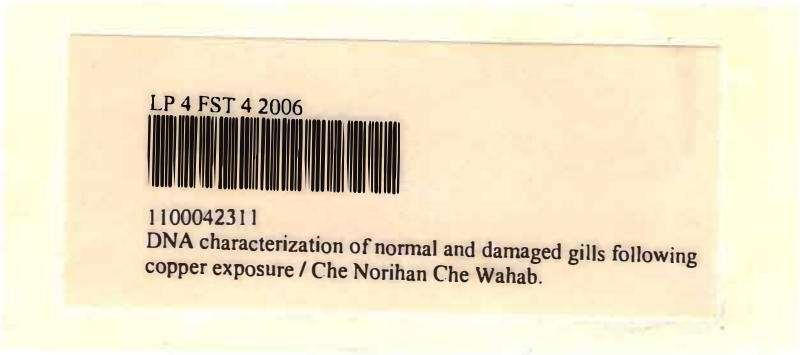

Lihat sebelah

DNA CHARACTERIZATION OF NORMAL AND DAMAGED GILLS FOLLOWING COPPER EXPOSURE

By
Che Norihan binti Che Wahab

Research Report submitted in partial fulfillment of the requirements for the degree of Bachelor of Science (Marine Science)

DEPARTMENT OF MARINE SCIENCE
FACULTY OF SCIENCE AND TECHNOLOGY
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
KUSTEM
2006

JABATAN SAINS SAMUDERA
FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA

PENGAKUAN DAN PENGESAHAN LAPORAN PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk :

DNA characterization of normal and damaged gills following copper exposure oleh **Che Norihan bt Che Wahab, No. Matrik: UK 8918** telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Samudera sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains – Sains Samudera, Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

Penyelia Utama

Nama: PROF. MADYA DR. KAMARUZZAMAN YUNUS
Timbalan Pengarah
Institut Oseanografi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu

Tarikh: 26/4/06

Cop Rasmi:

Penyelia kedua

Nama:

Tarikh:

Cop Rasmi:

Ketua Jabatan Sains Samudera

Nama

Tarikh 8/5/06

Cop Rasmi PROF. MADYA DR. HJ. ROSNAN HJ. YAACOB
Ketua
Jabatan Sains Samudera
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu.

This report should be cited as:

Che. C. C. W. 2006. DNA characterization of normal and damaged gills following copper exposure. Undergraduate thesis, Bachelor of Science (Marine Science), Faculty of Science and Technology, KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA, KUSTEM. 71p.

ACKNOWLEDGEMENT

First of all, Alhamdullillah i'm grateful thanks to Almighty Allah because give me strength, very good patient and his blessing for me to finish this final year project. My big thanks goes to my family especially my mother Zaiton bt Chik and my father Che Wahab b. Che Mat because be always besides me to give a support continuously.

I would like to take this opportunity to thanks my first supervisor Assoc. Prof. Dr. Kamaruzzaman Yunus for his concerns, comments and suggestion for me in order to accomplish this project, my second supervisor Assoc. Prof. Dr. Mohd Effendy Abd. Wahid for giving the advice and permission to use the equipments in biotechnology laboratory, FST. My thanks also goes to Mr. Vijayendran Govindasamy for guide me to do this final year project especially for his patient, caring and understanding personality and Mr Ong Meng Chuang for giving encouragement and helping me in this final year project.

Besides that, I would like to thank to my friends who really give me a hand on this DNA project especially my housemates Nor Ziana and Siti Noor Aini, my best friends, Wan Ahmad Shahrir Azlan and also my partner in doing the same project, Maznah. Not to forget to my others friends, my course mates and those who helped me directly or indirect.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS ii

LIST OF TABLES viii

LIST OF FIGURES x

LIST OF APPENDIXS xii

LIST OF SYMBOLS xi

ABSTRACT xiv

ABSTRAK xv

CHAPTER I

Introduction 1

CHAPTER II

Literature review 4

2.1. Red/Nile tilapia Fish (*Oreochromis niloticus*) 4

2.2. Copper in fish 5

2.3. 96-hr LC_{50} acute toxicity test 7

2.4. Heavy metal in tissue gills 9

2.5. Fish as a bio-indicator 10

2.6. Heavy metal –copper 11

2.7. Fish gill 12

2.8. RAPD-PCR 13

CHAPTER III

Material and methodology

3.1. Study site 19

3.1.1. Experimental design 19

3.2. Part A: Determination of 96 hours LC50 of Cu2+ on Tilapia fingerlings 21

3.2.1. Statistical analysis 22

3.2.2. Uptake and accumulation of copper in tilapia fish 22

3.3. Part B: Determination of metal concentration in tissue of Tilapia fingerlings at 7, 14 and 21 days exposed to copper 24

3.3.1. Tissue sampling 24

3.3.2. Tissue preparation for determination of metal Concentration the gills 24

3.3.3. Open acid digestion 25

3.3.4. Metal recovery measurement 25

3.3.5. Heavy metal measurement in the tissue gills of tilapia Fingerlings 26

3.4. Part C: DNA analysis of normal and exposure tilapia Fingerlings at 7, 14 and 21 days exposure to copper 27

3.4.1. DNA extraction 27

3.4.2. Purification and quantification of DNA 28

3.4.2.1. Biophotometer 28

3.4.2.2. Electrophoresis method 29

3.4.3. DNA amplification by RAPD 29

3.4.4. Database establishment 31

3.4.5. Data analysis 32

CHAPTER IV

Results

4.1. Part A: Determination of 96 hours LC_{50} Cu^{2+} for red nile/tilapia fish 33

4.2. Part B: Uptake and accumulation of copper on tilapia Fingerlings's gill for the period of 21 days 35

4.2.1. Metal recovery measurement 35

4.2.2. Accumulation of copper on tilapia 36

4.3. Part C: Effect of copper on the genomic of the red tilapia at 7, 14 and 21 days of exposure. 39

4.3.1. DNA extraction 39

4.3.2. RAPD screening test 41

4.3.3. PCR data analysis 42

CHAPTER V

Discussion **46**

CHAPTER VI

Conclusion **53**

References **54**

Appendix **60**

Curriculum Vitae **71**

LIST OF TABLES

2.8.1a.	Universal Primers	18
3.2.2a.	Concentration design for uptake and accumulation of Cu^{2+} in tilapia fingerlings for 21 days period	23
3.3.4a.	Summary of DOLT-3, certified Metal concentration	26
3.4.3a.	Primer sequences selected	28
3.4.3b.	Final concentration of RAPD mixture	30
3.4.3c.	Amplification profile	31
4.1.1.	96-h LC50 using spearman-karber computer programme	33
4.1.2.	Comparison 96HLC50 of copper on tilapia by using different types of method	34
4.1.3.	96-h median lethal concentration at different percentage	35
4.2.2a.	The statistical correlation and regression for the metals accumulated in nile tilapia within 21 days	37
4.3.1a.	Absorbance reading by biophotometer at 260 and 280 wavelength and concentration of DNA	39

4.3.3a. RAPD fingerprinting pattern of tilapia fish of 7 days exposed to copper 42

4.3.3b. RAPD fingerprinting pattern of tilapia fish of 14 days exposed to copper 44

4.3.3c. RAPD fingerprinting pattern of tilapia fish of 21 days exposed to copper 45

LIST OF FIGURE

2.1.1.	Red Nile tilapia	5
2.7.1.	Diagram of gill filament Vasculature	13
2.8.2.	Illustration on PCR-RAPD technique work	17
4.2.2a.	Copper accumulation in gills tissue at 7, 14 and 21 days of exposure	36
4.2.2b.	Metal Concentration according to size for 7, 14 and 21 days Exposure.	38
4.3.1a.	The electrophoresis pattern of gills tilapia fish genomic DNA	40
4.3.2a.	RAPD screening test for selective primers	41
4.3.3a.	RAPD fingerprinting profiles of tilapia fish at 7 days exposed to copper	42
4.3.3b.	RAPD fingerprinting profiles of tilapia fish at 14 days exposed to copper	43
4.3.3c.	RAPD fingerprinting profiles of tilapia fish at 21 days exposed to copper	44

LIST OF SYMBOLS

ICP-MS	Inductively Coupled Plasma – Mass Spectrophotometry
cm	Centimeter
g	Gram
mg	milligram
µg	microgram
Cu	Copper
Cu^{2+}	Copper in ionic form
HNO_3	Nitric Acid
H_2SO_4	Sulfuric Acid
HCL	Hydrocloric Acid
H_2O_2	Hydrogen Peroxide
ppm	parts per million or equivalent to mgL^{-1}
mgL^{-1}	milligram per liter
L	Liter
d.w.	Dry weight
PCR	Polymerase Chain Reaction
PCR-RAPD	Randomly Amplified Polymerase Chain Reaction

LIST OF APPENDIXES

I	Standard stock solution of copper (Cu^{2+})	60
II	Toxicity testing-percentage of mortality of nile tilapia (*Oreochromis niloticus*) at different time exosed to different metal concentration (Copper replicate 1)	61
III	Toxicity testing-percentage of mortality of nile tilapia (*Oreochromis niloticus*) at different time exosed to different metal concentration (Copper replicate 2)	62
IV	Toxicity testing-percentage of mortality of nile tilapia (*Oreochromis niloticus*) at different time exosed to different metal concentration (Copper replicate 3)	63
V	Toxicity Testing-Summary on calculating the LC 50 values (Probit and Spearman karber method) using Spearman-karber Computer programme (Replicate 1)	64
VI	Toxicity Testing-Summary on calculating the LC 50 values (Probit and Spearman karber method) using Spearman-karber Computer programme (Replicate 2)	65
VII	Toxicity Testing-Summary on calculating the LC 50 values (Probit and Spearman karber method) using Spearman-karber Computer programme (Replicate 3)	66

VIII Volume of copper at each concentration for the uptake copper for 21 days. 67

IX Total Length, Body Length, Total Body Weight, Wet Weight and Dry Weight of *Oreochromis niloticus* at 7, 10, 21 Days Exposure. 68

X Concentration of Metal in the Gills Tissue (ppb), Standard Length and Total Body Weight (g) of Nile Tilapia Fingerlings at 7, 14 and 21 Days Exposure 69

XI Statistical Analysis (ANOVA 2 ways without replication): Mean Accumulation of copper in gill tissues of nile tilapia fingerlings for 30 days. 70

ABSTRACT

The ultimate aim of this study is to predict how genomic of aquatic organisms respond to heavy metal exposure. Red tilapia fingerlings siblings (*Oreochromis niloticus*) (0.5 cm-5.0 cm) were exposed to copper (Cu^{2+}). The study was conducted in three parts whereby each part is interrelated. The first part was the copper acute toxicity test. Using probit method, the 96-h LC50 value for tilapia fingerlings was 1.82 ppm. This value was then used to design a sub-lethal concentration (1.82 ppm, 1.36 ppm, 0.91 ppm, 0.45 ppm and 0 ppm), which the fishes were exposed to a period of 21 days. At end of 7 days, 6 fishes were killed. The changes of the genomic of tilapia's gill was detected by using the RAPD-PCR technique. Heavy metal analysis (open acid digestion technique and ICP-MS) was conducted to determine the accumulation of copper in the dry weight of the gills. No significant changes occurred in the genomic of the gills after 21 days exposure of copper in all of the exposure concentration.0.0878 ppb and 0.00242 ppb of Cu^{2+} was found from the highest to the lowest concentration. After 7, 14 and 21 days exposure to the copper, no genomic changes were obtained. As a conclusion, the genomic of the tilapia gills shows no changes for all concentration in the exposure period.

ABSTRAK

Ciri-ciri DNA normal dan rosak ekoran pendedahan terhadap kuprum.

Tujuan utama kajian ini adalah untuk meramal perubahan genomik organisma akuatik terhadap pendedahan kepada logam berat. Anak ikan tilapia merah (*Oreochromis niloticus*) (0.5 cm-5.0 cm) telah didedahkan kepada kuprum. Kajian ini telah dijalankan dalam tiga peringkat yang saling berkaitan di antaranya. Bahagian pertama ialah ujian penentuan ketoksikan akut kuprum. Dengan menggunakan kaedah probit, 96-h LC_{50} Cu^{2+} adalah 1.82 ppm. Nilai ini kemudian di gunakan untuk membentuk 5 jenis kepekatan (1.82 ppm, 1.36 ppm, 0.91 ppm, 0.45 ppm dan 0 ppm) yang mana anak ikan didedahkan selama 21 hari. 6 ikan di bunuh pada setiap 7 hari. Perubahan pada genomik insang tilapia dikesan dengan menggunakan kaedah RAPD-PCR. Analisis logam berat (kaedah asid terbuka dan ICP-MS) juga dijalankan untuk menentukan akumulasi Cu^{2+} pada tisu insang yang kering. Tidak ada sebarang perubahan didapati setelah didedahkan kepada semua kepekatan selama 21 hari itu. 0.00242 sehingga 0.0878 ppb berat kering akumulasi Cu^{2+} telah dijumpai dalam ikan yang didedahkan dari kepekatan rendah ke kepekatan tertinggi. Selepas 21 hari didedahkan dengan kuprum, didapati tiada perubahan genomik terhadap ikan tilapia. Hal ini di sebabkan oleh kuprum yang merupakan logam perlu didalam semua tisu hidupan. Secara kesimpulannya, tiada perubahan genomik yang ditunjukkan dalam semua kepekatan dalam tempoh 21 hari tersebut.