การศึกษาระดับโมเลกุลของยีนที่ชักนำโดยเอสโทรเจนในปลากระบอกดำ, *Liza subviridis*

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MOLECULAR STUDY ON ESTROGEN INDUCIBLE GENES IN GREENBACK MULLET, *Liza subviridis*

Mr. Arttasit Tangserisuksan

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Biotechnology
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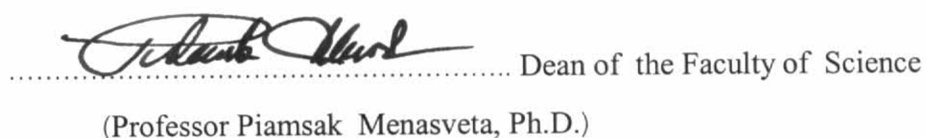
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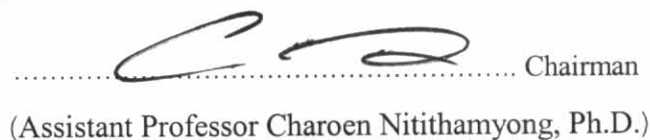
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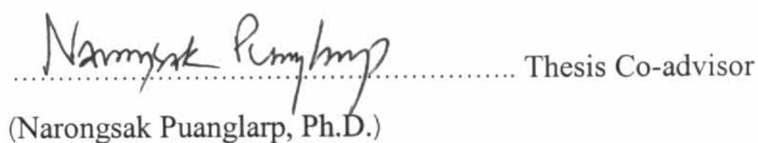
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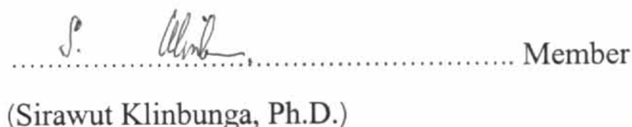
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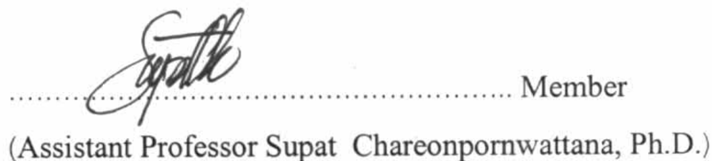
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อรรถสิทธิ์ ตั้งเสรีสุขสันต์ : การศึกษาระดับโมเลกุลของยีนที่ชักนำโดยเอสโทรเจนในปลากระบอกดำ, *Liza subviridis* (MOLECULAR STUDY ON ESTROGEN INDUCIBLE GENES IN GREENBACK MULLET, *Liza subviridis*) อ. ที่ปรึกษา: ศ.ดร. เปี่ยมศักดิ์ เมนะเศวต, อ. ที่ปรึกษา ร่วม: ดร.ณรงค์ศักดิ์ พ่วงลาภ 183 หน้า.

ซีโนเอสโทรเจนสามารถเหนี่ยวนำให้เกิดการแสดงออกของยีนไวเทลโลเจนิน และคอริโอเจนินในตับของปลาเพศผู้ และปลาวัยอ่อนซึ่งโดยปกติแล้วการแสดงออกของยีนทั้ง 2 ยีนจะเกิดขึ้นในตับของปลาเพศเมียวัยเจริญพันธุ์ภายใต้การควบคุมของฮอร์โมนเอสโทรเจน งานวิจัยครั้งนี้ได้ทำการโคลน และศึกษาลักษณะสมบัติของยีนเอสโทรเจนรีเซปเตอร์ (ER), คอริโอเจนิน (*chg*) และ ไวเทลโลเจนิน (*vtg*) จากตับของปลากระบอกดำ *Liza subviridis* และศึกษาผลของการกระตุ้นด้วยฮอร์โมนเอสโทรเจนต่อระดับการแสดงออกของยีน *chg* และ *vtg* ด้วยเทคนิค semi-quantitative RT-PCR เพื่อนำไปประยุกต์ใช้เป็นดัชนีวัดการปนเปื้อนของสารซีโนเอสโทรเจนในแหล่งน้ำ จากการทดลองพบว่า open reading frame ของยีน ERα, ERβ, *chg*-L และ *vtg*-1 ประกอบด้วย 1863, 1431, 1260 และ 4653 bp ซึ่งควบคุมการสร้าง ERα, ERβ, Chg-L และ Vtg-1 ที่ประกอบด้วยกรดอะมิโน 620, 476, 419 และ 1,550 หมู่ ตามลำดับ นอกจากนี้ยังสามารถหา partial coding sequence ของยีน *chg*-H ซึ่งควบคุมการสร้างพอลิเพปไทด์ที่ประกอบด้วยกรดอะมิโน 310 หมู่ และ 96% ของ coding sequence ของยีน *vtg*-3 จากการศึกษาผลของการกระตุ้นด้วยฮอร์โมนเอสโทรเจนด้วยการฉีดเข้าช่องท้อง ที่ระดับ 0, 0.05, 0.1, 0.25, 0.5, และ 5 มิลลิกรัมต่อน้ำหนักปลา 1 กิโลกรัม ตามลำดับ ต่อระดับการแสดงออกของยีน *chg*-L, *chg*-H และ *vtg*-3 พบว่าการกระตุ้นด้วยฮอร์โมนเอสโทรเจนที่ระดับ 5 มิลลิกรัมต่อน้ำหนักปลา 1 กิโลกรัมทำให้ระดับการแสดงออกของยีน *chg*-L และ *chg*-H เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) ในวันที่ 3 และ 6 หลังการกระตุ้น และทำให้ระดับการแสดงออกของยีน *vtg*-3 เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) ในวันที่ 3 หลังการกระตุ้น ซึ่งการวัดระดับการแสดงออกของยีน *chg*-L, *chg*-H, และ *vtg*-3 ในตับของปลากระบอกดำ *Liza subviridis* วัยอ่อน และ/หรือ เพศผู้ ด้วยเทคนิค semi-quantitative RT-PCR ที่พัฒนาขึ้นมาในงานวิจัยครั้งนี้สามารถนำไปใช้ในการตรวจสอบการปนเปื้อนของสารซีโนเอสโทรเจนในแหล่งน้ำได้

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4672525723: MAJOR BIOTECHNOLOGY
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ARTTASIT TANGSERISUKSAN: MOLECULAR STUDY ON ESTROGEN INDUCIBLE GENES IN GREENBACK MULLET, *Liza subviridis*. THESIS ADVISOR: PROF. PIAMSAK MENASVETA, Ph.D., THESIS COADVISOR: NARONGSAK PUANGLARP, Ph.D., 183 pp.

Xenoestrogen can induce vitellogenin (*vtg*) and choriogenin (*chg*) genes expression in liver of male and juvenile fish which normally expressed in liver of spawning female fish under estrogen control. In this research we cloned and characterized estrogen receptor (ER), choriogenin (*chg*) and vitellogenin (*vtg*) genes in liver of greenback mullet, *Liza subviridis* and studied estrogen response at mRNA expression level of *chg* and *vtg* genes by semi-quantitative RT-PCR for application to biomarker for detecting xenoestrogen in water. The result showed open reading frame of ERα, ERβ, *chg*-L, and *vtg*-1 genes at size 1863, 1431, 1260 and 4653 bp that encode ERα, ERβ, Chg-L, and Vtg-1 which include 620, 476, 419, and 1,550 amino acid residues, respectively. We can determine partial coding sequence of *chg*-H that encode polypeptide which include 310 amino acid residues and 96 % of coding sequence of *vtg*-3. The result of estrogen response of *chg*-L, *chg*-H, and *vtg*-3 at mRNA expression level by injection estrogen intraperitoneally at dose 0, 0.05, 0.1, 0.25, 0.5, 1 and 5 mg/kg body weight show *chg*-L and *chg*-H expression level increase statistical significant ($P < 0.05$) after 3 and 6 days exposed with estrogen at dose 5 mg/kg and *vtg*-3 expression level increase statistical significant ($P < 0.05$) after 3 days exposed with estrogen at dose 5 mg/kg. Measurement of *chg*-L, *chg*-H, and *vtg*-3 expression level in liver of male and/or juvenile greenback mullet *Liza subviridis* by semi-quantitative RT-PCR that develop in this research can use for detecting xenoestrogen contamination in water.

Field of study...Biotechnology............Student's signature. Arttasit Tangserisuksan

Academic year......2006.................Advisor's signature.............

Co-advisor's signature. Narongsak Puanglarp

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LIST OF ABBREVATIONS

bp	Base pair
°C	Degree Celsius
cDNA	Complementary deoxyribonucleic acid
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytosine triphosphate
dGTP	Deoxyguanosine triphosphate
dNTP	Deoxyribonucleotide triphosphate
dTTP	Deoxythymidine triphosphate
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetate
g	Gram
g	Gravity (multiples of, as in centrifugal field)
HCl	Hydrochloric acid
h	hour
IPTG	Isopropyl-thiogalactoside
kb	Kilo base
kDa	Kilo dalton
LB	Luria-Bertani
M	Molar (mole per litres)
$MgCl_2$	Magnesium chloride
mg	Milligram
min	Minute
ml	Milliliter
mM	Millimolar
ng	Nanogram
OD	Optical density
PCR	Polymerase chain reaction

RNaseA	Ribonuclease A
rpm	Revolution per minute
SDS	Sodium dodecyl sulfate
Tm	Temperature, melting
Tris	Tris (hydroxy methyl) aminomethane
U	Unit
μg	Microgram
μl	Microlitre
μM	Micromolar
UV	Ultraviolet
v/v	Volume / volume (concentration)
w/v	Weight / volume (concentration)
λ	Lambda