การโคลนยีนและลักษณะสมบัติของยีนไซโคลเดกซ์ทรินไกลโคซิลทรานสเฟอเรสจากแบคทีเรียทนร้อน *Paenibacillus sp.* RB01 และ *Paenibacillus sp.* T16

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GENE CLONING AND CHARACTERIZATION OF THE CYCLODEXTRIN GLYCOSYLTRANSFERASE GENE FROM THERMOTOLERANT *Paenibacillus* sp. RB01 AND *Paenibacillus* sp. T16

Miss Ratiya Charoensakdi

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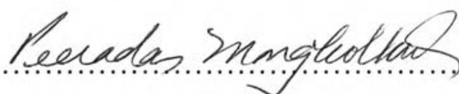
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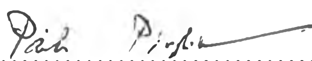
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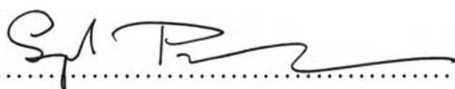
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ได้โคลนยีน CGTase จากแบคทีเรียทนร้อนที่คัดเลือกจากดินบริเวณแหล่งน้ำพุร้อนในจังหวัดราชบุรีและจังหวัดตาก คือ *Paenibacillus sp.* strain RB01 และ T16 โดยใช้โครโมโซมัลดีเอ็นเอเป็นต้นแบบในการเพิ่มจำนวนชิ้นยีน CGTase ได้มีการออกแบบสายไพรเมอร์ขนาด 24 นิวคลีโอไทด์ให้ครอบคลุมส่วนของโปรโมเตอร์และ CGTase ยีน ชิ้นยีนที่ได้จากวิธี PCR ถูกนำมาเชื่อมต่อกับเวคเตอร์และโคลนเข้าเชื้ออีโคไล ได้โคลนที่ให้ชื่อว่า pRB และ pT ตามลำดับ ผลการวิเคราะห์ลำดับนิวคลีโอไทด์ของ pRB และ pT ประกอบด้วยนิวคลีโอไทด์ขนาด 2194 และ 2139 คู่เบส ซึ่งกำหนดการสร้างโปรตีนที่ประกอบด้วย 732 และ 713 กรดอะมิโนตามลำดับ ซึ่งมีลำดับกรดอะมิโนที่ใกล้เคียงกับเชื้อ *Paenibacillus* sp. A11 ด้วยค่าความเหมือน 97% และ99% ผลการทดลองหลังทำเอนไซม์จากรีคอมบิแนนท์ทั้งสองให้บริสุทธิ์และศึกษาสมบัติพบว่า เอนไซม์จาก pRB และ pT มีน้ำหนักโมเลกุลประมาณ 65 และ 77 กิโลดาลตันจากการวิเคราะห์ด้วยอิเล็กโทรโฟเรซิสแบบเสียสภาพที่มีเอสดีเอสและค่า pI เท่ากับ 5.85 เอนไซม์ RB01 และ pRB มี pH และอุณหภูมิที่เหมาะสมในการเกิดปฏิกิริยาแตกต่างกัน โดยปฏิกิริยา dextrinizing มีค่า pH และอุณหภูมิที่เหมาะสมในช่วง 5-6, 60°C และ 5-9, 50-70°C ตามลำดับ ปฏิกิริยา cyclizing มี pH และอุณหภูมิที่เหมาะสมในช่วง 6.5, 60-70°C และ 6.5, 50-60°C ตามลำดับ ในขณะที่เอนไซม์ T16 และ pT ปฏิกิริยา dextrinizing มี pH และอุณหภูมิที่เหมาะสมในช่วง 5-9, 60°C และ 5-9, 40-70 °C. ปฏิกิริยา cyclizing มี pH และ อุณหภูมิที่เหมาะสมคือ 6.5, 60-70°C และ 6.5, 50-70°C. เอนไซม์ทั้งสี่มีเสถียรภาพในช่วง pH 6-10 และอุณหภูมิในช่วง 40-60°C ได้นาน 30 นาที และ เมื่ออยู่ที่ pH 7-9 อุณหภูมิในช่วง 45-55°C เอนไซม์มีเสถียรภาพได้นาน 60 นาที และการอยู่ในสภาพที่แวดล้อมด้วยสารตั้งต้น (แป้งเข้มข้น 6%) จะช่วยเพิ่มเสถียรภาพที่อุณหภูมิ 70 °C ได้นานขึ้น การศึกษาทางจลนพลศาสตร์ของเอนไซม์ทั้งสี่ด้วยปฏิกิริยาควบคู่ พบว่า RB01 และ pRB มีค่า k_{cat} ต่อ G-α-CD สูง ส่วนเอนไซม์ T16 และ pT มีค่า k_{cat} ต่อ α-CD และ γ-CD สูง พบว่า RB01 และ pRB มีค่าค่าสัมประสิทธิ์เอนไซม์ (k_{cat}/K_m) ต่อ HP-β-CD สูง ส่วนเอนไซม์ T16 และ pT มีค่า k_{cat}/K_m ต่อ β-CD สูง เอนไซม์ RB01 pRB และ pT สามารถย่อยแป้งให้เกิดผลิตภัณฑ์หลักเป็น β-CD เมื่อตรวจสอบด้วยวิธี HPLC พบว่ามีอัตราส่วนของ α:β:γ-CD เป็น 0.57 : 1 : 0.13 โดยเอนไซม์ RB01, 0.21 : 1 : 0.05 โดยเอนไซม์ pRB, 0.95 : 1 : 1.57 โดยเอนไซม์ T16 และ 0.25 : 1 : 0.51 โดยเอนไซม์ pT ตามลำดับ

ภาควิชา..........ชีวเคมี....................ลายมือชื่อนิสิต..

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RATIYA CHAROENSAKDI : GENE CLONING AND CHARACTERIZATION OF THE CYCLODEXTRIN GLYCOSYLTRANSFERASE GENE FROM THERMOTOLERANT PAENIBACILLUS SP. RB01 AND PAENIBACILLUS SP. T16. THESIS ADVISOR: ASSOCIATE PROFESSOR. TIPAPORN LIMPASENI, PH.D., THESIS COADVISOR: ASSOCIATE PROFESSOR VICHEIN RIMPHANITCHAYAKIT, PH.D. 150 P. ISBN 974-17-6430-8

Genes encoding cyclodextrin glycosyltransferase (CGTase), from the thermotorelant *Paenibacillus* sp. RB01 and *Paenibacillus* sp. T16 isolated from the hot spring area in Ratchaburi and Tak provinces were cloned into *Escherichia coli*. Genomic DNA was extracted and used as the template for CGTase gene amplification using a pair of designed primers that amplified a whole CGTase gene including its promoter. The PCR product was ligated to pGEM®-T vector and then transformed into *E. coli* JM109. The transformants of *Paenibacillus* sp. RB01 and *Paenibacillus* sp. T16 were pRB and pT, respectively. Their nucleotide sequences from pRB and pT consisted of 2194 and 2139 bp open reading frame with 732 and 713 deduced amino acid residues, respectively. The deduced amino acid sequences of pRB and pT showed 97% and 99% identity with the CGTase of *Paenibacillus* sp. A11, respectively. The recombinants required one-third culture time of wild types and a neutral pH for culture medium to produce compatible amount of CGTase. The recombinant and wild type CGTases were purified and characterized in parallel. Both enzymes showed almost similar biochemical characteristics in terms of molecular weight, optimum pH and temperature. There were some significant differences in pH, temperature stability and kinetic parameters. The recombinant enzymes were more stable at higher temperature and low pH. Molecular weight of GCTase from pRB and pT were estimated to be 65 and 77 kDa by SDS-PAGE and pI of 5.85. The optimum pH and temperature for dextrinizing activities of CGTases from RB01 and pRB were 5-6, 60°C and 5-9, 50-70°C. The optimum pH and temperature for cyclization activity of CGTases from RB and pRB were 6.5, 60-70°C and 6.5, 50-60°C. The optimum pH and temperature for dextrinizing activity of CGTases from T16 and pT were 5-9, 60°C and 5-9, 40-70°C. The optimum pH and temperature for cyclization activity of CGTases from T16 and pT were 6.5, 60-70°C and 6.5, 50-70°C. The enzymes were stable in a wide pH range of 6-10 and temperature at 40-60°C within 30 minutes. The enzymes were stable at pH 7-9 and temperature of 45-55°C within 60 min. The enzymes were specific for substrates with α-1,4 glycosidic bonds, with minimum of 3 glucose units. The turnover numbers (k_{cat}) of the CGTases from both RB01 and pRB with G-α-CD were higher than those of β-CD and its derivatives. The turnover numbers (k_{cat}) of the CGTases from T16 and pT with natural CD (α-CD and γ-CD) were higher than those of β-CD and its derivatives.. The efficiency (k_{cat}/K_m) of CGTases from both RB01 and pRB were highest with HP-β-CD and the efficiency (k_{cat}/K_m) of CGTases from T16 and pT were highest with β-CD. The dominant end-products obtained were β-CDs. CGTases catalyzed the conversion of starch to CDs with a ratio of α– : β– : γ–CD of 0.57 : 1 : 0.13 for RB01 and 0.21 : 1 : 0.05 for pRB and 0.95 : 1 : 1.57 for T16 and 0.25 : 1 : 0.51 for pT, respectively.

Department ....Biochemistry............Student's signature.......................................

Field of study..............Biochemistry.............Advisor's signature.......................................

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ABBREVIATIONS

A	absorbance
BSA	bovine serum albumin
CD	cyclodextrin
CGTase	cyclodextrin glycosyltransferase
cm	centimeter
^{o}C	degree Celsius
Da	Dalton
DEAE	diethylaminoethyl
DNA	deoxyribonucleic acid
DTT	dithiothreitol
EDTA	ethylenediamine tetraacetic acid
et al.	Et. Alii (latin), and others
g	gram
IPTG	isopropylthiogalactoside
kb	kilobase
k_{cat}	catalytic constant
k_{cat}/K_m	specific constant
K_m	Michaelis constant
l	litre
μg	microgram
μl	microlitre
M	molar
mA	milliampere

ml	milliliter
mM	millimolar
mol	mole
MW	molecular weight
nm	nanometer
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
pI	isoelectric point
PMSF	phenylmethylsulfonyl fluoride
rpm	revolution per minute
SDS	sodium dodecyl sulfate
sec	second
V	volt
V_{max}	maximal velocity,
V/V	volume by volume
W/V	weight by volume