ISOLATION AND IDENTIFICATION OF PHYTOCHELATIN-PRODUCING FUNGI ASSOCIATED WITH MANGROVES

NOOR AFIZA BADALUDDIN

MASTER OF SCIENCE
UNIVERSITI MALAYSIA TERENGGANU
MALAYSIA
2011

ISOLATION AND IDENTIFICATION OF PHYTOCHELATIN-PRODUCING FUNGI ASSOCIATED WITH MANGROVES

NOOR AFIZA BADALUDDIN

Thesis Submitted in Fulfillment of the Requirement for the Degree of Master of Science in the Faculty of Science and Technology
Universiti Malaysia Terengganu

2011

ABSTRAK

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

PEMENCILAN DAN PENGENALPASTIAN KULAT PENGELUAR FITOKELATIN YANG BERKAITAN DENGAN BAKAU

NOOR AFIZA BINTI BADALUDDIN

Pengerusi : **Mariam Taib, Ph.D.**

Ahli : **Profesor Madya Aziz Ahmad, Ph.D**
Jamilah Mohd. Salim @ Halim, Ph.D

Fakulti : **Sains dan Teknologi**

Kulat memainkan peranan penting dalam beberapa proses ekosistem utama termasuk penyahtoksikan logam berat. Peranan fitokelatin dalam penyahtoksikan logam berat oleh kulat masih belum dikaji secara meluas. Selain itu, enzim yang bertanggung jawab untuk sintesis fitokelatin daripada glutation juga kurang dikaji. Oleh kerana pokok bakau diketahui mempunyai toleransi terhadap ketoksikan logam berat, kajian ini dilakukan untuk memencilkan dan mengenalpasti kulat penghasil fitokelatin yang berasosiasi dengan bakau. Kajian ini juga bertujuan untuk memencilkan gen yang mengkodkan enzim tersebut di dalam kulat yang terpilih. Kulat yang berasosiasi dengan bakau di Universiti Malaysia Terengganu dan Tok Bali, Kelantan dipencilkan dan dikenalpasti dengan menggunakan teknik 'Direct Plating' dan 'Slide Culture'. Secara keseluruhan, 25 spesis kulat telah dikenalpasti termasuk 19 Ascomycota, lima Zygomycota dan satu Deuteromycota. Daripada 25 kulat

yang telah dipencilkan, hanya tujuh dikenalpasti sebagai kulat akuatik: *Koralionastes augustus, K. violaceus, Haloguignardia cystoseirae, Pontogeneia calospora, Trematosphaeria mangrovei, Savoryella paucispora* dan *Trichoderma atroviride*. Kulat akuatik ini disaring untuk ciri-ciri toleransi terhadap kadmium dan *T. atroviride* didapati mempunyai toleransi tertinggi terhadap kadmium. *T. atroviride* dipilih untuk eksperimen selanjutnya selepas disahkan identiti kulat tersebut secara teknik molekul. Kulat tersebut dikulturkan dalam pelbagai kepekatan kadmium untuk merangsang pengeluaran fitokelatin. Amaun penghasilan fitokelatin, dianggarkan oleh ujian Ellman untuk penyukatan glutation. Penghasilan fitokelatin didapati bertambah dengan peningkatan kepekatan kadmium, yang menunjukkan bahawa penghasilan fitokelatin adalah bergantung kepada kepekatan kadmium. Jenis fitokelatin dikaji menggunakan HPLC fasa songsang dengan kolum oktadesil. Satu puncak baru telah didapati menunjukkan ianya produk glutation. Analisis SDS-PAGE terhadap protein yang bertindak balas terhadap kadmium menunjukkan bahawa keamatan jalur ~55 kDa meningkat dengan peningkatan kadmium. Kaedah konvensional dan 'real-time' PCR digunakan untuk memencilkan gen putatif fitokelatin sintase dan mengkaji ekspresi gen, setiap satunya. Multipleks PCR daripada enam pasang primer menghasilkan hanya satu produk PCR daripada pasangan primer Cad dan Bj. Produk ini seterusnya diklon dan dijujukkan. Namun keputusan jujukan menunjukkan bahawa produk PCR bersaiz ~150 bp tidak mempunyai persamaan dengan gen fitokelatin sintase yang telah dikenalpasti. Keputusan 'real-time' PCR menunjukkan bahawa peningkatan kadmium tidak mempengaruhi ekspresi gen putatif fitokelatin sintase. Peranan fitokelatin

sintase atau enzim-enzim lain dalam biosintesis fitokelatin sebagai pengikat logam berat perlu diselidiki lebih lanjut. Oleh kerana implikasi fitokelatin dalam penyahtoksikan logam berat boleh membawa kepada potensi nilai komersial yang sangat besar kepada banyak industri terutama dalam rawatan sisa air, *T. atroviride* boleh dieksploitasi selanjutnya sebagai sumber alternatif fitokelatin.

ABSTRACT

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

ISOLATION AND IDENTIFICATION OF PHYTOCHELATIN-PRODUCING FUNGI ASSOCIATED WITH MANGROVES

NOOR AFIZA BINTI BADALUDDIN

Chairperson: **Mariam Taib, Ph.D.**

Members : **Associate Professor Aziz Ahmad, Ph.D.**
Jamilah Mohd. Salim @ Halim, Ph.D.

Faculty : **Science and Technology**

Fungi play important roles in some of the major ecosystem processes including heavy metal detoxification. The roles of phytochelatins in heavy metal detoxification by fungi have not been widely investigated. Furthermore, the enzyme that is responsible for the synthesis of phytochelatins from glutathione, has not been studied well. As mangroves are known to tolerate heavy metal toxicity, this study was conducted to isolate and identify the phytochelatin-producing fungi associated with mangrove. This study also intended to isolate the gene that encodes the related enzyme in selected fungus. Fungi associated with mangrove in Universiti Malaysia Terengganu and Tok Bali, Kelantan were isolated and identified using Direct Plating and Slide Culture techniques. Altogether, 25 fungal species have been identified including 19 Ascomycota, five Zygomycota and one Deuteromycota. Out of 25 isolated fungi, only seven were

identified as aquatic fungi: *Koralionastes augustus, K. violaceus, Haloguignardia cystoseirae, Pontogeneia calospora, Trematosphaeria mangrovei, Savoryella paucispora* and *Trichoderma atroviride.* These aquatic fungal isolates were screened for cadmium tolerance property where *T. atroviride* was found to show the highest tolerance to cadmium. *T. atroviride* was selected for further experiments upon confirmation of its identity through molecular technique. It was cultured in different concentration of cadmium to induce phytochelatin production. The amount of phytochelatins produced was estimated by Ellman's test for glutathione measurement. The production of phytochelatins increased as the cadmium concentration increased, indicating that the production of phytochelatins is dependent on the cadmium concentration. The phytochelatin type was investigated using reversed-phase HPLC with an octadecyl column. A new sole peak was observed indicating a glutathione product. SDS-PAGE analysis of cadmium-responsive protein showed that the band intensity of ~55 kDa increased, as the cadmium concentration increased. The conventional and real-time PCR methods were used to isolate the putative phytochelatin synthase gene and study the gene expression, respectively. Multiplex PCR of six primer pairs generated only one PCR product from Cad forward and Bj reverse primer pair. The product was further cloned and sequenced. The sequencing results however showed that the ~150 bp product has no similarity with any established phytochelatin synthase gene. Real-time PCR results indicated that the increment of cadmium concentration did not affect the expression of putative phytochelatin synthase gene. The role of phytochelatin synthase or other enzymes in the biosynthesis of phytochelatins as heavy metal chelators should

be investigated further. As the implication of phytochelatins in heavy metal detoxification causes a huge potential commercial value towards many industries especially in wastewater treatment. *T. atroviride* can be exploited further as an alternative source of phytochelatins.