

การโคลน การแสดงออก และการจัดเรียงตัวของยีนโปรตีนหลัก 1 และ 2
ในรอยัลเจลลีของผึ้งโพรง *Apis cerana*



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CLONING, EXPRESSION AND GENOMIC ORGANIZATION OF MAJOR
ROYAL JELLY PROTEINS 1 AND 2 GENES OF THE HONEY BEE *Apis cerana*

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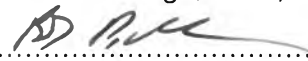

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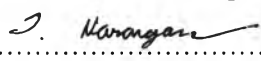

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จันทร์ประภา อิ่มจงใจรัก : การโคลน การแสดงออก และการจัดเรียงตัวของยีนโปรตีนหลัก 1 และ 2 ในรอยัลเจลลีของผึ้งโพรง *Apis cerana* (CLONING, EXPRESSION AND GENOMIC ORGANIZATION OF MAJOR ROYAL JELLY PROTEINS 1 AND 2 GENES OF THE HONEY BEE *Apis cerana*) อ. ที่ปรึกษา : รศ.ดร. ศิริพร สิทธิประณีต, อ. ที่ปรึกษาร่วม : ดร. ศิราวุธ กลิ่นนุหงา 252 หน้า. ISBN 974-53-1452-8.

ในงานวิจัยนี้ได้โคลน cDNA ของโปรตีนหลัก 1 และ 2 (AcMRJP1 and AcMRJP2) ในรอยัลเจลลี จากส่วนหัวของผึ้งโพรง (*Apis cerana*) โดยใช้เทคนิค reverse transcription-PCR (RT-PCR) จากการวิเคราะห์ลำดับนิวคลีโอไทด์ของ AcMRJP1 และ AcMRJP2 cDNA พบว่าประกอบด้วยนิวคลีโอไทด์ขนาด 1,302 และ 1,392 คู่เบส ซึ่งกำหนดการสร้างโปรตีนที่ประกอบด้วย 433 และ 463 กรดอะมิโนตามลำดับ ลำดับนิวคลีโอไทด์ของ AcMRJP1 และ AcMRJP2 มีความคล้ายกับลำดับนิวคลีโอไทด์ของยีนดังกล่าวในผึ้งพันธุ์ *A. mellifera* 93 และ 92 เปอร์เซ็นต์ตามลำดับ และมีกรดอะมิโน จำเป็น เป็นองค์ประกอบ 47.4 และ 45 เปอร์เซ็นต์

จากการโคลนยีนของโปรตีนหลัก 1 และ 2 และนำมาวิเคราะห์การจัดเรียงตัวของยีน พบว่ายีนโปรตีนหลัก 1 และ 2 ประกอบด้วยนิวคลีโอไทด์ขนาด 3,663 และ 3,963 คู่เบส ตามลำดับ โดยยีนทั้งสองมี 6 exon และ 5 intron ซึ่งรอยต่อเป็นไปตาม GT/AG rule จากการวิเคราะห์ลำดับนิวคลีโอไทด์ทางด้าน 5' upstream พบลำดับนิวคลีโอไทด์ที่คาดว่าจะเป็นส่วนของโปรโมเตอร์โดยมี putative TATA box ที่บริเวณ 31-32 นิวคลีโอไทด์เหนือบริเวณจุดเริ่มต้นของการถอดรหัสของแต่ละยีน นอกจากนี้ยังพบ putative binding site ของ transcription factor เช่น Ultraspiracle (USP) transcription factor

เมื่อนำ cDNA ของโปรตีนหลัก 1 และ 2 มาทำการแสดงออกในเชื้อ *E. coli* โดยใช้เวกเตอร์ pET17b พบว่าการแสดงออกของโปรตีนที่มีขนาดประมาณ 50 และ 55 กิโลดาลตัน ซึ่งสอดคล้องกับขนาดของโปรตีนที่คำนวณจากลำดับนิวคลีโอไทด์ของ cDNA ของโปรตีนหลัก 1 และ 2 (47.9 และ 51.7 กิโลดาลตัน) และพบว่าการแสดงออกสูงสุดในช่วง 4 ชั่วโมง หลังจากชักนำด้วย IPTG โปรตีนหลัก 1 และ 2 มีการแสดงออกในลักษณะเป็นโปรตีนที่ไม่ละลายน้ำ ซึ่งสามารถทำให้บริสุทธิ์ได้โดยใช้ affinity chromatography โดยโปรตีนถูกชะออกมาด้วยอิมิดาโซลความเข้มข้น 250 มิลลิโมลาร์ จากนั้นยืนยันว่าโปรตีนที่ได้เป็นโปรตีนหลัก 1 และ 2 ด้วยการหาลำดับกรดอะมิโนทางด้าน N-terminal และเทคนิค Western blot

ได้สร้างรีคอมบิแนนท์พลาสมิดที่สามารถแสดงออกเพื่อผลิตโปรตีนหลัก 1 ของรอยัลเจลลี โดยรีคอมบิแนนท์พลาสมิดที่สร้างขึ้นประกอบด้วย AcMRJP1 cDNA เพื่อใช้ทรานส์ฟอร์มเข้าสู่มันฝรั่ง (*Solanum tuberosum* L.) และข้าว (*Oryza sativa* L.) ด้วยการใช้ *Agrobacterium* รีคอมบิแนนท์พลาสมิดที่สร้างเพื่อส่งทอดสู่มันฝรั่ง AcMRJP1 cDNA จะเชื่อมต่อภายใต้การควบคุมของโปรโมเตอร์ 3 ชนิด cauliflower mosaic virus 35S promoter, granule bound starch synthase (GBSS) promoter และ patatin B33 promoter ส่วนรีคอมบิแนนท์พลาสมิดที่สร้างเพื่อส่งทอดสู่ข้าว AcMRJP1 cDNA จะเชื่อมต่อภายใต้การควบคุมของ 35S promoter หลังทำการส่งทอดรีคอมบิแนนท์พลาสมิดทั้ง 4 เข้าสู่มันฝรั่ง และข้าว พบว่าการแสดงออกให้ทั้งโปรตีน และ mRNA ของโปรตีนหลัก 1 ของรอยัลเจลลี ในพืชทั้งสอง ทั้งนี้โดยการตรวจวิเคราะห์ด้วย RT-PCR และ Western blot analysis

ภาควิชา.....ชีวเคมี.....

สาขาวิชา.....ชีวเคมี.....

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ลายมือชื่อผู้ผลิต.....*จันทร์ประภา อิ่มจงใจรัก*.....

ลายมือชื่ออาจารย์ที่ปรึกษา.....*ศิริพร สิทธิประณีต*.....

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KEY WORD : ROYAL JELLY PROTEIN / *Apis cerana* / EXPRESSION

CHANPRAPA IMJONGJIRAK : CLONING, EXPRESSION AND GENOMIC ORGANIZATION OF MAJOR ROYAL JELLY PROTEINS 1 AND 2 GENES OF THE HONEY BEE *Apis cerana*. THESIS ADVISOR: ASSOC. PROF. SIRIPORN SITTIPRANEED, Ph.D., THESIS CO-ADVISOR: SIRAWUT KLINBUNGA, Ph.D., 252 pp. ISBN 974-53-1452-8.

Major Royal Jelly Protein (AcMRJP) cDNAs of *Apis cerana* were isolated from head of *Apis cerana* nurse bee by Reverse transcription-PCR (RT-PCR). The open reading frames (ORFs) of AcMRJP1 and AcMRJP2 were 1,302 and 1,392 bp encoding 433 and 463 amino acid residues protein, respectively. Nucleotide sequence of AcMRJP1 and AcMRJP2 cDNA showed high homology with those of AmMRJP1 (93%) and AmMRJP2 (92%), respectively. Deduced amino acids showed high essential amino acid content of AcMRJP1 and AcMRJP2 (47.4% and 45%, respectively).

The genomic organization of AcMRJP1 and AcMRJP2 genes were determined by PCR. The AcMRJP1 and AcMRJP2 gene sequence spans over 3,663 bp and 3,963 bp, respectively. Both AcMRJP1 and AcMRJP2 genes contain six exons separated by five introns. All intron-exon boundaries followed the GT/AG rule. Sequence analysis of the 5' upstream regions revealed a putative TATA-box, locating approximately 31-32 bps upstream of the predicted transcription start sites of each gene. The presence of potential recognition sequences for ultraspiracle (USP) transcription factors were observed.

The AcMRJP1 and AcMRJP2 cDNAs were cloned into expression vectors for expression in *E. coli*. SDS-PAGE analysis revealed protein band of 50 and 55 kDa corresponding to the expected molecular weight of approximately 47.9 kDa and 51.7 kDa for AcMRJP1 and AcMRJP2, respectively. The expression of AcMRJP1 and AcMRJP2 in *E. coli* was maximal at 4 hours after IPTG induction. The AcMRJP1 and AcMRJP2 were expressed as inclusion body and purified using affinity chromatography. These bands were eluted with 250 mM imidazole and confirmed by N-terminal sequencing and Western blot analysis.

The expressed recombinant plasmids containing AcMRJP1 cDNA were constructed and introduced into potato (*Solanum tuberosum* L.) and rice (*Oryza sativa* L.) using *Agrobacterium*-mediated transformation method. The constructed recombinant plasmid for rice, AcMRJP1 cDNA was inserted under the control of the cauliflower mosaic virus 35S promoter whereas for potato the cDNA was inserted under the control of the cauliflower mosaic virus 35S, or granule bound starch synthase (GBSS) or patatin B33 promoter. Successful expression of AcMRJP1 mRNA and AcMRJP1 in both potato and rice were obtained when all four recombinant plasmids were transformed as analyzed by RT-PCR and Western blot analysis.

Department.....Biochemistry..... Student's signature.....*Chanprapa Imjongjirak*.....
 Field of study..... Biochemistry..... Advisor's signature.....*Siriporn Sittipraneed*.....
 Academic year.....2004..... Co-advisor's signature.....*S. Klinbunga*.....

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LIST OF ABBREVIATION

AcMRJP	=	<i>Apis cerana</i> major royal jelly protein
AmMRJP	=	<i>Apis mellifera</i> major royal jelly protein
ATP	=	adenosine triphosphate
bp	=	base pair
°C	=	degree celcius
cDNA	=	complementary deoxyribonucleic acid
CTAB	=	cetyltrimethylammonium bromide
dATP	=	deoxyadenosine triphosphate
dCTP	=	deoxycytosine triphosphate
dGTP	=	deoxyguanosine triphosphate
dTTP	=	deoxythymine triphosphate
DNA	=	deoxyribonucleic acid
GUS	=	β-glucuronidase
hpt	=	hygromycin phosphotransferase gene
HygR	=	hygromycin resistant
IPTG	=	isopropyl-thiogalactoside
KanR	=	kanamycin resistant
Kb	=	kilobase
KCl	=	potassium chloride
KDa	=	kilodalton
MgCl ₂	=	magnesium chloride
mg	=	milligram
MRJP	=	major royal jelly protein
ml	=	millilitre
mM	=	millimolar
M	=	molar
ng	=	nanogram
nptII	=	neomycin phosphotransferase gene
OD	=	optical density
PCR	=	polymerase chain reaction
RJ	=	royal jelly
RNaseA	=	ribonuclease A
rpm	=	revolution per minute
SDS	=	sodium dodecyl sulfate
μg	=	microgram
μl	=	microlitre
μM	=	micromolar
U	=	unit
UV	=	ultraviolet
vir	=	virulence
X-Gluc	=	5-Bromo-4-chloro-3-indolyl-β-D-glucuronic acid