

**USE OF MICROORGANISMS AS BIOINDICATORS FOR DETECTION OF  
HEAVY METALS**

**By**

**FAZURIANA BINTI AHMAD**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirement for the Degree of Master of Science**

**May 2006**

*Dedicated to my beloved family and friends.....*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**USE OF MICROORGANISMS AS BIOINDICATORS FOR DETECTION OF HEAVY METALS**

By

**FAZURIANA BINTI AHMAD**

**May 2006**

**Chairman : Professor Mohd Arif Syed, PhD**

**Faculty : Biotechnology and Biomolecular Sciences**

In this study, soil bacteria were isolated and were then screened for their sensitivity to heavy metals. This study employs the tetrazolium dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) where bacteria reduced the dye, causing the dye to precipitate and to become intensely coloured. In the presence of heavy metals, the reduction will be inhibited and become colourless. A total of 250 bacterial isolates were successfully obtained from 10 different locations in Peninsular Malaysia which were then screened with six selected heavy metals in the presence of common divalent cations such as calcium and magnesium at the highest concentration of 25 mg/L and 50mg/L respectively using a MTT assay. An isolate designated as isolate SC27 at 8 hours growth and isolate S8 at 12 hours growth were found to be most sensitive to mercury and silver respectively. The IC<sub>50</sub> (50% inhibitory concentration) of mercury and silver are 0.2698 mg/L and 0.073 mg/L respectively after data was analyzed using the Graphpad Prism™ version 4.0 software. The assay was found to be unaffected by interference from other tested xenobiotics. Preliminary field study tests showed the ability of these two bacterial

isolates to detect mercury and silver after comparison with AAS analysis. Isolate SC27 was identified as Uncultured bacterium strain Dr.Y13 (DQ 226214) which is related to *Enterobacter* sp. using Microbact™ kit and was confirmed using 16S rRNA gene analysis while isolate S8 was identified as *Serratia* sp. with 90.79 % similarity using the Microbact™ kit.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PENGGUNAAN MIKROORGANISMA SEBAGAI BIOINDIKATOR UNTUK MENGESAN LOGAM-LOGAM BERAT**

Oleh

**FAZURIANA BINTI AHMAD**

**Mei 2006**

**Pengerusi : Profesor Mohd Arif Syed, PhD**

**Fakulti : Bioteknologi dan Sains Biomolekul**

Dalam kajian ini, bakteria tanah dipencilkan dan kemudian disaringkan untuk melihat tahap kesensitifan terhadap logam berat. Kajian ini menggunakan pewarna tetrazolium MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) di mana bakteria ini akan menurunkan pewarna ini menyebabkan pewarna termendak dan sebatian menjadi berwarna. Dengan kehadiran logam berat, penurunan ini akan direncat dan sebatian menjadi tidak berwarna. Sebanyak 250 isolat bakteria berjaya diperolehi dari 10 kawasan yang berlainan di Semenanjung Malaysia dan seterusnya disaring dengan enam logam berat yang dipilih dengan kehadiran kation divalen seperti kalsium dan magnesium pada kepekatan 25 mg/L dan 50 mg/L dengan menggunakan asai MTT. Isolat yang dikenali sebagai isolat SC27 pada pertumbuhan 8 jam dan isolat S8 pada pertumbuhan 12 jam didapati masing-masing sensitif kepada merkuri dan argentum.  $IC_{50}$  (50% kepekatan perencat) merkuri dan argentum masing-masing pada 0.2698 mg/L dan 0.073 mg/L setelah data dianalisa menggunakan perisian Graphpad Prism™ versi 4.0. Dalam kajian ini didapati asai

ini tidak dipengaruhi oleh lain-lain xenobiotik yang dipilih. Kajian percubaan awal terhadap sampel air menggunakan asai ini menunjukkan kedua-dua isolat bakteria ini berupaya untuk mengesan merkuri dan argenterium setelah dibandingkan dengan analisa AAS. Isolat SC27 dikenalpasti sebagai Uncultured bacterium strain Dr. Y13 (DQ 226214) dengan menggunakan analisa molecular filogenetik 16S rRNA walaupun kit Microbact™ mengelaskan bakteria ini sebagai *Enterobacter* sp. Isolat S8 pula dikelaskan kepada *Serratia* sp. dengan kepercayaan sebanyak 90.70% dengan menggunakan kit Microbact™.

## ACKNOWLEDGEMENTS

First and foremost, I am most grateful to Allah S.W.T. for enabling me to come this far. Life has its ups and downs, but in time of needs, You have always been there for me. And in one way or another, I have always managed to go through. I could not have done it without Your help and blessings.

After what I thought to be the most challenging time of my master years, I managed to summarize it all into this black book. And there are many people out there I most thank for that. Firstly, my deepest gratitude to my lovable supervisor, Prof. Mohd. Arif Syed for his invaluable guidance throughout the completion of this project. Special thanks also dedicated to my co-supervisor, Dr. Mohd Yunus Abd. Shukor, who has always, always been there to guide, teach and support me throughout my project. He has made me a better person in many, many ways. I'm sure anybody who has had the honor to know him will say the same. I pray that this respectful man will make it big out there, which I'm sure is not very far ahead.

I would also like to take this opportunity to thank all the wonderful people in the lab, who are Abg. Ariff, Kak Sue, Kak Ilah, Surini and Sim. You guys are the most labmates anyone can ask for. Not forgetting all lab members 204 as well as the undergraduates of Enzymology and Bioremediation Lab (115 and 204) for their kind assistance and for sharing their experiences and knowledge, directly or indirectly.

And most of all, I owe it all to my beloved parents and family for their undying support and faith in me. Thank you for being understanding even though you don't really understand what on earth I was working on. Only God has the wealth to

reward these wonderful people. I shall not go out there and be successful without being grateful to each and everyone of them.

*“Always aim for the sky, for if u fail, at least u can reach the clouds”*



I certify that an Examination Committee has met on 9 May 2006 to conduct the final examination of Fazuriana Binti Ahmad on her Master of Science thesis entitled “Use of Microorganisms as Bioindicators for Detection of Heavy Metals” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**Norhani Abdullah, PhD**

Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Chairman)

**Abu Bakar Salleh, PhD**

Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Mohammad Ismail Yaziz, PhD**

Associate Professor  
Faculty of Environmental Studies  
Universiti Putra Malaysia  
(Internal Examiner)

**Wan Azlina Ahmad, PhD**

Associate Professor  
Faculty of Science  
Universiti Technology Malaysia  
(External Examiner)

---

**HASANAH MOHD. GHAZALI, PhD**

Professor/Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date :

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

**Mohd Arif Syed, PhD**

Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Chairman)

**Mohd Yunus Abdul Shukor, PhD**

Lecturer

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Member)

---

**AINI IDERIS, PhD**

Professor/Dean

School of Graduate Studies

Universiti Putra Malaysia

Date :

## **DECLARATION**

I hereby declare that the thesis is based on my original work except for quotation and citations, which have been duly acknowledged. I also declare that it has been not been previously or concurrently for any other degree at UPM or other institutions.

---

**FAZURIANA BINTI AHMAD**

Date:

## TABLE OF CONTENTS

	<b>Page</b>
<b>DEDICATION</b>	ii
<b>ABSTRACT</b>	iii
<b>ABSTRAK</b>	v
<b>ACKNOWLEDGEMENTS</b>	vii
<b>APPROVAL</b>	ix
<b>DECLARATION</b>	xi
<b>LIST OF TABLES</b>	xiv
<b>LIST OF FIGURES</b>	xv
<b>LIST OF ABBREVIATIONS</b>	xvii
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
<b>2 LITERATURE REVIEW</b>	<b>4</b>
2.1 Heavy metal Pollutions	4
2.2 Heavy metals in the Malaysian Environment	6
2.3 Definition of Heavy Metals	10
2.4 The Chemistry of Several Heavy Metals and Their Toxicity	12
2.4.1 Silver (Ag)	15
2.4.2 Mercury (Hg)	16
2.4.3 Arsenic (As)	17
2.4.4 Cadmium (Cd)	19
2.4.5 Lead (Pb)	20
2.4.6 Copper (Cu)	21
2.5 Biochemistry of Heavy Metals	21
2.5.1 Heavy Metals in Soils and Plants	21
2.5.2 Heavy Metals in Animals	23
2.6 Uses of Heavy Metals	24
2.7 Determination/Bioassay of Heavy Metals in the Environment	27
2.7.1 Classical Bioassay/ Bioindicator	29
2.7.2 Modern Bioassay/ Bioindicator of Heavy Metals	30
2.7.3 Microtox™ Bioluminescence Assay	32
2.7.4 Polytox™ Bioassay	33
2.7.5 DeltaTox™ Bioassay	34
2.7.6 <i>Lux-Fluoro</i> Test Bioassay	34
2.7.7 Enzyme Bioassay	36
2.7.8 Metabolic/ Microbial Bioassay	37
2.7.9 Bioassay Using Antibodies	45
2.7.10 Biosensors for Heavy Metals Detection	46
<b>3 MATERIALS AND METHODS</b>	<b>48</b>
3.1 Chemicals and Equipments	48
3.2 Preparation of Solutions	48
3.2.1 MTT Dye Stock Solution	48

3.2.2	Heavy Metals Stock Solutions	48
3.2.3	Pesticides and Miscellaneous Xenobiotics Stock Solutions	49
3.3	Sample Collection	49
3.4	Isolation and Culture of Bacteria	51
3.5	MTT Assay of Bacterial Inhibition Studies	52
3.5.1	Preliminary Screening of Bacterial Respiration Inhibited by Divalent Cations and Heavy Metals	52
3.6	Effect of Different Stages of Microbial Growth on Inhibitory Effect of Heavy Metals	54
3.7	Determination of the IC <sub>50</sub> Value of the Mercury and Silver	55
3.8	Effect of Different Buffers System	56
3.9	Interfering Effects of Other Xenobiotics on Selective Bacteria	57
3.10	Preliminary Testing on Water Samples from Polluted Areas	58
3.11	Identification of Bacteria	59
3.11.1	Biochemical Test Using Microbact™ Kit	59
3.11.2	Partial Sequence of 16S rRNA for the Identification of Bacteria	61
<b>4</b>	<b>RESULTS AND DISCUSSION</b>	<b>68</b>
4.1	Sample Collection	68
4.2	MTT Assay of Bacterial Inhibition Studies	70
4.2.1	Preliminary Screening of Bacterial Respiration Inhibited by Divalent Cations and Heavy Metals	70
4.3	Effect of Different Stages of Microbial Growth on Inhibitory Effect of Heavy Metals	74
4.4	Determination of the IC <sub>50</sub> Value of Mercury and Silver	83
4.5	Effect of Different Buffers System	89
4.6	Interfering Effect of Other Xenobiotics on the MTT Assay by Isolate SC27 and S8	92
4.6.1	Interfering Effects of Xenobiotics on Isolate SC27	92
4.6.2	Interfering Effects of Xenobiotics on Isolate S8	96
4.7	Preliminary Testing on Water Samples from Polluted Areas	100
4.8	Identification of Bacteria	104
4.8.1	Colony Examination of Isolate SC27 and S8	104
4.8.2	Biochemical Test Using Microbact™ Kit	107
4.8.2.1	Identification of Isolate SC27 and S8	107
4.8.3	Partial Sequence of 16S rRNA for the Identification of Bacteria	111
<b>5</b>	<b>CONCLUSION</b>	<b>123</b>
	<b>REFERENCES</b>	<b>125</b>
	<b>APPENDICES</b>	<b>136</b>
	<b>BIODATA OF THE AUTHOR</b>	<b>146</b>