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PREPARATION OF *N*-ACETYL-D-GLUCOSAMINE AND *N,N*-DIACETYLCHITOBIOSE
BY ENZYMATIC HYDROLYSIS OF SQUID PEN CHITIN

Miss Thitima Maneekul

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for the Degree of Master of Science Program in Chemistry

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By Thitima Maneekul
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Thesis Advisor Associate Professor Mongkol Sukwattanasinitt, Ph.D.
Thesis Co-advisor Associate Professor Hunsa Punnapayak, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree


..... Dean of the Faculty of Science
(Professor Piamsak Menasveta, Ph.D.)


THESIS COMMITTEE


..... Chairman
(Professor Udom Kokpol, Ph.D.)


..... Thesis Advisor
(Associate Professor Mongkol Sukwattanasinitt, Ph.D.)


..... Thesis Co-advisor
(Associate Professor Hunsa Punnapayak, Ph.D.)


..... Member
(Assistant Professor Voravee P. Hoven, Ph.D.)


..... Member
(Rath Pichyangkura, Ph.D.)

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การย่อยไคตินจากแกนหมึกด้วยเอนไซม์จากรา *Aspergillus fumigatus* และโคลนแบคทีเรีย *Serratia sp.* สามารถผลิตเอ็น-แอซีทิล-ดี-กลูโคซามีน (GlcNAc) และเอ็น,เอ็น-ไดแอซีทิลโคโทไบโอส [(GlcNAc)₂] อย่างเฉพาะเจาะจงได้ เอนไซม์จากรา *Aspergillus fumigatus* (4 U/1 g of chitin) สามารถย่อยไคติน (3% w/v) ที่ pH เป็น 3 อุณหภูมิ 40 °C ได้ผลิตภัณฑ์เป็น GlcNAc ด้วยเปอร์เซ็นต์ผลผลิต 72% ภายในเวลา 2 วัน การย่อยไคติน (3% w/v) ด้วยเอนไซม์จากโคลนแบคทีเรีย *Serratia sp.* (1 U/1 g of chitin) ที่ pH เท่ากับ 6 อุณหภูมิ 37 °C ทำการบ่มเป็นเวลา 6 วันให้ผลิตภัณฑ์เป็น (GlcNAc)₂ และ GlcNAc ด้วยเปอร์เซ็นต์ผลผลิต 43% และ 2.6% ตามลำดับ การแยก GlcNAc สามารถทำได้โดยการตกตะกอนจากสารละลาย GlcNAc ที่มีความเข้มข้นสูงด้วยเอทานอล ตามด้วยการกำจัดสีด้วยผงถ่านให้ GlcNAc บริสุทธิ์ด้วยเปอร์เซ็นต์ผลผลิต 64% การย่อยไคตินด้วยเอนไซม์จากแบคทีเรีย (5 U/1 g of chitin) ตามด้วยการแยก (GlcNAc)₂ โดยใช้คอลัมน์ที่มี activated charcoal เป็นเฟสคงที่ชะด้วยเฟสเคลื่อนที่ที่มีเปอร์เซ็นต์เอทานอลในน้ำตั้งแต่ 0-30% ให้ (GlcNAc)₂ บริสุทธิ์ด้วยเปอร์เซ็นต์ผลผลิต 40%

ภาควิชา : เคมี

สาขาวิชา : เคมีอินทรีย์

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ลายมือชื่อนิสิต.....*จิตติมา มณีกุล*.....

ลายมือชื่ออาจารย์ที่ปรึกษา.....*ดร.มงคล สุขวัฒนาสินธิ์*.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....*ดร.หรรษา ปุณณะพยัคฆ์*.....

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KEY WORD: N-ACETYL-D-GLUCOSAMINE /N,N'-DIACETYLCHITOBIOSE / *Aspergillus fumigatus* / *Serratia sp.*

THITIMA MANEEKUL : PREPARATION OF N-ACETYL-D-GLUCOSAMINE AND N,N'-DIACETYLCHITOBIOSE BY ENZYMATIC HYDROLYSIS OF SQUID PEN CHITIN.
 THESIS ADVISOR : ASSOC. PROF. MONGKOL SUKWATTANASINITT, Ph.D. THESIS
 COADVISOR : ASSOC. PROF. HUNSA PUNNAPAYAK, Ph.D. 61 pp. ISBN 974-14-2528-7.

Squid pen chitin (β -chitin) was hydrolyzed by crude enzymes from two sources; fungal enzyme from *Aspergillus fumigatus* and bacterial enzyme from cloned *Serratia sp.* to selectively produced N-acetyl-D-glucosamine (GlcNAc) and N,N'-diacetylchitobiose ((GlcNAc)₂) respectively. The crude enzyme (4 U/1 g of chitin) from *A. fumigatus* hydrolyzed chitin (3% w/v) at pH 3, 40 °C and gave 72% HPLC yield of GlcNAc within 2 days. The hydrolysis of chitin (3% w/v) with the crude enzyme (1 U/1 g of chitin) from cloned bacteria *Serratia sp.* at pH 6, 37 °C for 6 days gave 43% and 2.6% HPLC yield of (GlcNAc)₂ and GlcNAc, respectively. The isolation of GlcNAc by ethanol precipitation from the highly concentrated solution of crude product followed by the decoloration with the activated charcoal gave pure GlcNAc with 64% isolated yield. The hydrolysis of chitin by bacterial enzyme 5 U/1 g chitin followed by isolation of (GlcNAc)₂ by activated charcoal column chromatography using stepwise-gradient elution of water up to 30% ethanol gave pure (GlcNAc)₂ in 40% yield.

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Student's Signature.....Thitima Maneekul
 Advisor's Signature.....Assoc. Prof. Mongkol Sukwattanasinitt
 Co-advisor's Signature.....Assoc. Prof. Hunsa Punnapayak

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List of Abbreviations

CCMM	colloidal chitin minimum medium	mL	milliliter (s)
cm	centimeter	mM	millimolar
°C	degree celsius	MRM	multiple reaction monitoring
DI-water	deionized water	mU	milliunit
DP	degree of polymerization	M_w	molecular weight
ESI	electrospray ionization	m/z	mass to charge ratio
g	gram (s)	PDA	potato dextrose agar
GlcNAc	<i>N</i> -acetyl-D- glucosamine	PDB	potato dextrose broth
(GlcNAc) ₂₋₇	<i>N,N'</i> - diacetylchito(biose, ..., heptaose)	ppm	part per million
GPC	gel permeation chromatography	rpm	round per minute
HPLC	high performance liquid chromatography	sec	second
LC	liquid chromatography	TCA	trichloroacetic acid
MS	mass spectrometry	U	unit
mg	milligram	Å	angstrom
M	molar	α	alpha
min	minute	β	beta
		γ	gamma
		μ L	microliter
		μ m	micrometer
		%	percent
		%DA	percent degree of acetylation