



**UNIVERSITI PUTRA MALAYSIA**

***DEVELOPMENT OF A TOXICITY BIOASSAY SYSTEM USING  
PHOTOBACTERIUM SP. STRAIN MIE***

**MOHD IZUAN EFFENDI BIN HALMI**

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**By**

**MOHD IZUAN EFFENDI BIN HALMI**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
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**March 2013**

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

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**March 2013**

**Chairman: Mohd Yunus Abd Shukor, PhD**

**Faculty: Biotechnology and Biomolecular Sciences**

In Malaysia there are about 180 river basins with about 5% of them severely polluted with toxicants. Current costs of instrument-based monitoring prevent regular toxicity monitoring of these polluted rivers. The global trend is using bioassays coupled with instrumental analysis that dramatically reduces the costs of monitoring. Toxicity bioassays using bioluminescent bacteria provide a rapid and sensitive method to monitor the presence of toxicants in the environment and are now globally accepted as the gold standard in environmental monitoring. Unfortunately, the most popular commercial bioassay system uses a bacterial strain that has a low (15°C) and narrow band of optimal working temperature which requires the use of a refrigerated water bath, preventing field work and near real time results. To solve this problem, a novel bioluminescent bacterium *Photobacterium* sp. strain MIE has been isolated from Indian mackerel, (*Rastrelliger kanagartha*). The optimal condition for bioluminescence production of this bacterium occurs within a broad temperature range of 24 to 30°C, allowing for easier application in the field. Other optimal

luminescence conditions are incubation at pH 5.5-7.5 with 30-50 g/L of tryptone as the nitrogen source, salinity with 15-20 g/L of sodium chloride and 4 g/L of glycerol as the sole carbon source. Experimental results showed that a bioassay system using this bacterium can be used to detect selected toxicants such as heavy metals, xenobiotics and solvents under a broad range of tropical temperature conditions. *Photobacterium* sp. strain MIE responded sensitively towards mercury, silver, copper, nickel, zinc and chromium with IC<sub>50</sub> values (mg/L) of 0.05, 0.12, 0.85, 12.32, 18.72 and 26.02, respectively. In addition, this bacterium could be used to detect the xenobiotics paraformaldehyde, phenol red, cycloheximide, p-nitroaniline, 2,4-dinitrophenol, 2,6-dichloroindophenol, ethanolamine and sodium dodecyl sulfate with IC<sub>50</sub> values 20.70, 15.66, 351.40, 64.20, 95.64, 11.34, 629.70 and 55.03 respectively. The IC<sub>50</sub> for the solvents hexane, formaldehyde, dimethyl sulfoxide, ammonia, chloroform, tween 80, ethyl acetate, hexanal, methanol are 20.41, 21.10, 28.73, 307.50, 555.30, 591.20, 1687, 3663 and 59,418, respectively. Based on these results, *Photobacterium* sp strain MIE was sensitive enough to detect various toxicants in the environment. Bioassay using this bacterium is suitable to be applied in the monitoring of toxicants in tropical countries compared to other commercial bioluminescent bacteria which have optimum assay temperatures of less than 25°C. The ability of this bioassay system to detect toxicants was proven in field trials at different polluted locations in Malaysia. The field trial showed promising results with six water samples collected from Juru River and Prai Industrial Estate giving more than 30% inhibitory effect on bioluminescence production. Validation using ICP-OES showed the presence of copper and zinc in these samples that exceeded the limits of permissible pollutants allowed by the Malaysian Department of Environment.

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

**PEMBINAAN SISTEM BIOASSAI TOKSIK MENGGUNAKAN  
*PHOTOBACTERIUM SP. STRAIN MIE***

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Di Malaysia, terdapat kira-kira 180 lembangan sungai dengan kira-kira 5% daripadanya tercemar teruk dengan sisa-sisa toksik. Kos semasa pemantauan berasaskan instrumen merencatkan pemantauan yang kerap ke atas sungai-sungai yang tercemar. Trend global menggunakan bioasai yang digabungkan dengan analisis instrumental dapat mengurangkan kos pemantauan. Bioasai bahan toksik menggunakan bakteria pendarkilau menyediakan satu kaedah yang cepat dan sensitif untuk memantau kehadiran bahan toksik dalam persekitaran dan kini diterima di seluruh dunia sebagai piawaian emas dalam pemantauan alam sekitar. Malangnya, sistem bioasai komersil yang paling popular menggunakan strain bakteria ini yang mempunyai suhu yang rendah (15°C) dan julat suhu optimum kecil yang memerlukan penggunaan mesin penyejuk, Keadaan ini menghalang kerja pemantauan dikawasan tercemar. Untuk menyelesaikan masalah ini, novel bakteria pendarkilau, *Photobacterium* sp. strain MIE telah diasingkan daripada ikan kembung, (*Rastrelliger kanagurta*). Keadaan optimum bagi pengeluaran pendarkilau bagi bakteria ini berlaku dalam julat suhu daripada 24 hingga 30°C, membolehkan penggunaan di kawasan tercemar. Keadaan optimum bagi penghasilan pendarkilau

adalah pada pH 5.5-7.5 dengan 30-50 g/L tripton sebagai sumber nitrogen, kemasinan dengan 15-20 g/L natrium klorida dan 4 g/L gliserol sebagai sumber karbon tunggal. Keputusan eksperimen menunjukkan bahawa sistem bioasai menggunakan bakteria ini boleh digunakan untuk mengesan bahan toksik terpilih seperti logam berat, xenobiotik dan pelarut organik di bawah pelbagai keadaan suhu tropika. *Photobacterium* sp. strain MIE sensitif terhadap merkuri, argentum, kuprum, nikel, zink dan kromium dengan nilai  $IC_{50}$  (mg/L) masing-masing adalah 0.05, 0.12, 0.85, 12.32, 18.72 dan 26.02. Di samping itu, bakteria ini boleh digunakan untuk mengesan xenobiotik paraformaldehid, fenol merah, sikloheksimid, p-nitroanilina, 2,4-dinitrofenol, 2,6-dikloroindofenol, etanolamina dan natrium sulfat dodesil dengan nilai  $IC_{50}$  masing-masing adalah 20.70, 15.66, 351.40, 64.20, 95.64, 11.34, 629.70 dan 55.03. Sementara itu,  $IC_{50}$  pelarut seperti heksana, formaldehid, sulfoksid dimetil, amonia, klorofom, tween 80, etil asetat, heksanal, metanol adalah 20.41, 21.10, 28.73, 307.50, 555.30, 591.20, 1687, 3663 dan 59,418, masing-masing. Berdasarkan keputusan ini, *Photobacterium* sp. strain MIE adalah cukup sensitif untuk mengesan pelbagai bahan toksik dalam persekitaran. Bioasai menggunakan bakteria ini adalah sesuai digunakan dalam pemantauan bahan toksik di negara-negara tropika berbanding dengan bakteria pendarkilau komersial lain yang mempunyai suhu asai optimum kurang daripada 25°C. Keupayaan sistem bioasai ini untuk mengesan bahan toksik telah terbukti dalam ujian percubaan di beberapa lokasi-lokasi yang tercemar di Malaysia. Ujian percubaan menunjukkan hasil yang memuaskan dengan enam sampel air diambil dari Sungai Juru dan kawasan perindustrian Prai memberikan lebih daripada 30% kesan yg perencatan terhadap pengeluaran pendarkilau. Pengesanan menggunakan ICP-OES menunjukkan

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I certify that a Thesis Examination Committee has met on, (18 March 2013) to conduct the final examination of Mohd Izuan Effendi Bin Halmi on his thesis entitled, “Development of a Toxicity Bioassay System using *Photobacterium sp.* Strain Mie” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded with the Master of Science degree.

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## **DECLARATION**

I declare that the thesis is my original work except for quotations and citations which has been duly acknowledged. I also declare that it has not been previously, and not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

---

**U P M**

**MOHD IZUAN EFFENDI BIN HALMI**

Date: 18 March 2013



## TABLES OF CONTENTS

	<b>Page</b>
<b>ABSTRACT</b>	ii
<b>ABSTRAK</b>	iv
<b>ACKNOWLEDGEMENTS</b>	vii
<b>APPROVAL</b>	viii
<b>DECLARATION</b>	x
<b>LIST OF TABLES</b>	xiv
<b>LIST OF FIGURES</b>	xv
<b>LIST OF ABBREVIATIONS</b>	xi
 <b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
1.1 Objectives	3
<b>2 LITERATURE REVIEW</b>	
2.1 Global water pollution	4
2.2 Water pollution in Malaysia	5
2.3 Toxicity of toxicants	8
2.3.1 Heavy metals	9
2.3.2 Xenobiotics	10
2.3.3 Pesticides	11
2.4 Monitoring of toxicants in the environment	12
2.4.1 Conventional detection of toxicants	12
2.4.1.1 Atomic Absorption Spectrometry and Induced Coupled Plasma	13
2.4.1.2 Chromatography techniques	14
2.4.2 Bioindicators/ bioassay of toxicants	15
2.4.2.1 <i>Daphnia magna</i>	16
2.4.2.2 <i>Rainbow trout</i>	17
2.4.2.3 An inhibitive enzyme assay using proteases enzyme	17
2.4.2.4 Microbial bioindicators/bioassay	18
2.4.2.4.1 MTT assay	18
2.4.2.4.2 Polytox™	19
2.4.2.4.3 Microtox®	19
2.5 Bioluminescence Inhibition assay.	20
2.5.1 Bioluminescent bacteria	22
2.5.2 <i>Vibrio fischeri</i>	22
2.5.3 <i>Photobacterium phosphoreum</i> .	24
2.5.4 Bioluminescence and the mechanisms	24
2.5.5 Monitoring of toxicants using bioluminescent bacteria	25

<b>3</b>	<b>METHODOLOGY</b>	
3.1	Material	27
3.1.1	Chemicals and equipments	27
3.1.2	Preparation of culture medium	27
3.1.2.1	Luminescence broth	27
3.1.2.2	Luminescence agar	28
3.1.2.3	Preparation of minimal salt medium	28
3.1.3	Preparation of buffer	28
3.1.3	Preparation of toxicants	29
3.1.3.1	Preparation of metals solutions	29
3.1.3.2	Preparation of pesticides	29
3.1.3.3	Preparation of other xenobiotics	30
3.2	Method	30
3.2.1	Statistical Analysis	30
3.2.2	Measurement of luminescence	29
3.2.3	Isolation and screening of bioluminescent bacteria	31
3.2.3.1	Isolation of bioluminescent bacteria	31
3.2.3.2	Screening of the bioluminescent bacteria	32
3.2.3.3	Maintenance and growth of bacterial isolates	32
3.2.4	Identification of bioluminescent bacterium	33
3.2.4.1	Biochemical Test	33
3.2.4.2	16S rRNA analysis	33
3.2.4.3	Genomic extraction	34
3.2.4.4	Polymerase chain reaction (PCR)	34
3.2.4.5	Purification of amplified PCR products	35
3.2.4.6	Quantification of DNA concentration	35
3.2.4.7	Sequence analysis	36
3.2.4.8	Phylogenetic analysis	37
3.2.5	Characterization of bioluminescence production	38
3.2.5.1	Screening of carbon sources	38
3.2.5.2	Effects of carbon source concentration	39
3.2.5.3	Screening of nitrogen sources	39
3.2.5.4	Effects of nitrogen source concentration	40
3.2.5.5	Effect of salinity	40
3.2.5.6	Effect of pH	41
3.2.5.7	Effect of temperature	41
3.2.6	Bacterial growth and bioluminescence production	42
3.2.7	Effect of toxicants toward bioluminescence production	43
3.2.7.1	Preparation of bacterial cells for toxicity assay	43
3.2.7.2	Bioluminescence inhibition studies	43
3.2.7.3	Effect of metals	44
3.2.7.4	Effect of pesticides	45
3.2.7.5	Effect of other xenobiotics and solvents	45
3.2.8	Field trials	46
3.2.8.1	Collection of environmental samples	46
3.2.8.1.1	Water samples	46
3.2.8.1.2	Fish tissue	46
3.2.8.2	Digestion of environmental samples	47
3.2.8.2.1	Water samples	47
3.2.8.2.2	Fish tissue	47

3.2.8.3	Method for assaying environmental samples	48
3.2.8.4	Instrumental analysis of samples	48
3.2.8.5	Analysis of data	49
<b>4.0</b>	<b>RESULT AND DISCUSSION</b>	<b>50</b>
4.1	Isolation and screening of bioluminescent bacteria.	50
4.2	Identification of the bioluminescent bacterium	53
4.2.1	Gram staining	54
4.2.2	Catalase test	54
4.2.3	16S rRNA Analysis	55
4.2.3.1	Genomic Extraction	55
4.2.3.2	Polymerase Chain Reaction (PCR)	56
4.2.3.3	16S rRNA Gene Sequencing	57
4.2.3.4	Tree View	59
4.3	Characterization of bioluminescence production	62
4.3.1	Effect of carbon source	62
4.3.2	Effect of nitrogen source	64
4.3.3	Effect of salinity	67
4.3.4	Effect of pH	69
4.3.5	Effect of temperature	71
4.4	Profile of Bioluminescence production and bacterial growth over time	73
4.5	Bioluminescence inhibition studies	74
4.5.1	Metals inhibition studies	74
4.5.2	Pesticides inhibition studies	81
4.5.3	Xenobiotics inhibition studies	82
4.5.4	Solvents inhibition studies	89
4.6	Field trials	94
4.6.1	Screening result of samples collected from Prai Industrial area	94
4.6.2	Screening result of water samples collected from Juru river	96
4.6.3	Screening result of samples collected from Perak river	98
4.6.4	Screening result of samples collected from Port Dickson Beach	100
4.6.5	Screening result of samples collected from Endau Rompin waterfall	102
4.6.6	Screening of digested fish tissue	104
<b>5.0</b>	<b>SUMMARY, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH</b>	<b>106</b>
	<b>REFERENCES</b>	<b>107</b>
	<b>APPENDICES</b>	<b>122</b>
	<b>BIODATA OF STUDENT</b>	<b>129</b>