

TABLE OF CONTENTS

| Page | |
|---|-------------|
| | Page |
| ABSTRACT..... | i |
| ABSTRAK..... | ii |
| ACKNOWLEDGEMENT..... | iii |
| CONTENTS..... | iv |
| LIST OF TABLES..... | viii |
| LISTS OF FIGURES..... | xii |
| ABBREVIATION..... | xvi |
| LIST OF APPENDECIS..... | |
| | |
| CHAPTER ONE: INTRODUCTION..... | |
| 1.1. Soil microbiology..... | 1 |
| 1.2. Soil microorganism..... | 3 |
| 1.3. Soil bacteriology..... | 3 |
| 1.4. Soil bacteria..... | 4 |
| 1.5. Indigenous microorganisms (IMO) | 6 |
| 1.6. Indigenous microorganism cultivation..... | 8 |
| 1.7. Indigenous microorganisms in Natural Farming..... | 9 |
| 1.8. Literature review..... | 11 |
| 1.9. Indigenous microorganisms soil research in Malaysia..... | 12 |
| 1.10. Biosurfactant..... | 14 |
| 1.11. Problem statement and importance of this study..... | 16 |

| | | |
|-------|-------------------------------|----|
| 1.12. | Objectives of this study..... | 17 |
|-------|-------------------------------|----|

CHAPTER TWO: MATERIALS AND METHODS

A. MATERIALS

| | | |
|-------|--|----|
| 2.1 | Soil sample collection..... | 19 |
| 2.2 | Bacterial cultures collection..... | 19 |
| 2.3 | Experimental animals..... | 20 |
| 2.4 | Chemicals, reagents, solvents and antibiotics..... | 20 |
| 2.5 | Media used..... | 21 |
| 2.5.1 | Brain Heart Infusion (BHI) broth..... | 21 |
| 2.5.2 | Brain Heart Infusion (BHI) agar..... | 21 |
| 2.5.3 | Mueller-Hinton (MH) agar medium..... | 21 |
| 2.6 | Solutions for plasmid DNA extractions..... | 21 |
| 2.6.1 | Sucrose-EDTA-Tris (SET) buffer, pH 8.0..... | 21 |
| 2.6.2 | Alkaline SDS (lysing) solution (1%)..... | 22 |
| 2.6.3 | Sodium acetate, 3 M, pH 4.8..... | 22 |
| 2.6.4 | Potassium acetate, 5 M (Sambrook et al., 1989)..... | 22 |
| 2.7 | DNA electrophoresis..... | 23 |
| 2.7.1 | Agarose gel (0.7%)..... | 23 |
| 2.7.2 | Tris-borate-EDTA (TBE) buffer (Peacock & Dingman, 1967)..... | 23 |
| 2.7.3 | Ethidium bromide (10 mg/ml)..... | 24 |
| 2.7.4 | Gel loading buffer (tracking dye)..... | 24 |
| 2.7.5 | Tris-EDTA (TE) buffer, pH 8.0..... | 24 |

B. METHODS

| | | |
|----------|---|----|
| 2.8 | Washing procedures and sterilization..... | 25 |
| 2.9 | Storage and maintenance of bacterial cultures..... | 25 |
| 2.10 | Bacterial colony screening and isolation..... | 26 |
| 2.11 | Colony morphology..... | 26 |
| 2.11.1 | Morphological Analysis..... | 26 |
| 2.12 | Bacterial Gram identification and differentiation..... | 27 |
| 2.12.1 | Gram staining..... | 27 |
| 2.12.2 | Aminopeptidase-strip test..... | 27 |
| 2.12.3 | Alkaline lysis solution..... | 28 |
| 2.13 | Haemolytic activity of isolated soil bacteria..... | 28 |
| 2.14 | Antibiotic susceptibility testing of isolated soil bacteria..... | 28 |
| 2.14.1 | Standardisation of soil bacterial broth culture..... | 29 |
| 2.14.2 | Inoculation of the susceptibility test media..... | 29 |
| 2.14.3 | Interpretation of zone inhibition..... | 29 |
| 2.15 | Preparation of soil bacterial filtrates..... | 30 |
| 2.16 | Biosurfactant activity assays – Drop collapsing test with milky activity... | 30 |
| 2.17 | Animal study..... | 31 |
| 2.17.1 | Mice lethality test..... | 31 |
| 2.17.2 | Mice toxicity test..... | 31 |
| 2.18 | Detection of plasmid DNA from soil bacteria..... | 32 |
| 2.18.1 | DNA extraction using kit..... | 32 |
| 2.18.2 | Small scale plasmid extraction..... | 33 |
| 2.18.2.1 | Alkaline lysis for Gram negative bacteria..... | 33 |

| | | |
|----------|---|----|
| 2.18.2.2 | Plasmid isolation for Gram positive bacteria..... | 33 |
| 2.19 | Agarose gel electrophoresis..... | 34 |

CHAPTER THREE: RESULTS

| | | |
|-------|--|----|
| 3.1 | Bacteriological characteristics of unknown colonies isolated from IMO4 soil | 36 |
| 3.1.1 | Colony morphology..... | 36 |
| 3.1.2 | Bacterial Gram identification..... | 41 |
| 3.2 | Preliminary screening of biosurfactant-producing bacteria..... | 47 |
| 3.3 | Antibiotic test..... | 52 |
| 3.4 | Presence of plasmid in soil bacterial isolates..... | 59 |
| 3.5 | Mouse pathogenicity studies on unknown soil bacterial isolates..... | 61 |
| 3.5.1 | Virulence of soil bacterial isolates in mice..... | 61 |
| 3.5.2 | Toxicity of cell-free supernatant in mice..... | 61 |

CHAPTER FOUR: DISCUSSION AND CONCLUSION

| | | |
|-------|--|----|
| 4.1 | Colony morphology and characterization of IMO4 soil bacteria..... | 64 |
| 4.2 | Bacterial gram differentiation using Gram staining, cell lysis and aminopeptidase-strip test..... | 65 |
| 4.3 | Screening for biosurfactant activity..... | 68 |
| 4.3.1 | Haemolysis assay..... | 69 |
| 4.3.2 | Drop collapse assay and emulsification ability of soil bacteria...71 | |
| 4.4 | Other tests – Antibiotic resistance, plasmid presence and | |

| | |
|-------------------------------------|--------------|
| pathogenicity of soil bacteria..... | 72 |
| 4.5 Conclusions..... | 73 |
| 4.6 Future study..... | 74 |
| References | 76-95 |