

แอกติวิตีของแอลคาไลน์ฟอสฟาเทสในระยะการเจริญของ
วรรณะผึ้งงานในผึ้งพันธุ์ *Apis mellifera* Linneaus, 1758

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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**ALKALINE PHOSPHATASE ACTIVITY IN DEVELOPMENTAL
STAGES OF HONEYBEE WORKER *Apis mellifera* Linneaus, 1758**

Miss Tipwan Suppasat

**A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Zoology**

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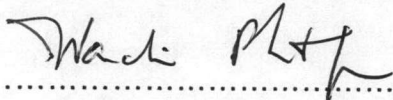
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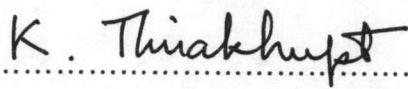
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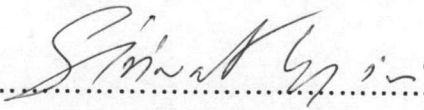
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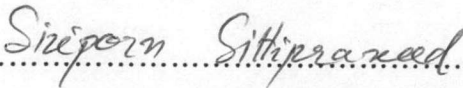

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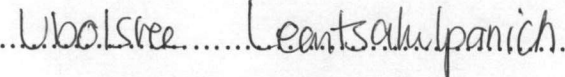
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ทิพย์วรรณ สรรพสัจย์: แอกติวิตีของแอลคาไลน์ฟอสฟาเทสในระยะเวลาเจริญของ
วรรณะผึ้งงานในผึ้งพันธุ์ *Apis mellifera* Linneaus, 1758 (ALKALINE PHOSPHATASE
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การศึกษาแอกติวิตีของแอลคาไลน์ฟอสฟาเทส (AP) ในผึ้งงานของผึ้งพันธุ์ เริ่มตั้งแต่ไข่อายุ
48 ชั่วโมง จนถึงตัวเต็มวัยที่จะออกจากเซลล์ โดยเก็บตัวอย่างระยะห่างกัน 48 ชั่วโมง ระยะเวลาเก็บตัว
อย่างทำโดยการเค็มสีที่สว่าง จากการศึกษากการแสดงออกของ AP ในเนื้อเยื่อของผึ้งงานโดยวิธีทาง
เนื้อเยื่อวิทยา พบแอกติวิตีสูงที่เนื้อเยื่อปกคลุม เนื้อเยื่อทางเดินอาหาร กล้ามเนื้อ ในเซลล์จะพบมากใน
ส่วนของเยื่อหุ้มเซลล์ และพบเป็นอิสระในเซลล์ ทำการเพิ่มปริมาณของดีเอ็นเอ โดยใช้ไพร์เมอร์ที่ออก
แบบมาจากบริเวณอนุรักษ์ของลำดับเบสของยีนส์ AP ที่ได้จากสิ่งมีชีวิตชนิดอื่นๆ ได้ลำดับเบสบาง
ส่วนที่มีความยาว 429 คู่เบส มีเปอร์เซ็นต์ของความเหมือนของยีนส์ AP ในผีเสื้อไหม 39.7% และใน
แมลงหวี่ (*Aph-4*) 39.2% ตรวจวัดแอกติวิตีที่จำเพาะของสารสกัดอย่างหยาบ จากปฏิกิริยาไฮโดรไลซิสกับ
สารตั้งต้นพาราไนโตรฟอสเฟต วัดการดูดกลืนแสงที่ 405 นาโนเมตร หลังจากทำการทดลองซ้ำ
จากผึ้ง 3 รัง พบว่าตัวอย่างผึ้งที่มีอายุ 144 ชั่วโมงมีแอกติวิตีที่จำเพาะของ AP สูงสุด (1.5×10^{-4} u/mg)
สภาวะที่เหมาะสมต่อการทำงานคือ ค่าความเป็นกรด-ด่างที่ 9.5-9.6 อุณหภูมิที่เหมาะสมคือ 50 องศา
เซลเซียส มีความสามารถในการทนความร้อนสูง นอกจากนี้ผลจากการย้อมสารสกัดอย่างหยาบด้วย
สารตั้งต้น NBT/BCIP โดยใช้ 12% Polyacrylamide gel ที่ไม่ถูกทำให้เสียสภาพพบว่าผลของแอกติวิตีของ
AP สอดคล้องกับผลของการไฮโดรไลซิส และพบว่ามวลโมเลกุลของ AP บนเจลที่กล่าวข้างต้นมีค่า
ประมาณ 150 กิโลดาลตัน การแสดงออกของ AP พบตั้งแต่ในระยะไข่ 48 ชั่วโมง และในระยะตัว
หนอนมีการเกิดแอกติวิตีมากที่สุด ในระยะดักแด้มีแอกติวิตีน้อยสุด และแนวโน้มของแอกติวิตีในตัว
เต็มวัยจะเพิ่มขึ้น ทั้งนี้คาดว่าน่าจะเกี่ยวข้องกับกระบวนการเจริญต่างๆ

ภาควิชา.....ชีววิทยา.....ลายมือชื่อนิสิต..... จันทิพน..... สิริวัฒน์
สาขาวิชา.....สัตววิทยา.....ลายมือชื่ออาจารย์ที่ปรึกษา.....
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KEYWORD: Alkaline phosphatase/*A. mellifera*/workers/para-nitrophenol phosphate/NBT/BCIP/specific activity/non-specific AP/AP gene

TIPWAN SUPPASAT: ALKALINE PHOSPHATASE ACTIVITY IN DEVELOPMENTAL STAGES OF HONEYBEE WORKER *Apis mellifera* Linnaeus, 1758. THESIS ADVISOR: SIRIWAT WONGSIRI, Ph. D. THESIS CO-ADVISOR: CHANPEN CHANCHAO, Ph. D. 114 pp. ISBN 974-17-1452-1

Alkaline phosphatase (AP) of *A. mellifera* workers was determined from 48 h egg (1st) to emerging adult (10th). The interval of each stage was 48 h. The sample was marked with painting color. For histochemical study, high activity was in an integument, alimentary, or muscle, etc. Mostly, it was found at membrane and as free AP in cell. Genomic DNA was amplified by designed primers from conserved regions of the AP from various organisms. Partial DNA sequence of AP at the length of 429 bp was obtained. The sequence shows 39.7% homology to AP in *Bombyx mori* and 39.2% homology to APh-4 gene in *Drosophila melanogaster*. AP activity in crude extract was assayed by hydrolysis of para-nitrophenol phosphate as substrate. Samples were collected from 3 hives. The highest specific activity (1.5×10^{-4} unit/mg) was from the 144 h sample. The optimum conditions were pH at 9.5-9.6, temperature at 50°C. It is also heat tolerant. According to the activity stain on 12% native polyacrylamide gel (native PAGE), the AP activity was positive during the mentioned period at the molecular mass of 150 kDa. An expression of AP by this experiment was begun from egg. The highest AP activity was found in larva while the least AP activity was in pupa. In adult, the AP activity trended to be high again. The fluctuated level of AP activity is possible to be involved in developmental processes.

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ABBREVIATIONS

AP	Alkaline phosphatase
AC	Acid phosphatase
bp	Base pair
°C	Degree celcius
Kb	Kilobase
kDa	Kilodalton
LM	Light microscope
h	Hour
min	Minute
M	Molar
mM	Millimolar
μM	Micromolar
PCR	Polymerase Chain Reaction
PAGE	Polyacrylamide Gel Electrophoresis
pM	Picomolar
μg	Microgram
ng	nanogram
mg	Milligram
μl	Microlitre
ml	Millilitre
nm	Nanometre
rpm	Revolution per minute
RT	Room temperature
Sec	second
SM	Stereomicroscope