UNIVERSITI PUTRA MALAYSIA

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

MOHD IZUAN EFFENDI BIN HALMI

FBSB 2013 4

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

By

MOHD IZUAN EFFENDI BIN HALMI

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

March 2013

© COPYRIGHT UPM

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

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

By

MOHD IZUAN EFFENDI BIN HALMI

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^{o}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 kanagurta*). 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

© COPYRIGHT UPM

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_{50} 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_{50} values 20.70, 15.66, 351.40, 64.20, 95.64, 11.34, 629.70 and 55.03 respectively. The IC_{50} 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.

© COPYRIGHT UPM

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

Oleh

MOHD IZUAN EFFENDI BIN HALMI

Mac 2013

Pengerusi: Mohd Yunus Abd Shukor, PhD

Fakulti: Bioteknologi dan Sains Biomolekul

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^{o}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

© COPYRIGHT UPM

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. Pengesahan menggunakan ICP-OES menunjukkan

© COPYRIGHT UPM

kehadiran kuprum dan zink dalam sampel yang melebihi had pencemaran yang dibenarkan oleh Jabatan Alam Sekitar, Malaysia.

© COPYRIGHT UPM

ACKNOWLEDGEMENTS

I am very glad and thankful to God, for His blessings for me to complete this thesis completely and successfully.

The completion of this project would not have been successful without the assistance, help, and co-operation from a number of people. I would like to express my gratitude to my beloved parents Sam bin Bidin and Sabariah Bt. Din for their encouragements and continuous support to keep me going on with this task. I also would like to take this opportunity to thank my supervisor, Associate Professor Dr. Mohd. Yunus Abd. Shukor, my co-supervisor, Professor Dr. Mohd. Arif Syed and Mr Arif Khalid. I appreciate the guidance and suggestions from them during the duration of this project.

Not to forget, all my dear project team members Mariam Zaaba, Shafiq, Sallehu, Astab Khan and thank you also to Norzila binti Kusnin who always supported me. Also to my super seniors in bioremediation lab especially, Mr Baskaran, Miss Norliza and Mr Khalizan thank you very much for your help and thanks a lot for supporting me.

Finally, I would like to thank to all my lecturers and my friends and anyone who were involved in assisting me to complete this project. Thank you for your support and understanding that meant a lot to me.

© COPYRIGHT UPM

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.

Members of the Thesis Examination Committee were as follows:

Mohd. Puad bin Abdullah, Phd
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Wan Zuhainis binti Saad, Phd
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Muskhazli bin Mustafa, Phd
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Mahmoud Abdel Mongy Ahmed Ismail, Phd
Genetic Engineering and Biotechnology research
Minufiya UniversitY
(External Examiner)

SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

© COPYRIGHT UPM

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

Mohd Yunus Abd Shukor, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mohd Arif Syed, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

© COPYRIGHT UPM

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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.

_____

MOHD IZUAN EFFENDI BIN HALMI

Date: 18 March 2013

© COPYRIGHT UPM

TABLES OF CONTENTS

© COPYRIGHT UPM

© COPYRIGHT UPM

© COPYRIGHT UPM