



**MOLECULAR IDENTIFICATION OF *Streptomyces* spp. ISOLATED FROM
AGRICULTURAL SOIL AT PUNCAK ALAM, KUALA SELANGOR,
MALAYSIA**

By

FATIMAH ZAHRAH BINTI MUSTAFFA

**Thesis submitted in Partial Fulfillment of the Requirement for the Bachelor of
Medical Laboratory Technology (Honours) Faculty of Health Sciences,
Universiti Teknologi MARA**

2015

DECLARATION

I, hereby declare that this thesis is based on my original work. I also declare that this thesis has not previously or concurrently submitted by any other degree student at UiTM or other institutions.

JULY 2015

Fatimah Zahrah Binti Mustaffa
2011243618

ABSTRACT

Soil in ecological field was widely used for study and *Actinomycetes*, mainly *Streptomyces*, were exist in these environments and a good sources of bioactive metabolites. This genus was Gram-positive bacteria with filaments in branches, ubiquitous, aerobic, soil-like odour, and high G+C content. The aims of this study were to isolate *Streptomyces spp.* from the agricultural soil in Puncak Alam, Kuala Selangor, Malaysia. The isolates were examined for morphological characteristics, antimicrobial activity and genus confirmation. Soil samples were pre-heated and cultured aerobically on ISP-2 agar for 14 days at 37 °C. Primary screenings of the isolates for antimicrobial activity were conducted by using cross streak method against *Bacillus subtilis*, *Bacillus cereus*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Escherichia coli*. DNA of the isolates was extracted using boiling method and 16S rDNA gene was amplified by PCR for genus confirmation. A total of four isolates of *Streptomyces spp.* were identified based on colour of the aerial mycelium showed as grey, yellow, brown and white. Isolate with brown colour groups, C4 displayed moderate inhibition against *Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus*. No inhibition of C4 against Gram negative bacteria. Two isolates, C1 (yellow group) and C5 (orange group) showed weak inhibition against *Bacillus subtilis* and *Bacillus cereus*. Whereas, no inhibition of all bacteria tested for grey group isolated namely, C3. DNA fragment with 520 bp of the amplified 16s rDNA gene were observed on the Agarose gel. The blast sequenced of C1 isolate was identified as *Streptomyces davawensis* JCM 4913, *Streptomyces pratensis* ATCC 33331, and *Streptomyces griseus subsp. griseus* NBRC 13350 DNA with 99% similarity. C3 isolate also have 99 % similarity with *Streptomyces albus* J1074 and *Streptomyces coelicolor* A3 (2). Meanwhile, C4 isolate was identical to *Streptomyces bingchengensis* BCW-1 and *Streptomyces cattleya str. NRRL 8057* with similarity of 98%. C5 isolate has 99 % similarity with *Streptomyces collinus* Tu 365 and *Streptomyces roseochromogenus subsp. oscitans*. As a conclusion, the presence of the *Streptomyces spp.* was identified from this soil and has significant antimicrobial activities against tested bacteria.

TABLE OF CONTENT

Title page.....	i
Declaration.....	ii
Abstract.....	iii
Acknowledgement.....	iv
List of Contents.....	v-vii
List of Figures.....	viii
List of Tables.....	ix
List of Abbreviations.....	x
List of Plates.....	xi
Chapter 1: Introduction.....	1
1.1. Study Background.....	1-2
1.2. Problem Statement.....	6
1.3. Research Objective.....	6
1.4. Research Hypothesis.....	7
1.5. Study Background.....	8
Chapter 2: Literature Review.....	9
2.1. Human Pathogens.....	9
2.2. Pathogenic Bacteria.....	10
2.3. General Overview on <i>Streptomyces</i>	13
2.4. Distribution of <i>Actinomyces</i> in nature.....	22
2.5. Important of <i>Actinomyces</i>	23
2.6. Molecular Detection Methods.....	28
2.7. Actinomycetes related researches in Malaysia.....	29

CHAPTER 1

INTRODUCTION

1.1.1 Study Background

Antimicrobial agents are less effective nowadays due to many antibiotic resistances are increasingly widespread (Obi *et al.*, 2007). The rising of resistance of human pathogen populations to current available antibiotics will cause major effect to the business of pharmacological and medical community (Saadoun and Gharaibeh, 2003). The continuous occurrence of pathogens that are resistant to numerous antibiotics affecting the difficulty of many therapy or else simple infections, needed for the continuous research for the evolution of new antibiotics (Saadoun *et al.*, 2006). Therefore, the availability of novel bioactive compounds and new lead structures is very crucial for a continuing source of therapeutics to fight the new emergence of resistant pathogens (Vikineswary, 2004). Most of the antibiotics on the market today are produced from microbial natural products (Pelàez, 2006). Screening of microorganisms for the production of new antibiotics has been concentratedly pursued for many years by scientists (Oskay *et al.*, 2004). The novel antimicrobial agents with a large spectrum of activity against these multi-resistant pathogens are seriously sought, and one of the way is to maximise the screening activity of the *Actinomycetes* as major antibiotic producing organism (Saadoun *et al.*, 2006).

Soil in ecological field is widely used for the study of bacteria with the ability to produce good sources of bioactive metabolites (Thakur *et al.*, 2007). *Actinomycetes* constitute of a large phylogenetic group of Gram-positive bacteria (Thirup *et al.*, 2001). *Actinomycetes*, mainly *Streptomyces*, are ubiquitous and abundant in soil and tend to be well distributed through the surface-soil mass like many fungi (Singh and Mehrotra, 1980). *Actinomycetes* are widely recognized as industrially important microorganisms because of