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จากราเอนโดไฟต์สายพันธุ์ EF6

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PURIFICATION AND CHARACTERIZATION OF GLUCOAMYLASE FROM ENDOPHYTIC FUNGUS EF6

Miss Patcharaporn Tangngamsakul

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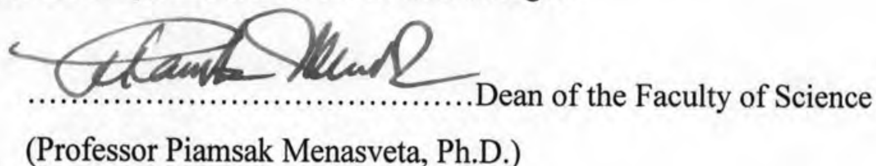
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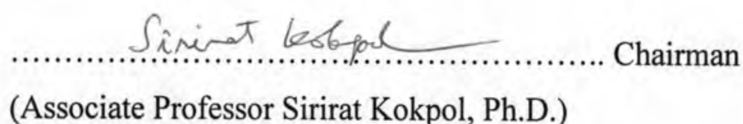
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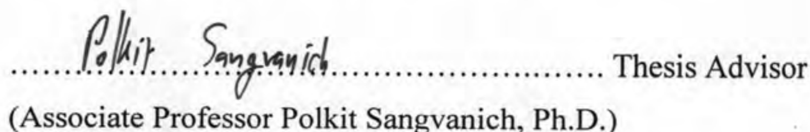
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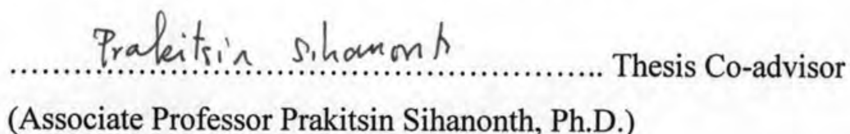
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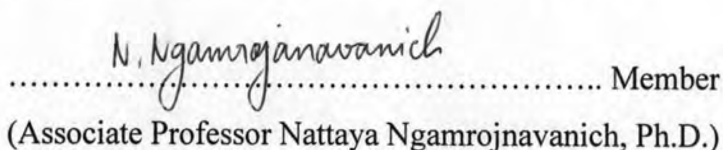
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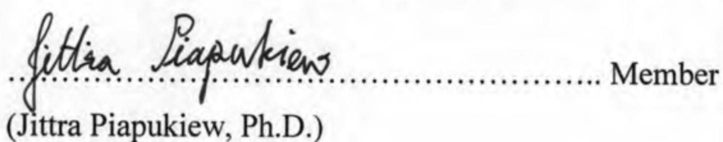
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จากการคัดเลือกราเอนโดไฟต์ทั้งหมด 25 ชนิด จากคลังเชื้อ RCBC พบว่า EF6 สามารถสร้างแอมีเลสได้มากที่สุดภายหลังการบ่มเป็นเวลา 8 วันในอาหารเหลวที่มีแป้งเป็นองค์ประกอบ จากนั้นทำการตกตะกอนโปรตีนด้วย 90% เกลือแอมโมเนียมซัลเฟต และนำไปแยกแอมีเลส ด้วยเทคนิค Ion Exchange Chromatography (Q Sepharose) และ Gel Filtration (Superdex 75) ตามลำดับ แอมีเลสที่ได้มีความ บริสุทธ์มากขึ้น 14.93 เท่า และมีกิจกรรมของเอนไซม์คิดเป็น 10.93 เปอร์เซ็นต์ เมื่อเทียบกับสารละลายโปรตีนเริ่มต้น โดยเอนไซม์แอมีเลสที่ได้นี้มีมวลโมเลกุลประมาณ 62 กิโลดาลตัน หลังจากนั้นทำการหาสภาวะที่เหมาะสมต่อการทำงานของเอนไซม์พบว่าเอนไซม์แอมีเลสสามารถทำงานได้ดีที่อุณหภูมิระหว่าง 50-60 องศาเซลเซียส และที่ pH 5.0-6.0 นอกจากนี้ความสามารถในการคงตัวของแอมีเลส ภายหลังการบ่มไว้เป็นเวลา 30 นาที พบว่ายังคงสามารถทำงานได้ดีที่อุณหภูมิต่ำกว่า 60 องศาเซลเซียส แต่ที่ 60 องศาเซลเซียส การทำงานของเอนไซม์จะลดลงเหลือ 43 เปอร์เซ็นต์ และยังพบว่าไอออนโลหะมีผลต่อการทำงานของเอนไซม์ซึ่ง Ca^{2+}, Ba^{2+} และ Mg^{2+} จะสามารถกระตุ้นการทำงานของแอมีเลสให้ดีขึ้น ในขณะที่ Al^{3+}, Ag^{2+} และ Cu^{2+} จะไปยับยั้งการทำงานของเอนไซม์ โดยแป้งมันสำปะหลังจะเป็นวัตถุดิบที่เอนไซม์สามารถย่อยได้ดีที่สุด โดยเอนไซม์มีค่า K_m และ V_{max} เท่ากับ 2.63 mg และ 1.25 mM/min ตามลำดับ จากนั้นนำเอนไซม์ที่ได้ไปพิสูจน์เอกลักษณ์ทาง Mass spectrometry ผลที่ได้พบว่าน่าจะเป็นกลูโคแอมีเลส เนื่องจากมีลำดับกรดอะมิโนบางส่วนคล้ายคลึงกับกลูโคแอมีเลส (3.2.1.3) ที่ได้จาก *Amorphotheca resinae*

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ปีการศึกษา...............2006...................

ลายมือชื่อนิสิต....พัชราภรณ์ ตั้งงามสกุล............
ลายมือชื่ออาจารย์ที่ปรึกษา.........................
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KEY WORD: GLUCOAMYLASE/ ENDOPHYTIC FUNGI /PURIFICATION/ STARCH

PATCHARAPORN TANGNGAMSAKUL: PURIFICATION AND CHARACTERIZATION OF GLUCOAMYLASE FROM ENDOPHYTIC FUNGUS EF6. THESIS ADVISOR: ASSOC. PROF. POLKIT SANGVANICH, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. PRAKITSIN SIHANONTH, Ph.D. 108 pp. ISBN : 974-14-2508-2.

Selective 25 endophytic fungi from stock culture of RCBC found that EF6 has maximum amylase activity was obtained after 8 days of incubation. Amylase from EF6 was extracted with 90% $(NH_4)_2SO_4$ and purified by ion exchange chromatography using Q sepharose and gel filtration using Superdex75, respectively. The purity of amylase was 14.93 fold over the original culture filtrate with a 10.93% recovery activity. The estimated molecular mass of purified amylase is about 62 kDa. Optimum conditions for this amylase are 50-60°C, pH 5.0-6.0. Amylase still functions with the temperature below 60°C. At this point the activity was decreased to 43%. The activity of this enzyme can be increased with the present of Ba^{2+}, Ca^{2+} and Mg^{2+} whereas Al^{3+}, Ag^{2+} and Cu^{2+} acted as enzyme inhibitors. For various type of starch, tapioca starch is the easiest material for the digestion process. The K_m and V_{max} value were 2.63 mg and 1.25 mM/min, respectively. The purified amylase was indentified by mass spectrometry. The result shown that purified amylase may be glucoamylase (3.2.1.3) because partial amino acid sequences of that enzyme similar to glucoamylase (3.2.1.3) from *Amorphotheca resinae*.

Field of Study......Biotechnology..... Student's Signature.Patcharaporn Tangngamsakul

Academic Year........2006............ Advisor's Signature....Polkit Sangvanich..........

Co-Advisor' Signature....Prakitsin Sihanonth

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LIST OF ABBREVIATIONS

μl	microliter
μmol	micromolar
ACN	Acetonitrile
Bis	*N,N'*-methylenebiscrylamide
BSA	Bovine serum albumin
°C	Degree Celsius
C	Crosslinking factor [%]
CCA	α-cyano-4-hydroxycinnamic acid
cm	centimeter
CTAB	Cetyl trimethyl ammonium bromide
Da	Dalton
DNA	Deoxyribonucleic acid
DNS	Dinirosalicylic Acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EtOH	Ethanol
ESI	Electrospray ionization
g	gram
h	hour
IAA	Isoamyl alcohol
IEC	Ion exchange chromatography
ITS	Internal transcribe spacer
kDa	Kilodalton
K_m	Michaelis constant for the substrate
L	liter
M	Molar
mA	Milliampare
MALDI	Matrix Assisted Laser Desorption Ionisation
min	Minute
mg	Milligram

mg/ml	Milligran per milliter
ml	Milliter
mm	Millimeter
mM	Millimolar
MS/MS	Tandem Mass spectrometry
m/z	Mass per charge
Native-PAGE	Native polyacrylamide gel electrophoresis
nm	Nanometer
PDA	Potato dextrose agar
rpm	Revolution per minute
S	Substrate
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
T	Total acrylamide concentration (%)
TEMED	*N,N,N',N'*- tetramethylenebiscrylamide
TFA	Trifluoroacetic acid
U	Unit activity
U/mg	Unit activity per milligram
v	velocity of the reaction
V	Volt
V_{max}	maximum velocity of the reaction
v/v	Volume by volume
w/v	Weight by volume