

การโคลนและการแสดงออกของยีน *picK* จาก *Streptomyces venezuelae*
ใน *Streptomyces* สปีชีส์

นางสาวนนุช บุญเดิม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาชีวเคมี ภาควิชาชีวเคมี

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2546

ISBN 974-17-5455-8

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

**CLONING AND EXPRESSION OF *picK* GENE FROM
Streptomyces venezuelae IN *Streptomyces* SPECIES**

Miss Nongnuch Boonderm

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

Department of Biochemistry

Faculty of Science


Chulalongkorn University

Academic Year 2001

ISBN 974-17-5455-8


Thesis Title CLONING AND EXPRESSION OF *picK* GENE FROM
Streptomyces venezuelae IN *Streptomyces* SPECIES
By Miss Nongnuch Boonderm
Field of Study Biochemistry
Thesis Advisor Suchart Chanama, 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
(Associate Professor Piamsook Pongsawadi, Ph.D.)


..... Thesis Advisor
(Suchart Chanama, Ph.D.)


..... Member
(Associate Professor Siriporn Sittipraneed, Ph.D.)


..... Member
(Assistant Professor Manee Chanama, Ph.D.)

นางนุช บุญเดิม : การโคลนและการแสดงออกของยีน *picK* จาก *Streptomyces venezuelae* เข้าสู่ *Streptomyces* สปีชีส์ (CLONING AND EXPRESSION OF *picK* GENE FROM *Streptomyces venezuelae* IN *Streptomyces* SPECIES) อาจารย์ที่ปรึกษา: ดร.สุชาติ ชะนะมา, 82 หน้า, ISBN 974-17-5455-8

PicK เป็น cytochrome P450 monooxygenase เร่งปฏิกิริยาไฮดรอกซิเลชันซึ่งเปลี่ยน narbomycin เป็น picromycin ที่พบใน *Streptomyces venezuelae* การวิจัยครั้งนี้ได้พยายามนำเอายีน *picK* ที่กำหนดการสังเคราะห์โปรตีน PicK โคลนในดีเอ็นเอพาหะลูกผสม pWHM3 ภายใต้การควบคุมการแสดงออกของยีนโดย *lac* promoter และนำเข้าสู่ *Streptomyces lividans* TK24, *S. narbonensis* และ *Escherichai coli* เมื่อทำการนำพลาสมิดลูกผสมเข้าสู่ *Escherichai coli* สายพันธุ์ XL1-Blue พบว่ามีการแสดงออกของยีน *picK* ได้โปรตีนที่น้ำหนักมวลโมเลกุลเท่ากับ 45 กิโลดาลตัน เมื่อวิเคราะห์ด้วย SDS-PAGE และตรวจพบแอกติวิตี้ของ P450 จากการทำ carbon monoxide difference spectra แต่ไม่สามารถตรวจพบการแสดงออกของยีน *picK* ใน *Streptomyces lividans* สายพันธุ์ TK24 ที่ได้รับพลาสมิดลูกผสมชนิดนี้ (pWHM3-*picK*) แสดงให้เห็นว่าโปรโมเตอร์ของ *lac* operon อาจจะไม่เหมาะสมที่จะนำมาใช้ในการแสดงออกของยีนในแบคทีเรียสกุล *Streptomyces*

ภาควิชา..... ชีวเคมี.....
สาขาวิชา..... ชีวเคมี.....
ปีการศึกษา.....2546.....

ลายมือชื่อนิสิต..... นอ นุช บุญเดิม.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....-

4372292123: MAJOR BIOCHEMISTRY

KEYWORD : *Streptomyces*/ picromycin/ *Streptomyces venezuelae*/ *Streptomyces narbonensis*/ PicK/ *picK*, cytochrome P450 monooxygenase/ cloning/ expression/ pWHM3/ *lac* promoter

NONGNUCH BOONDERM: CLONING AND EXPRESSION OF *picK* GENE FROM *Streptomyces venezuelae* IN *Streptomyces* SPECIES. THESIS ADVISOR: SUCHART CHANAMA, Ph.D., 82 pp. ISBN 974-17-5455-8

PicK, a cytochrome P450 monooxygenase encoded by *picK* gene of *Streptomyces venezuelae*, biocatalyzes the hydroxylation of narbomycin to picromycin. The objectives of the research are to clone *picK* in *Streptomyces* species and *Escherichai coli*. The recombinant *picK* could be expressed as protein detected at molecular weight of 45,000 daltons (SDS-PAGE) and the PicK cytochrome P450 monooxygenase activity could be determined by carbon monoxide (CO) difference spectra analysis. On the other hand, no sign of the *picK* expression and its P450 activity could be observed by SDS-PAGE and CO-difference spectra analyses in *Streptomyces* species. The results seem to imply that *lac* promoter is unsuitable for gene expression in *Streptomyces*.

Department Biochemistry.....
Field of study....Biochemistry.....
Academic year...2003.....

Student's signature.. Nongnuch Boonderm
Advisor's signature..... Suchart Chanama
Co-advisor's signature.....-

ACKNOWLEDGEMENTS

This thesis would not have been completed without a good advice from many people. I am indebted to my advisor, Dr. Suchart Chanama, for his valuable advice and encouragement throughout the course of the thesis.

Sincere thanks are due to Assist. Prof. Dr. Manee Chanama, for supporting the bacterial sample, plasmid vector, and recombinant *picK* gene, and for serving as thesis committee.

Also, I would also like to thank to Assoc. Prof. Dr. Piamsook Pongsawat and Assoc. Prof. Dr. Siriporn Sittipraneed for serving as thesis chairman and committee members.

The financially support of research scholarship, Chulalongkorn university, is also gratefully acknowledged.

Many thanks are given to graduate students from other laboratories (room 604, 618 and 708) at Department of Biochemistry for their helps and friendship during my study.

Finally, I would like to express my deepest appreciation and gratefulness to my parents, my sister and brother for their warmest love, care, understanding and encouragement extended throughout my study.

CONTENTS

	Page
THAI ABSTRACT.....	iv
ENGLISH ABSTRACT.....	v
ACKNOWLEDGEMENT.....	vi
CONTENTS.....	vii
LIST OF TABLES.....	x
LIST OF FIGURES.....	xi
ABBREVIATIONS.....	xii
CHAPTER ONE LITURATURE REVEIW.....	1
Introduction.....	1
Streptomycetes.....	2
Polyketide.....	5
Cytochrome P450 monooxygenase	11
Picromycin biosynthesis.....	16
Gene expression <i>Streptomyces</i>	18
Cloning vectors and systems.....	18
Expression vector and system.....	18
Boctechnological application.....	19
CHAPTER TWO MATERIALS AND METHODS.....	21
Materials and Equipments	21
Bacterial strains and plasmids.....	21
Chemicals, enzymes and culture media.....	21
Equipments.....	22

Methods.....	23
Preparation of plasmid pWHM3.....	23
Preparation of <i>picK</i> gene.....	24
Agarose gel electrophoresis.....	24
Cloning of <i>picK</i> in pWHM3.....	25
Transformation of pWHM3- <i>picK</i> into <i>E. coli</i> XL1-Blue.....	26
Screening of recombinant <i>E. coli</i> clone carrying pWHM3/ <i>picK</i>	29
DNA sequencing of <i>picK</i> in pWHM3- <i>picK</i> recombinant.....	29
Expression pWHM3/ <i>picK</i> in <i>E.coli</i> XL1-Blue.....	30
Induction of <i>picK</i> in <i>E.coli</i> XL1-Blue	30
Protein analysis by SDS-PAGE.....	30
CO-difference spectra analysis.....	31
Expression pWHM3/ <i>picK</i> in <i>Streptomyces</i> species.....	31
Preparation of <i>Streptomyces lividans</i> TK24 and <i>S. narbonensis</i> protoplasts.....	31
Transformation of pWHM3/ <i>picK</i> in <i>Streptomyces lividans</i> TK24 and <i>S. narbonensis</i> protoplasts	32
Screening of recombinant clone(s) carrying pWHM3/ <i>picK</i>	32
Expression of <i>picK</i> in <i>Streptomyces lividans</i> TK24 and <i>S. narbonensis</i> protoplasts	33

CHAPTER THREE RRESULTS.....	34
Construction of expression plasmid pWHM3/ <i>picK</i>	34
Expression of the <i>picK</i> gene in <i>E.coli</i> strain XL1-Blue.....	42
Expression of pWHM3- <i>picK</i> in <i>Streptomyces</i>	45
Expression of pWHM3- <i>picK</i> in <i>Streptomyces lividans</i> TK24	45
Expression of pWHM3- <i>picK</i> in <i>Streptomyces narbonensis</i>	52
CHAPTER FOUR DISCUSSION.....	53
CHAPTER FIVE CONCLUSIONS.....	57
REFERENCES.....	58
APPENDICES.....	65
BIOGRAPHY.....	82

LIST OF TABLES

Table	Page
1.1 Approximate numbers of secondary metabolites produced by different groups of organisms, as of 1994.....	3
1.2 The polyketides are produced from Stretomycetes.....	4
3.1 The transformation efficiency of <i>Streptomyces lividans</i> TK24 protoplasts	47
3.2 The transformation efficiency of <i>Streptomyces narbonensis</i> protoplasts	52

LIST OF FIGURES

Figure	Page
1.1 The chemical structures of polyketide	6
1.1 (continued) The chemical structures of polyketide	7
1.2 Polyketide synthesis.....	9
1.3 The action of cytochrome P450.....	13
1.4 The catalytic cycle of cytochrome P450.....	15
1.5 Biosynthesis of picromycin.....	17
2.1 Plasmid maps of pWHM3 and pMC- <i>picK</i>	27
2.2 Construction of pWHM3- <i>picK</i>	28
3.1 Agarose gel electrophoresis of recombinant plasmid extraction.....	36
3.2 Restriction enzyme digestion of constructed plasmids, pWHM3- <i>picK</i>	38
3.3 The restriction map of constructed plasmid, pWHM3/ <i>picK</i>	39
3.4 Nucleotide sequences of <i>picK</i> gene in pWHM3- <i>picK</i>	40
3.5 Deduced amino acid sequences with PicK.....	41
3.6 SDS-PAGE analysis of protein pattern of <i>E. coli</i> XL1-Blue carrying pWHM3- <i>picK</i>	43
3.7 The carbon monoxide difference spectra of whole cell of the <i>E. coli</i> XL1-Blue transformant carrying pWHM3- <i>picK</i> of 24 hours expression.....	44
3.8 Agarose gel electrophoresis of isolated plasmid from <i>S. lividans</i> TK24 transformant.....	48
3.9 Restriction enzymes digestion of <i>S. lividans</i> TK24 transformant.....	49
3.10 Protein pattern of <i>S. lividans</i> TK24 transformant on SDS-PAGE.....	50
3.11 The carbon monoxide difference spectra of whole cell of <i>S. lividans</i> TK24 transformant.....	51
4.1 SDS-PAGE analysis of protein pattern of <i>E. coli</i> BL21(DE3)/pMC- <i>picK</i>	55
4.2 The carbon monoxide difference spectra of the <i>E. coli</i> BL21(DE3)pLysS carrying pMC- <i>picK</i> at 6 hours induction.....	56

ABBREVIATIONS

A	absorbance, 2'-deoxyadenosine (in a DNA sequence)
bp	base pairs
BSA	bovine serum albumin
C	2'-deoxycytidine (in a DNA sequence)
°C	degree Celsius
cm	centrimetre
Da	Dalton
DNA	deoxyribonucleic acid
EDTA	ethylene diamine tetraacetic acid
G	2'-deoxyguanosine (in a DNA sequence)
IPTG	isopropyl-thiogalactoside
kb	kilobase pairs in duplex nucleic acid, kilobases in single-standed nucleic acid
kDa	kiloDalton
l	liter
LB	Luria-Bertani
µg	microgram
µl	microlitre
µM	micromolar
M	mole per liter (molar)
mg	milligram
min	minute
ml	millilitre
mM	millimolar
MW	molecular weight
N	normal
ng	nanogram
nm	nanometer
OD	optical density

ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
pmol	picomole
RBS	ribosome-binding site
rpm	revolution per minute
RNase	ribonuclease
rRNA	ribosomal ribonucleic acid
SDS	sodium dodecyl sulfate
T	2'-deoxythymidine (in a DNA sequence)
TE	Tris-EDTA buffer
UV	ultraviolet
V	voltage
X-gal	5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside