

การระบุเครื่องหมายสนิปที่เกี่ยวข้องกับการเติบโตในกึ่งกลาดำ *Penaeus monodon*



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IDENTIFICATION OF GROWTH-RELATED SNP MARKERS
IN THE GIANT TIGER SHRIMP *Penaeus monodon*

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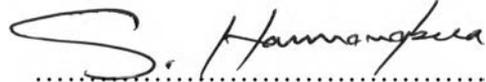
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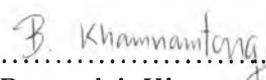
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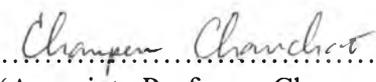

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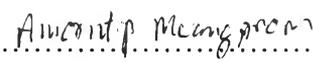
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ศิริธร จานพุม: การระบุเครื่องหมายสลับที่ที่เกี่ยวข้องกับการเติบโตในกุ้งกุลาดำ *Penaeus monodon*.

(IDENTIFICATION OF GROWTH-RELATED SNP MARKERS IN THE GIANT TIGER SHRIMP *Penaeus monodon*)

อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ศ.ดร. เปี่ยมศักดิ์ เมณะเสวต, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : ดร. บวรลักษณ์ คำน้ำทอง, 140 หน้า.

ตรวจสอบภาวะพหุสัณฐานของจีนที่มีหน้าที่เกี่ยวข้องกับการเติบโตของกุ้งกุลาดำ ประกอบด้วย *calponin1 (PmCnn1)* *cyclin C (PmCyC)* และ *cdc25 (PmCdc25)* ในกุ้งวัยรุ่นอายุสามเดือน (BUM03 และ SNP3A) และอายุ 5 เดือน (PM05) ด้วยวิธี PCR-SSCP พบความสัมพันธ์ระหว่างรูปแบบ SSCP ของจีน *PmCnn1*, *PmCyC* และ *PmCdc25* กับปัจจัยการเติบโตของกุ้งกุลาดำ (น้ำหนักตัว, ความยาว, น้ำหนักตับ และค่าดัชนีของตับ) ในกลุ่มตัวอย่าง SNP3A อย่างมีนัยสำคัญทางสถิติ โดยกุ้งวัยรุ่นที่มีรูปแบบ SSCP แบบที่ I และ II ของจีน *PmCnn1*₅₃₀ มีน้ำหนักตัวและความยาวเฉลี่ยมากกว่ากุ้งรูปแบบที่ III ($N = 156, P < 0.05$) สำหรับจีน *PmCyC* พบรูปแบบ SSCP จำนวนสามรูปแบบ โดยกุ้งวัยรุ่นซึ่งมี SSCP รูปแบบที่ II พบว่ามีน้ำหนักตัวและน้ำหนักตับเฉลี่ยมากกว่ากุ้งที่มีรูปแบบ SSCP แบบที่ I และ III อย่างมีนัยสำคัญทางสถิติ ($N = 145, P < 0.05$) นอกจากนี้ยังพบว่ากุ้ง SNP3A ที่มีรูปแบบ SSCP แบบที่ I ของจีน *PmCdc25* มีน้ำหนักตัว ความยาว และน้ำหนักตับเฉลี่ยมากกว่ากุ้งที่มี SSCP รูปแบบที่ II ($N = 144, P < 0.05$) นอกจากนี้ยังพบความสัมพันธ์ระหว่างรูปแบบ SSCP ของจีน *PmCnn1*₄₂₅ กับ น้ำหนักตัวและความยาวเฉลี่ยของกุ้ง BUM03

เมื่อนำตัวแทนของแต่ละรูปแบบ SSCP ของทั้งสามจีนดังกล่าวในกุ้งวัยรุ่น SNP3A มาหาลำดับนิวคลีโอไทด์ ผลการวิเคราะห์ลำดับนิวคลีโอไทด์พบสลับที่จำนวน 6 ตำแหน่งที่บริเวณ intron ในจีน *PmCnn1*₅₃₀ มีความสัมพันธ์กับปัจจัยการเติบโตของกุ้งวัยรุ่นอย่างมีนัยสำคัญทางสถิติ โดยผลการวิเคราะห์ความสัมพันธ์ระหว่างสลับที่ของจีนดังกล่าวกับลักษณะการเติบโตของกุ้งพบว่าสลับที่ G/G₂₀₉T/T₂₁₀ -/-₂₁₂-/-₂₁₁C/C₂₁₈G/G₂₄₀ และสลับที่ G/A₂₀₉T/A₂₁₀-/G₂₁₂-/T₂₁₃C/T₂₁₈G/A₂₄₀ ซึ่งพบในกุ้งที่มี SSCP รูปแบบที่ I และ II จะพบว่ามีน้ำหนักตัว ความยาวและน้ำหนักตับเฉลี่ยมากกว่ากุ้งที่มีสลับที่เป็น A/A₂₀₉A/A₂₁₀G/G₂₁₂T/T₂₁₃T/T₂₁₈A/A₂₄₀ ซึ่งพบในกุ้งที่มี SSCP รูปแบบที่ III สำหรับจีน *PmCyC* พบสลับที่ทั้งหมดจำนวนห้าตำแหน่ง โดยสามตำแหน่ง (A/G₃₁, G/A₃₇₉, และ T/C₃₈₂) เป็นสลับที่ที่เกิดบริเวณ exon แต่ไม่ทำให้เกิดการเปลี่ยนแปลงชนิดของกรดอะมิโน ส่วนอีกสองตำแหน่ง (T/C₁₃₄ และ T/C₁₈₈) เป็นสลับที่ที่เกิดบริเวณ intron โดยกุ้งที่มีสลับที่เป็น G/G₃₁C/T₁₃₄C/C₁₈₈A/A₃₇₉C/C₃₈₂ ซึ่งพบในกุ้งที่มีรูปแบบ SSCP แบบที่ II พบว่าเติบโตเร็วกว่ากุ้งที่มีสลับที่เป็น A/A₃₁C/C₁₃₄T/T₁₈₈G/G₃₇₉T₃₈₂ และ A/G₃₁C/T₁₃₄T/C₁₈₈G/A₃₇₉T/C₃₈₂ ซึ่งพบในกุ้งที่มีรูปแบบ SSCP แบบที่ I และ III ตามลำดับ สำหรับจีน *PmCdc25* นั้นพบสลับที่แค่เพียงหนึ่งตำแหน่ง (A/C₂₄₃) โดยกุ้งที่มีสลับที่เป็น A/C₂₄₃ นั้นพบว่ามีน้ำหนักตัว ความยาวและน้ำหนักตับเฉลี่ยมากกว่ากุ้งที่มีสลับที่เป็น C/C₂₄₃ อย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) ทำการพัฒนาวิธีการตรวจสอบอย่างง่ายโดยจากข้อมูลลำดับนิวคลีโอไทด์ของจีน *PmCnn1*₅₃₀ และ *PmCdc25* พบว่าสลับที่ของจีนดังกล่าวสามารถตรวจสอบได้ด้วยวิธี PCR-RFLP

เมื่อตรวจสอบระดับการแสดงออกของจีน *PmCnn1* และ *PmCdc25* mRNA ในตับของกุ้งวัยรุ่น SNP3A ด้วยวิธี quantitative real-time PCR พบว่าระดับการแสดงออกของจีน *PmCnn1* ของกุ้งที่มีรูปแบบ SSCP แบบที่ III มีระดับการแสดงออกของจีนสูงกว่ากุ้งที่มีรูปแบบ SSCP แบบที่ I และ II อย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) สำหรับระดับการแสดงออกของจีน *PmCdc25* นั้นพบว่างุ้งที่มีรูปแบบ SSCP แบบที่ I นั้นมีระดับการแสดงออกของจีนสูงกว่ากุ้งที่มีรูปแบบ SSCP แบบที่ II อย่างมีนัยสำคัญทางสถิติ ($P < 0.05$)

หาลำดับนิวคลีโอไทด์ที่สมบูรณ์ของจีน *PmCyC* พบว่ามีความยาว 1443 bp มี ORF ยาว 804 bp สามารถแปลรหัสเป็นโปรตีนที่มี 267 กรดอะมิโน นอกจากนี้ยังสร้างโปรตีนลูกผสมของ *PmCnn1* และผลิตภัณฑ์โคลนอลแอนติบอดีของ r*PmCnn1* ดังกล่าว

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SIRITHORN JANPOOM: IDENTIFICATION OF GROWTH-RELATED SNP MARKERS
IN THE GIANT TIGER SHRIMP *Penaeus monodon*.

ADVISOR: PROF. PIAMSAK MENASVETA, Ph.D.

CO-ADVISOR: BAVORNLAK KHAMNAMTONG, Ph.D., 140 pp.

Polymorphism of growth-related genes; *calponin1* (*PmCnn1*), *cyclin C* (*PmCyC*) and *cdc25* (*PmCdc25*) in 3- (BUM03 and SNP3A) and 5-month-old (PM05) juveniles of the giant tiger shrimp (*Penaeus monodon*) were identified by polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) analysis. Relationships between SSCP patterns and growth parameters (average body weight, BW; total length, TL; hepatopancreatic weight, HPW and/or hepatosomatic index, HSI) of the examined shrimp were examined. In the SNP3A sample, shrimp carrying SSCP patterns I and II of *PmCnn1*₅₃₀ (primers Cnn1-F/R) had a greater average BW and TL than those exhibiting pattern III ($N = 156$, $P < 0.05$). Likewise, juveniles shrimp carrying SSCP pattern II of *PmCyC* possessed a significantly greater average BW and HPW than those of shrimp carrying patterns I and III ($N = 145$, $P < 0.05$). For *PmCdc25*, the BW, TL and HPW of shrimp carrying SSCP pattern I was significantly greater than those of shrimp carrying SSCP pattern II ($N = 144$, $P < 0.05$). Moreover, significant relationships between SSCP patterns of *PmCnn1*₄₂₅ and average BW and TL were found in the BUM03 sample ($P < 0.05$).

Nucleotide sequences of cloned *PmCnn1*₅₃₀, *PmCyC* and *PmCdc25* gene segments of representative individuals carrying each SSCP genotype were determined. Six intronic SNPs of *PmCnn1*₅₃₀ were significantly related with growth parameters. Of these, shrimp with each of G/G₂₀₉T/T₂₁₀-/-₂₁₂-/-₂₁₃C/C₂₁₈G/G₂₄₀ (SSCP pattern I) and each of (G/A)₂₀₉(T/A)₂₁₀(-/G)₂₁₂(-/T)₂₁₃(C/T)₂₁₈(G/A)₂₄₀ (SSCP pattern II) had a greater average BW, TL and HPW than those with each of A/A₂₀₉A/A₂₁₀G/G₂₁₂T/T₂₁₃T/T₂₁₈A/T₂₄₀ (SSCP pattern III). For *PmCyC*, three exonic (A/G₃₁, G/A₃₇₉, and T/C₃₈₂) and two intronic (T/C₁₃₄ and T/C₁₈₈) SNPs corresponding to SSCP pattern I, II and III were observed, respectively. Each SNP of shrimp with SSCP pattern I: G/G₃₁C/T₁₃₄C/C₁₈₈A/A₃₇₉C/C₃₈₂ had a significantly greater average growth parameters (except HSI) than those with each SNP of shrimp found in SSCP pattern I: A/A₃₁C/C₁₃₄T/T₁₈₈G/G₃₇₉T/T₃₈₂ and III: A/G₃₁C/T₁₃₄T/C₁₈₈G/A₃₇₉T/C₃₈₂. Only one SNP (A/C₂₄₃) was found in *PmCdc25* for which shrimp exhibiting A/C₂₄₃ had a significantly greater average BW, TL and HPW ($P < 0.05$) than those carrying C/C₂₄₃. Simplification of SNP detection of *PmCnn1*₅₃₀ and *PmCdc25* gene segments was successfully developed based on PCR-RFLP.

The relative expression level of *PmCnn1* and *PmCdc25* in hepatopancreas of juvenile shrimp (SNP3A) carrying different SSCP pattern were significantly different ($P < 0.05$). The expression level of *PmCnn1* in shrimp exhibiting SSCP pattern III was significantly greater than those exhibiting pattern I and II ($P < 0.05$) while the expression level of *PmCdc25* in shrimp exhibiting SSCP pattern I was significantly greater than those exhibiting genotypes II ($P < 0.05$).

The full-length cDNA of *PmCyC* was successfully characterized. It was 1443 bp in length containing and ORF of 804 bp corresponding to a polypeptide of 267 amino acids. Moreover, recombinant PmCnn1 protein was successfully expressed as the soluble protein in *E.coli*. The polyclonal antibody against rPmCnn1 was successfully produced in rabbit.

Field of Study : Biotechnology..... Student's Signature..... *Sirithorn Janpoom*

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LIST OF ABBREVIATIONS

bp	base pair
°C	degree celsius
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
dGTP	deoxyguanosine triphosphate
dTTP	deoxythymidine triphosphate
DNA	deoxyribonucleic acid
HCl	hydrochloric acid
IPTG	isopropyl-thiogalactoside
M	Molar
MgCl ₂	magnesium chloride
mg	milligram
ml	milliliter
mM	millimolar
ng	nanogram
OD	optical density
PCR	polymerase chain reaction
RACE	Rapid Amplification of cDNA Ends
RNA	Ribonucleic acid
RNase A	Ribonuclease A
rpm	revolutions per minute
RT	reverse transcription
SDS	sodium dodecyl sulfate
Tris	tris (hydroxyl methyl) aminomethane
µg	microgram
µl	microliter
µM	micromolar
UV	ultraviolet