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

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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-demethyl-thiazol-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_{50} (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-demethyl-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 argentum 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™ mengkelaskan bakteria ini sebagai *Enterobacter* sp. Isolat S8 pula dikelaskan kepada *Serratia* sp. dengan kepercayaan sebanyak 90.70% dengan menggunakan kit Microbact™.

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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.

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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:

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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:

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DECLARATION

I hereby declare that the thesis in 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.

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FAZURIANA BINTI AHMAD

Date:

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