UNIVERSITI TEKNOLOGI MARA

SCREENING FOR β-LACTAMASE INHIBITORS FROM MANGROVE SOIL ACTINOMYCETES

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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on 18th June 2015 to conduct the final examination of Suhaidi bin Ariffin on his Doctor of Philosophy thesis entitled "Screening for β-Lactamase Inhibitors from Mangrove Soil Actinomycetes" in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The panel of Examiners was as follows:

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ABSTRACT

Marine-derived actinomycetes possess distinct and complex metabolic capabilities, resulting in the wide diversity of their secondary metabolites in terms of chemical structure and biological activity. The bioactive compounds of actinomycetes isolated from mangrove soils are reported to possess various biological activities such as antimicrobial, cytotoxicity, anticancer and antioxidant. The aims of this study are to isolate and characterize diverse populations of marine actinomycetes from mangrove soils, to identify selected morphologically distinct isolates by using 16S rRNA sequence analysis, to screen their potential biological activities as antimicrobial as well as β-lactamase inhibitor agents, and to elucidate the structure of selected bioactive compounds. A total of 73 actinomycete strains were isolated from 11 mangrove locations in Malaysia. Of these, morphological observations and 16S rRNA sequence analysis indicate the presence of representative species from at least 3 genera *Streptomyces*, *Pseudonocardia* and *Saccharomonospora*. The majority of the isolates belong to genus *Streptomyces*. It was found that the most productive medium for isolation is starch casein nitrate agar (SCNA). It was also found that addition of 3% sodium chloride (NaCl) improved the isolation rate of mangrove actinomycetes. Disc diffusion assay showed that 9.6% (direct broth culture) and 8.2% (ethyl acetate extract) of these isolates were able to produce antimicrobial compounds active against *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans* as well as *Saccharomyces cerevisiae*. Hexane extracts however exhibited no activity. A modified microdilution plate assay showed that 16.4% (ethyl acetate extract) of these strains were able to generate antimicrobial compounds active against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Candida albicans*. A rapid screening assay for β-lactamase inhibitors activity by resazurin microdilution plate assay was developed and showed that 4.9% of actinomycete isolates were able to produce inhibitor compounds against *Staphylococcus aureus* ATCC 43300. The KMS1 isolate was chosen as a lead candidate because this strain showed consistent production of antimicrobial and inhibitor active compounds throughout the screening experiments. Nuclear magnetic resonance (NMR) spectroscopy analysis showed that the active compound (KMS1-2B) was 4-hydroxybenzoic acid.

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CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF STUDY

Actinomycetes are filamentous and sporulating Gram-positive bacteria characterized by a complex life cycle belonging to the phylum Actinobacteria. Actinobacteria are widely distributed in both terrestrial and aquatic ecosystems mainly soil. They are known as degrader and also as decomposer because they recycle the nutrients associated with intractable polymers such as chitin, keratin and lignocelluloses in soil biodegradation and humus formation (Sharma, 2014; Stach and Bull, 2005; McCarthy and Williams, 1992; Goodfellow and Williams, 1983). These activities resulted in the production of volatile substances like geosmin which is responsible for the 'wet earth odour' characteristic (Wilkins, 1996) and exhibited diverse physiological and metabolic properties, for example the manufacture of extracellular enzymes (Schrempf, 2001; McCarthy and Williams, 1992). They are all reported to have high guanine and cytosine content in their DNA and identified as the most economically and biotechnologically valuable bacteria that provide many important bioactive compounds (Gulve and Deshmukh, 2012).

Natural products are chemical compounds originated from living organisms such as plants, animals as well as microorganisms. They are known to be the most consistently successful source of drug leads. Traditionally, higher plants used to be the most productive sources of drugs from nature (Ginsburg and Deharo, 2011) and the uses of medicinal plants are well reported (Pan et al., 2014). Microorganisms heralded the era of natural products after the discovery of penicillin in 1929 by Sir Alexander Fleming and started to be used clinically in the 1940s (Peláez, 2006). Since then, the discovery of antibiotics from microbes has been intensively studied. After a few decades, the discovery of secondary metabolites from microorganisms has been slowly decreased. The researchers started to focus on synthesizing existing compounds. However, due to the increase in resistance to current antibiotics and other antimicrobial compounds (Spížek et al., 2010), the search of new compounds particularly from microorganisms is regaining favour among scientists.