

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

นางสาวนงนุช บุญเดิม

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**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**

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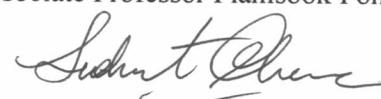
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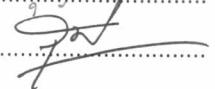

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นางนุช บุญเดิม : การโคลนและการแสดงออกของยีน *picK* จาก *Streptomyces venezuelae* เข้าสู่ *Streptomyces* สปีชีส์ (CLONING AND EXPRESSION OF *picK* GENE FROM *Streptomyces venezuelae* IN *Streptomyces* SPECIES) อาจารย์ที่ปรึกษา: ดร.สุชาติ อะนะมา, 82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

PicK เป็น cytochrome P450 monooxygenase เร่งปฏิกิริยาไฮดรอกซิเลชันซึ่งเปลี่ยน *narbomycin* เป็น *picromycin* ที่พบใน *Streptomyces venezuelae* การวิจัยครั้งนี้ได้พยายามนำเขายีน *picK* ที่กำหนดการสังเคราะห์ไปร่วมกับ *lac promoter* และนำเข้าสู่ *Streptomyces lividans* TK24, *S. narbonensis* และ *Escherichai coli* เมื่อทำการนำพลาสมิดลูกผสมเข้าสู่ *Escherichai coli* สายพันธุ์ XL1-Blue พบว่ามีการแสดงออกของยีน *picK* ได้ไปร่วมที่น้ำหนักมวลไม่เกิน 45 กิโลดัลตัน เมื่อวิเคราะห์ด้วย SDS-PAGE และตรวจพบเอนโคดิวิตี้ของ P450 จากการทำ carbon monoxide difference spectra แต่ไม่สามารถตรวจพบการแสดงออกของยีน *picK* ใน *Streptomyces lividans* สายพันธุ์ TK24 ที่ได้รับพลาสมิดลูกผสมชนิดนี้ (pWHM3-*picK*) แสดงให้เห็นว่าโปรไนเตอร์ของ *lac operon* อาจจะไม่เหมาะสมที่จะนำมาใช้ในการแสดงออกของยีนในแบคทีเรียสกุล *Streptomyces*

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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:
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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*.

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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