THE GENOTOXIC EVALUATION AND GENE EXPRESSION STUDIES OF CADMIUM UPTAKE IN HYBRID TILAPIA (*Oreochromis sp.*)

VIJAYENDRAN GOVINDASAMY

DOCTOR OF PHILOSOPHY
UNIVERSITI MALAYSIA TERENGGANU
MALAYSIA

2010

THE GENOTOXIC EVALUATION AND GENE EXPRESSION STUDIES OF CADMIUM UPTAKE IN HYBRID TILAPIA (*Oreochromis* sp.)

VIJAYENDRAN GOVINDASAMY

Thesis Submitted in Fulfillment of the Requirement for Doctorate of Philosophy in the Institute of Marine Biotechnology University Malaysia Terengganu

June 2010

I dedicate this thesis to my Mother, siblings and my life partner

Abstract of thesis presented to the Senate of University Malaysia Terengganu in fulfillment of the requirement for the doctorate of philosophy

THE GENOTOXIC EVALUATION AND GENE EXPRESSION STUDIES OF CADMIUM UPTAKE IN HYBRID TILAPIA (*Oreochromis* sp.)

VIJAYENDRAN GOVINDASAMY

June 2009

Chairperson : Professor Dr Mohd Effendy Abd Wahid, Ph.D.

Members : Dr. Mariam Taib, Ph.D.
: Dr. Cha Thye San, Ph.D.

Institute : Institute of Marine Biotechnology

Toxic heavy metals especially cadmium are growing threat to global environmental health. To respond to these problems, there has been large demand for the new ways for assessment of environment pollution, which are fast, reliable and accurate. Fish is becoming an important organism for the assessment of cadmium toxicity and traditionally the bioaccumulation effect of cadmium in fish was used as an indicator to address the metal toxicity. However, the effects of cadmium especially cellular and molecular levels are poorly studied in which metals induce various genes, proteins and enzymes which play roles in the cellular stress response. Because of this, this dissertation set out to examine the affect of cadmium accumulation, genotoxic evaluation and gene expression studies at various cadmium treatments in hybrid tilapia. Firstly, this work examined the 96 hours acute median lethal concentration of cadmium in tilapia that was found to be 4.688 mgL^{-1}. The results were used to design a set of concentrations (0, 0.469, 0.938, 1.875 and 2.312 mgL^{-1}) in which tilapia fish were exposed for 21 days. At certain

days point, the viscera, intestine, gills and muscle tissues were dissected to study the accumulation and genotoxic effects. Cadmium uptake studies revealed that viscera, intestine and gills exhibited significant elevation of cadmium concentration ($p<0.05$) as compared to control and muscle in the 21 days of experimental period. The cadmium accumulation pattern was in an increment manner in viscera, intestine and muscle, however a mixed pattern was observed in the gills in which the concentration was recorded the highest at dat 14 before reducing at day 21. Next the comet assay was used to study the genotoxic effect of cadmium in the tilapia tissues which successfully showed high degree of DNA damages in the viscera ($p<0.05$), intestine ($p<0.05$), gills ($p<0.05$) as compared to control and muscle tissue. While DNA damage from viscera and intestine cells decreased at the end of the experiment, the DNA damages in gills continued to rise. To understand the phenomenon behind this observation, the gills samples were further analysed at the gene level by identifying at genes that might regulare the cadmium toxicity. The mRNA differential display was employed to understand this phenomenon as it can clearly distinguish the differently expressed genes between a control and treatment sample. A set of cadmium concentration which is approximately 2, 4, 10 and 16 fold of 96-h LC_{50} value were chosen as the purpose is only to excised the genes which is induced due to cadmium toxicity. This resulted in ten partial cDNA being expressed which were then cloned, sequenced and characterized. Only partial cDNA showed similar amino acid homology to the available data in GenBank. These partial cDNA were divided into proto-oncogenes/ proliferation (pre-B leukemia transcription factor/Ribosomol S10); apoptosis (TANK kinase 1) and anti oxidants (Cythochrome P450 Aramotase and Manganase Superoxide Dismutase). Further analysis of these partial cDNA by real time PCR showed

that only mRNA from pre-B leukemia and TANK-kinase 1 increased proportionally with the increase of Cd concentration, making these transcripts as potential markers for the assessment of cadmium toxicity, with gills as an ideal toxicology assessment tissue. From the present study, it is postulated that gills lost many viable cells during the accumulation process and thus

Abstrak thesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk ijazah doktor falsafah.

EVALUSI KESAN GENOTOKSIK DAN PENGEKPRESAN GEN BERIKUTAN PENGAMBILAN KADMIUM DALAM HIBRID TILAPIA (*Oreochromis* sp.)

VIJAYENDRAN GOVIDASAMY

Jun 2010

Pengerusi **:Profesor Dr. Mohd Effendy Abd Wahid, Ph.D.**

Ahli **:Dr. Mariam Taib, Ph.D.**
:Dr. Cha Thye San, Ph.D.

Institut **: Institut Bioteknologi Marin**

Logam berat yang toksik terutama kadmium adalah ancaman yang semakin berkembang terhadap kesihatan global. Untuk mengatasi masalah ini terdapat permintaan yang tinggi terhadap cara terkini dan untuk menilai pencemaran persekitaran yang cepat, diyakini dan tepat. Ikan merupakan antara organisma yang penting untuk mengukur ketoksikan kadmium dan secara tradisinya kesan kadmium ke atas fisiologi ikan telah digunakan sebagai petunjuk kepada ketoksikan logam. Namum begitu, kajian kesan kadmium terutama kerosakan dalam sel and molekul ikan masih tidak mencukupi. Justeru itu, kajian ini dijalankan untuk mengaji kesan pengambilan kadmium, penilaian genotoksik dan ekspresi gen susulan daripada rawatan kadmium yang berbeza di dalam ikan tilapia. Bahagian pertama ialah penentuan kepekatan akut median "lethal" 96-jam kadmium dalam tilapia dan nilai didapati ialah 4.688mgL^{-1}. Nilai ini digunakan untuk merekabentuk satu set kepekatan kadmium (0, 0.469, 0.938, 1.875 and 2.312 mgL^{-1}) di

mana ikan didedahkan selama 21 hari. Pada hari-hari tertentu, tisu viscera, usus, insang dan otot diasingkan dan digunakan dalam penentuan akumulasi kadmium dan ujikaji genotoksik. Kajian ini mendapati kadar peningkatan kadmium yang signifikan dalam viscera ($p<0.05$) usus ($p<0.05$) dan insang ($p<0.05$) berbanding dengan kawalan dan tisu otot. Akumulasi kadmium didapati secara berkadar untuk viscera, perut dan insang tetapi berubah-ubah untuk insang yang mana akumulasi kadmium menunjukan nilai yang tinggi pada hari ke 14 sebelum nilainya menurun pada hari ke 21.

Seterusnya, ujian komet telah dijalankan untuk mengenalpasti tahap kerosakan DNA oleh kadmium dalam tisu tilapia yang menunjukan tahap kerosokkan yang tinggi dalam viscera, usus dan insang berbanding dengan kawalan dan tisu otot. Sel viscera dan usus menunjukan pengurangan kerosakan DNA di akhir ujikaji tetapi sel insang menunjukan kerosakan DNA yang teruk secara berterusan. Untuk memahami phenomena ini, sampel insang teah dianalisis seterusnya di peringkat mRNA untuk mengenalpasti gen yang terlibat dalam ketoksikan kadmium. Keadah pempaparan berbeza mRNA telah digunakan untuk memahami phenomena ini keran ia dapat membezakan gene daripada sampel kawalan dan sampel yang didedahkan kepada kadmium. Satu siri kepekatan yang nilainya lebih kurang 2, 4, 10 dan 16 kali dari nilai 96-hLC_{50} telah digunakan untuk mengenalpasti gen yang diekpreskan oleh ketokisikan kadmium. Sebagai hasilnya, sepuluh separa gen telah diekspreskan, yang kemudian diklon, dijujukkan dan dibuat perbandingan pencirian di GenBank. Hanya lima separa gen yang menunjukan homologi asid amino dengan data sedia ada di GenBank. Separa gen ini dibahagikan kepada gen proto-onkogen (pre-B leukemia transcription factor; Ribosomol S10), apoptosis (TANK

kinase 1) dan anti oksida (Cythochrome P450 Aramotose; Manganase Superoxide Dismutase). Kajian selanjutnya menggunakan "Real Time PCR" mendapati bahawa pre-B leukemia transcription factor dan TANK kinase 1 menunjukkan peningkatan berkadar langsung dengan kadmium, menjadikan gen ini sebagai penanda berpotensi dalam penentuan ketoksikan kadmium dengan insang sebagai tisu toksikologi yang ideal. Hasil kajian ini membolehkan kita memahami lebih lanjut tentang ketoksikan kadmium dalam persekitaran akuatik dan menekankan kepentingan mengambilkira kedua-dua aspek kimia dan fisiologi dalam menganalisis dan mentaksir ketoksikan kadmium. Diharapkan hasil kajian ini dapat menyumbang kepada perkembangan seterusnya dalam penentuan kualiti air.