# GENE EXPRESSION STUDY ON CADMIUM STRESS IN *Chlorella vulgaris* (UMT-M1) BY USING mRNA DIFFERENTIAL DISPLAY POLYMERASE CHAIN REACTION (DD-PCR)

CHEW ENG HOW

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# GENE EXPRESSION STUDY ON CADMIUM STRESS IN *Chlorella vulgaris* (UMT-M1) BY USING mRNA DIFFERENTIAL DISPLAY POLYMERASE CHAIN REACTION (DD-PCR)

CHEW ENG HOW

Thesis Submitted in Fulfillment of the Requirement for the Degree of Master of Science in the School of Marine Science and Environment
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Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Master of Science

**GENE EXPRESSION STUDY ON CADMIUM STRESS IN *Chlorella vulgaris* (UMT-M1) BY USING mRNA DIFFERENTIAL DISPLAY POLYMERASE CHAIN REACTION (DD-PCR)**

**CHEW ENG HOW**

**March 2014**

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**Faculty** : **School of Marine Science and Environment**

Cadmium is a non-essential metal which is toxic to all organisms and was classified as carcinogen by several agencies. This study utilizes *Chlorella vulgaris* (UMT-M1) as a model to study cadmium induced stress in cells. *C. vulgaris* were exposed to 0.26 mg/L and 2.6 mg/L of cadmium and a total of nine cadmium-inducible genes were identified using differential display polymerase chain reaction (DD-PCR). The sizes of isolated DNA fragments ranged from 242 to 1009 bp which was grouped into three categories based on their function. There were; i) antioxidant or oxidation damage repair proteins (pCad-2, pCad-6 and pCad-7); ii) growth proteins (pCad-1 and pCad-4) and; iii) proteins with unknown function or novel protein (pCad-3, pCad-5, pCad-8 and pCad-9). The expression of clone pCad-2 (MsrB), pCad-3 (hypothetical protein) and pCad-4 (Gcd 10p-domain-containing protein) in *C. vulgaris* were further evaluated under 0.26 mg/L of cadmium treatment at 0, 2, 6, 12 and 24 hours. The Real-Time PCR result shows that pCad-2 was up-regulated at 6 hour after treatment (HAT) indicates the activation of repair mechanism. On the other hand, clone pCad-4 was down-

regulated at 24 HAT which indicates the inhibition of cells growth. While clone pCad-3, which is a predicted protein with unknown function, was up-regulated shortly after cadmium exposure at 2 HAT, suggests that it may function as an antioxidant in response to oxidative stress caused by cadmium. The gene expression pattern showed that cells response immediately after cadmium treatment and antioxidant was produced to repair oxidative damage and ensure normal cell growth. However, prolonged exposure to cadmium decreases the production of antioxidant which leads to inhibition of cell growth. The characterizations of these cadmium-inducible genes show that they are suitable to be developed into biomarker to monitor or detect cadmium pollutions in the environment.

Abstrack thesis yang dikemukakan kepada Senat University Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah master Sains.

**KAJIAN EKSPRESI GEN DI ATAS TEKANAN KADMIUM BAGI *Chlorella vulgaris* (UMT-M1) DENGAN MENGGUNAKAN TINDAK BALAS RANGKAIAN POLIMERAS PERBEZAAN PAPARAN mRNA**

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**March 2014**

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Kadmium adalah unsur logam yang tidak diperlukan malah toxik terhadap semua organism dan diklasifikasi sebagai karsinogen oleh beberapa agensi. Kajian ini menggunakan *Chlorella vulgaris* (UMT-M1) sebagai model untuk mengkaji tekanan rangsangan kadmium dalam sel. *C. vulgaris* didedahkan kepada 0.26 mg/L dan 2.6 mg/L kadmium dan sejumlah sembilan gen teraruhkan kadmium telah dikenal pasti. Size fragmen DNA yang diasingkan adalah antara 242 bp hingga 1009 bp dimana ia boleh dibahagi kepada tiga kumpulan, iaitu i) bahan antioksida dan protein pembaikan kerosakan pengoksidaan (pCad-2, pCad-6 and pCad-7), ii) protein pertumbuhan (pCad-1 and pCad-4) dan iii) protein dengan fungsi yang belum diketahui atau protein baru (pCad-3, pCad-5, pCad-8 and pCad-9). Ekspresi klon pCad-2 (Methionien-R-Sulfoxide reductase), pCad-3 (protein ramalan) dan pCad-4 (Gcd 10p-domain-containing protein) dalam *C. vulgaris* telah dinilai selanjutnya bawah 0.26 mg/L olahan kadmium pada 0, 2, 6, 12 dan 24 jam. Keputusan tindak balas rantai polimeras in situ (Real-Time PCR) menunjukkan bahawa ekspresi klon pCad-2 telah meningkat pada masa 6 jam olahan menandakan pengaktifan mekanisma pembaikan sel. Manakala

ekspresi klon pCad-4 menurun pada masa 24 jam selepas didedahkan kadmium menunjukkan kerencatan pertumbuhan sel. Ekspresi klon pCad-3, iaitu protein ramalan yang belum diketahui fungsi telah meningkan pada jam kedua didedahkan kepada kadmium mencadangkan bahawa ia mungkin berfungsi sebagai bahan antioksida yang bergerak balas dengan tekanan pengoksidaan yang disebabkan kadmium. Corak ekspresi gen menunjukkan sel bergerak balas dengan segera setelah olahan kadmium dan bahan antioksida dihasilkan untuk membaiki kerosakan disebabkan pengoksidaan dan memastikan pertumbuhan sel. Walau bagaimanapun, pendedahan kadmium jangka panjang akan menurunkan penghasilan bahan antioksida dan menyebabkan kerencatan pertumbuhan sel. Ciri-ciri gen teraruhkan kadmium menunjukkan bahawa mereka sesuai untuk dikembangkan untuk menjadi biopenunjuk bagi mengawasi atau mengesan pencemaran kadmium dalam alam sekitar.