces*, we believe that there is still much more to be found from these marine bacteria collected from new sources like Samed Island. In this study, Samed Island is chosen because of its environment is still preserved from man-caused pollution, and has not been studied by other researchers in Thailand as shown in all documented records.

2

Samed Island is T-shaped island near Rayong province, Thailand. Most part of the island is covered by mangrove forest, with a modest degree of development mostly for tourism industry. The soil is full of decayed organic matter origin from the leaf encouraging a grow environment of microorganism (สนิท, 2542). There is a variety of soil such as marine sand, loam and soil, with a pH value ranging from neutral to alkaline (กรมทรัพยากรธรณี, 2535).

We study the secondary metabolites of actinomycetes strains isolated from the shore environments where its soils contain highest organic matters. The highlight of the study comes when one of the isolates, coded as PC 4-3, which had been isolated from soil sample collected from Samed Island showed high antimicrobial activity and believe to be a new strain. It is determined to be a genus of *Streptomyces* based on morphological and chemotaxonomic characteristics. Preliminary bioactivity screening on the ethyl acetate extract of the fermentation broth from this actinomycetes strain showed antimicrobial activity in a disc diffusion assay against *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 and *Candida albicans* ATCC 10231 with 28.2, 22.0 and 12.0 mm zone of inhibition at 0.5 mg/6 mm disc, respectively.

The objectives of this study are:

- 1. To isolate and screen the actinomycetes from soil at Samed island which produced antimicrobial substances.
- 2. To identify and characterize the selected actinomycetes.
- 3. To compare the fermentation and antimicrobial activities of the selected actinomycetes.
- 4. To isolate antimicrobial substances from the selected actinomycetes.