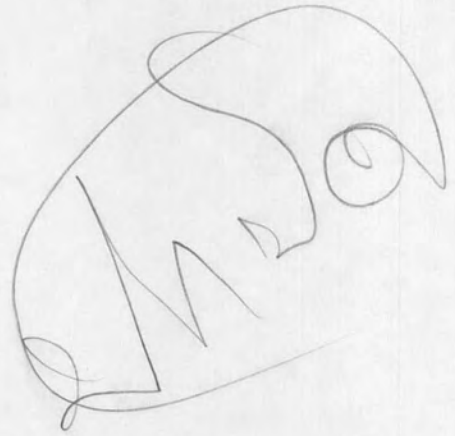


การทำให้บริสุทธิ์บางส่วนและลักษณะสมบัติของเอนไซม์ที่สามารถสลายพอลิเอทิลีนไกลคอล
และพอลิโพรพิลีนไกลคอลจาก *Pseudomonas* sp. PE-2



นางสาว สุานิดา วุฒิมุกดาภิบาล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเทคโนโลยีชีวภาพ

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2549

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

PARTIAL PURIFICATION AND CHARACTERIZATION OF
POLYETHYLENE GLYCOL/POLYPROPYLENE GLYCOL-
DEGRADING ENZYMES FROM *Pseudomonas* sp. PE-2

Miss Thanida Vuthikulvanich

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Biotechnology
Faculty of Science
Chulalongkorn University
Academic Year 2006
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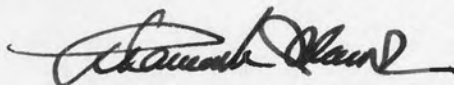
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Pseudomonas sp. PE-2

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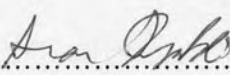
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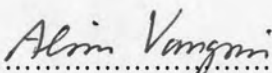
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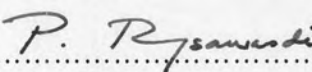
Accepted by the Faculty of Science, Chulalongkorn University in
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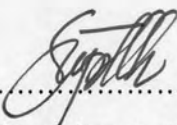

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ฐานันดา วุฒิมิกุลวานิช : การทำให้บริสุทธิ์บางส่วนและลักษณะสมบัติของเอนไซม์ที่สามารถสลายพอลิเอทิลีนไกลคอลและพอลิโพรพิลีนไกลคอลจาก *Pseudomonas* sp. PE-2. (PARTIAL PURIFICATION AND CHARACTERIZATION OF POLYETHYLENE GLYCOL/POLYPROPYLENE GLYCOL-DEGRADING ENZYMES FROM *Pseudomonas* sp. PE-2) อ. ที่ปรึกษา : ผศ.ดร. อลิสา วังโน, 200 หน้า.

พอลิเอทิลีนไกลคอล (PEG) และพอลิโพรพิลีนไกลคอล (PPG) เป็นวัสดุที่ปลูกใช้อย่างกว้างขวางในอุตสาหกรรมหลายประเภท ยังผลให้เกิดการรั่วไหลสู่ระบบบำบัดน้ำเสียและสิ่งแวดล้อม ได้มีการศึกษากระบวนการย่อยสลายทางชีวภาพของสารดังกล่าวใน *Pseudomonas* sp. PE-2 พบแอกติวิตีของพอลิเอทิลีนไกลคอลดีไฮโดรจีเนส (PEG-DH) และพอลิโพรพิลีนไกลคอลดีไฮโดรจีเนส (PPG-DH) ในสารสกัดปลอดเซลล์ *Pseudomonas* sp. PE-2 เอนไซม์ทั้งสองชนิดถูกเหนี่ยวนำเมื่อใช้ PEG และ PPG เป็นแหล่งคาร์บอนและแหล่งพลังงาน ในการเจริญช่วงปลายของระยะการเพิ่มจำนวนเชื้อ พบแอกติวิตีส่วนใหญ่ของ PEG-DH (76.6%) ในส่วนของเพอริพลาซึม ขณะที่แอกติวิตี PPG-DH (82%) อยู่ในส่วนของไซโทพลาซึม จากการทำให้บริสุทธิ์บางส่วนโดยการตกตะกอนด้วยเกลือแอมโมเนียมซัลเฟตอิ่มตัว คอลลิมน์ แลกเปลี่ยนประจุลบ และคอลลิมน์ไฮโดรโฟบิก ได้ความบริสุทธิ์เพิ่มขึ้น 11.8 และ 51.4 เท่า ตามลำดับ PEG-DH บริสุทธิ์บางส่วนประกอบด้วย 1 หน่วยย่อยที่มีน้ำหนักโมเลกุล 73.6 กิโลดาลตัน สำหรับ PPG-DH มีน้ำหนักโมเลกุล 66.2 กิโลดาลตัน ประกอบด้วย 2 หน่วยย่อยที่มีน้ำหนักโมเลกุลเท่ากันคือ 36 กิโลดาลตัน pH และ อุณหภูมิที่เหมาะสมในการทำงานของเอนไซม์ PEG-DH และ PPG-DH คือ 9.0 และ 25 องศาเซลเซียส และ 7.5 และ 25 องศาเซลเซียส ตามลำดับ เอนไซม์ PEG-DH และ PPG-DH ที่บริสุทธิ์บางส่วนมีความเสถียรต่อ pH ในช่วง 8.0-9.5 และ 7.0-8.0 ตามลำดับ นอกจากนั้นเอนไซม์ทั้งสองมีความเสถียรต่ออุณหภูมิที่ต่ำกว่า 30 องศาเซลเซียส และมีไพโรไลควิโนลีนควิโนน (PQQ) ทำหน้าที่เป็นโคแฟกเตอร์ ไอออนโลหะที่นำมาทดสอบที่ความเข้มข้น 2 มิลลิโมลาร์ สามารถยับยั้งแอกติวิตี 8-100% ต่อเอนไซม์ PEG-DH และ 5-30% ต่อเอนไซม์ PPG-DH ตามลำดับ แต่ Ni^{2+} สามารถเพิ่มประสิทธิภาพแอกติวิตีเอนไซม์ PPG-DH ประมาณ 38% เอนไซม์ทั้งสองสามารถออกซิไดส์ PEG และ PPG ได้หลากหลายชนิด โดยมี PEG 600 และ PPG 725 เป็นสับสเตรทที่ดีที่สุดสำหรับ PEG-DH และ PPG-DH ตามลำดับ ค่า K_m ของ PEG-DH สำหรับ PEG 600, PEG 2000, PEG 4000, PEG 6000 และ PEG 8000 เท่ากับ 0.7, 3.3, 4.6, 30.5 และ 28.3 มิลลิโมลาร์ ตามลำดับ ขณะที่ค่า K_m ของ PPG-DH สำหรับ PPG 725, PPG 1000 และ PPG 2000 เท่ากับ 5.1, 4.6 และ 12.6 มิลลิโมลาร์ ตามลำดับ

สาขาวิชา.....เทคโนโลยีชีวภาพ.....ลายมือชื่อนิสิต.....*Tinon Dan*.....
ปีการศึกษา.....2549.....ลายมือชื่ออาจารย์ที่ปรึกษา.....*D.N.*.....

4672254623 : MAJOR BIOTECHNOLOGY

KEY WORD : POLYETHYLENE GLYCOL DEHYDROGENASE /

POLYPROPYLENE GLYCOL DEHYDROGENASE / *Pseudomonas* sp.

THANIDA VUTHIKULVANICH : PARTIAL PURIFICATION AND CHARACTERIZATION OF POLYETHYLENE GLYCOL/POLYPROPYLENE GLYCOL-DEGRADING ENZYMES FROM *Pseudomonas* sp PE-2. THESIS ADVISOR : ASST. PROF. ALISA VANGNAI, Ph.D., 200 pp.

Polyethylene glycol (PEG) and polypropylene glycol (PPG) have been widely used as raw materials for many industries. Consequently, they are released into wastewater system and environment. Their biodegradation have been studied in *Pseudomonas* sp. PE-2. Polyethylene glycol dehydrogenase (PEG-DH) and polypropylene glycol dehydrogenase (PPG-DH) activities were found in cell-free extract of *Pseudomonas* sp. PE-2. Both enzymes were inducible when PEGs and PPGs served as a sole carbon and energy sources. In the late phase of logarithmic growth, most of the PEG-DH activities (76.6%) were found in the periplasm, while PPG-DH activities (82%) were in the cytoplasm. The PEG-DH and PPG-DH in cell-free extract were partially purified by ammonium sulfate precipitation, anion-exchange column and hydrophobic column to 11.8 and 51.4 fold, respectively. The partially purified PEG-DH is a monomer of 73.6 kDa. PPG-DH appeared to consist of two identical subunits of 36 kDa. The optimum pH and temperature of the PEG-DH and PPG-DH were at pH 9.0, 25°C and pH 7.5, 25°C, respectively. The partially purified PEG-DH and PPG-DH were stable in the pH range of 8.0 to 9.5 and 7.0 to 8.0, respectively. Moreover, both enzymes were stable below 30°C and had pyrroloquinoline quinone (PQQ) as cofactor. The metal ions at 2 mM inhibited the PEG-DH and PPG-DH activities by 8-100% and 5-30%, respectively, while Ni²⁺ could enhance the PPG-DH activity by 38%. The enzymes oxidized various kinds of PEGs and PPGs, among which, PEG 600 and PPG 725 were the most active substrates for PEG-DH and PPG-DH, respectively. The apparent K_m values of PEG-DH for PEG 600, PEG 2000, PEG 4000, PEG 6000 and PEG 8000 were 0.7, 3.3, 4.6, 30.5 and 28.3 mM, respectively, whereas those of PPG-DH for PPG 725, PPG 1000 and PPG 2000 were 5.1, 4.6 and 12.6 mM, respectively.

Field of study.....Biotechnology.....Student's signature.....*Thanida Vuthi*.....

Academic year.....2006.....Advisor's signature.....*Alisa Vangnai*.....

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my advisor, Assistant Professor Alisa Vangnai for her excellent instruction, guidance, encouragement, attention and support throughout this thesis. Without her kindness and understanding, this work could not be accomplished.

My gratitude is also extended to Associate Professor Aran Incharoensakdi, Associate Professor Piamsook Pongsawasdi and Assistant Professor Supat Chareonpornwattana for serving as thesis committee, for valuable comments and also for useful suggestions.

My appreciation is also expressed to Professor Fusako Kawai, of the Research Institute for Bioresources, Okayama University, Japan for her kindness to give a gift, *Pseudomonas* sp. PE-2, and provide the most useful information for this research. I am very grateful to Mr. Surachai Yaiyen for his support in the techniques involving purification and characterization. Moreover, I would like to thank Mr. Thanade Paoin who gives me a hand whenever I need help.

There are so many friends to mention from so little space to fill. I would like to give a deep thank to all staff members of room 604 and friends in the Department of Biochemistry and Program in Biotechnology for their friendship, kind assistance and support.

Finally, the greatest gratitude is expressed to my lovely family (my parents, my brother and my dog) for their infinite love, willpower, support, understanding, encouragement and everything giving to my life.

This work was financially supported by Graduate School and Program in Biotechnology, Faculty of Science, Chulalongkorn University.

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LIST OF ABBREVIATIONS

°C	Degree of Celsius
cm	centimeter
cP	centipoise
Da	daltons
DCIP	2,6-Dichlorophenol-indophenol sodium salt dehydrate
DEAE	diethylaminoethyl
EDTA	Ethelenediaminetetraacetic acid
<i>et al.</i>	Et. Alii (latin), and others
FAD	Flavin adenine dinucleotide
g	Gravitational acceleration
h	hour
HCl	Hydrochloric acid
kDa	kilo Dalton
K_m	Michaelis constant
l	liter
M	molar
mA	milliampere
mg	milligram
min	minute
ml	milliliter
mM	millimolar
mol	mole
MW	molecular weight

NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NBT	nitroblue tetrazolium
nm	nanometer
OD	Optical density
PAG	polyalkylene glycol
PAGE	polyacrylamide gel electrophoresis
PBG	polybutylene glycol
PEG	Polyethylene glycol
PEG-DH	Polyethylene glycol dehydrogenase
PMSF	Phenylmethylsulfonyl fluoride
PPG	Polypropylene glycol
PPG-DH	Polypropylene glycol dehydrogenase
PQQ	pyrroloquinoline quinone
psi	pounds per square inch
PTMG	polytetramethylene glycol
rpm	revolution per minutes
SDS	Sodium dodecyl sulfate
TEMED	<i>N,N,N',N'</i> -Tetramethylene ethylene diamine
v/v	volume by volume
V_{max}	maximal velocity
w/v	weight by volume
μg	microgram
μl	microliter