

การพิสูจน์เอกลักษณ์สารเมตาบอไลต์ที่เกิดจากการย่อยสลายโพรีนและฟลูออแรนธิน
ในวิถีโคเมตาบอลิซึมกับพีแนนทริน โดย *Sphingomonas* sp. P2 และการศึกษาเบื้องต้นของยีน
ที่เกี่ยวข้องกับการย่อยสลายพีแนนทริน

นางสาว อรุทัย ภิญญาคง



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IDENTIFICATION OF METABOLITES FROM DEGRADATION OF PYRENE AND
FLUORANTHENE VIA CO-METABOLISM WITH PHENANTHRENE BY *Sphingomonas* sp.
STRAIN P2 AND PRELIMINARY STUDY ON GENES INVOLVED IN PHENANTHRENE
DEGRADATION



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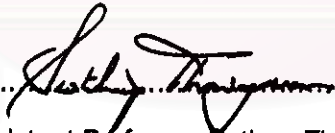
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อรรถยัย ภิญญาคง : การพิสูจน์เอกลักษณ์สารเมตาบอไลต์ที่เกิดจากการย่อยสลายไพรีน และฟลูออแรนทีน ในวิถีโคเมตาบอลิซึมกับฟีนแอนทรีน โดย *Sphingomonas* sp. P2 และการศึกษาเบื้องต้นของ ยีนที่เกี่ยวข้องกับการย่อยสลายฟีนแอนทรีน (IDENTIFICATION OF METABOLITES FROM DEGRADATION OF PYRENE AND FLUORANTHENE VIA CO-METABOLISM WITH PHENANTHRENE BY *Sphingomonas* sp. STRAIN P2 AND PRELIMINARY STUDY ON GENES INVOLVED IN PHENANTHRENE DEGRADATION) อ. ที่ปรึกษา : ผศ. ดร. อุเทพ ธนียวัน, อ. ที่ปรึกษาร่วม : รศ. ดร. กาญจนา จันทองจีน, 139 หน้า, ISBN 974-333-575-7

การพิสูจน์เอกลักษณ์สารเมตาบอไลต์ที่เกิดจากการย่อยสลายไพรีนและฟลูออแรนทีน ในวิถีโคเมตาบอลิซึมกับฟีนแอนทรีนโดย *Sphingomonas* sp. P2 ทำโดยเลี้ยงเชื้อในอาหารเหลว carbon free mineral medium (CFMM) ที่มีฟีนแอนทรีนกับไพรีน หรือฟีนแอนทรีนกับฟลูออแรนทีน ในถังหมักขนาด 30 ลิตร เป็นเวลา 4 วัน ใช้ ส่วนน้ำหมักมาแยกและทำสารเมตาบอไลต์ให้บริสุทธิ์โดยใช้ silica gel open column, thin-layer และ high performance liquid chromatography และจำแนกชนิดสารเมตาบอไลต์โดยเทคนิค GC-MS และ NMR ไม่พบสารเมตาบอไลต์ที่เกิดจากการย่อยสลายไพรีนและฟลูออแรนทีน แต่ตรวจพบและพิสูจน์เอกลักษณ์สารเมตาบอไลต์ที่เกิดจากการย่อยสลายฟีนแอนทรีนในอาหารที่มีไพรีนอยู่ด้วยได้ 5 ชนิด พบว่ามีสารเมตาบอไลต์ที่สำคัญ 2 ชนิดคือ 5,6-เบนโซคูมาริน (5,6-benzocoumarin) ซึ่งเกิดจากการปฏิกิริยาออกซิเดชันที่คาร์บอนตำแหน่ง 1 และ 2 ของวงฟีนแอนทรีน และ 1,5-ไดไฮดรอกซี-2-แนฟโธอิก แอซิด (1,5-dihydroxy-2-naphthoic acid) โดยทั้ง 2 ชนิดเป็นสารเมตาบอไลต์ชนิดใหม่ในวิถีการย่อยสลายฟีนแอนทรีน นอกจากนี้อีก 3 ชนิด ได้แก่ 7,8-เบนโซคูมาริน (7,8-benzocoumarin) 1-ไฮดรอกซี-2-แนฟโธอิก แอซิด (1-hydroxy-2-naphthoic acid) และคูมาริน (coumarin) อย่างไรก็ตามคูมาริน 5,6-เบนโซคูมาริน และ 7,8-เบนโซคูมาริน อาจเกิดจากการเปลี่ยนรูปของ สารเมตาบอไลต์ในวิถีการย่อยสลายฟีนแอนทรีนโดยปฏิกิริยาที่ไม่ต้องอาศัยเอนไซม์ จากผลการทดลองที่ได้แสดง ว่า *Sphingomonas* sp. P2 สามารถเริ่มปฏิกิริยาออกซิจีเนชันฟีนแอนทรีนได้ทั้งที่คาร์บอนตำแหน่งที่ 1,2 และ 3,4 ในการย่อยสลายฟีนแอนทรีน โดย *Sphingomonas* sp. P2 จากการศึกษาเบื้องต้นของยีนที่ประมวลผล ของเอนไซม์ไดออกซิจีเนสใน *Sphingomonas* sp. P2 พบว่ายังไม่สามารถเพิ่มจำนวนยีนไดออกซิจีเนสได้โดยวิธี PCR ซึ่งใช้ nahAc, phnAc, modified pPAH, and Rieske type เป็นไพรเมอร์ และยังไม่สามารถได้โคลนที่มียีน ไดออกซิจีเนสโดยวิธี shot gun cloning เช่นกัน

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Metabolites from pyrene and fluoranthene degradation via co-metabolism with phenanthrene by *Sphingomonas* sp. P2. were investigated. After 4-day cultivation of *Sphingomonas* sp. P2 in carbon free mineral medium (CFMM) supplemented with phenanthrene and pyrene or fluoranthene in 30 l-fermenters, no metabolite from pyrene and fluoranthene was found, whilst five metabolites in phenanthrene degradation pathway in the presence of pyrene were detected and purified by silica gel open column, thin-layer and high performance liquid chromatography. Based on gas chromatography-mass spectral, ^1H and ^{13}C nuclear magnetic resonance spectral analyses, two novel metabolites in phenanthrene degradation were characterized. One was identified as 5,6-benzocoumarin deriving from catabolism initiated by dioxygenation at the 1 and 2 positions of phenanthrene ring and the other one as 1,5-dihydroxy-2-naphthoic acid. Other metabolites from phenanthrene degradation, including 7,8-benzocoumarin, 1-hydroxy-2-naphthoic acid, and coumarin were also identified. The detection of coumarin, 5,6-benzocoumarin and 7,8-benzocoumarin suggested that these three compounds were formed from nonenzymatic conversion of certain metabolites in phenanthrene degradation pathway. Therefore, the results obtained suggested that phenanthrene could be degraded by *Sphingomonas* sp. P2 via dioxygenation at both 1,2 and 3,4 positions and subsequently undergone *meta*-cleavage. Pathway for phenanthrene degradation is proposed. Preliminary study of gene encoding dioxygenase revealed that this gene could not be amplified by PCR using nahAc, phnAc, modified pPAH and Rieske type primers while *Escherichia coli* clones containing dioxygenase gene from shot gun cloning have not yet obtained.

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LIST OF ABBREVIATIONS

Ap	ampicillin
Ap ^r	resistance to ampicillin
bp	base pair
δ	chemical shift
$^{\circ}\text{C}$	degree Celsius
CDCl_3	deuterated chloroform
CD_3OD	deuterated methanol
CFMM	carbon free mineral medium
COSY	Correlated spectroscopy
CTAB	hexadecyl trimethyl ammoniumbromide
d	doublet
dd	doublet of doublet
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside triphosphate
EDTA	ethylene diamine tetraacetic acid
<i>et al.</i>	Et alii (latin), and other
FAD	flavine adenine dinucleotide
Fe	Iron
flu	fluoranthene
g	gram
GC-MS	Gas chromatography-Mass Spectrometry
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum correlation
HPLC	High performance liquid chromatography
hr	hour

IPTG	isopropylthio- β -D-galactoside
<i>J</i>	coupling constant
kb	kilobase
kDa	kilodalton
kg	kilogram
l	litre
LD ₅₀	Medial lethal dose fifty
M	Molar
M ⁺	molecular ion
m	multiplet
m ³	cubic metre
m/z	mass to charge ratio
MES	2 [N-morpholino]ethanesulfonic acid
mg	milligram
min	minute
ml	millilitre
mM	millimolar
MHz	megahertz
MW	molecular weight
μ g	microgram
μ l	microlitre
μ m	micrometre
NADH	nicotinamide adenine dinucleotide
NOESY	Nuclear overhauser enhancement spectroscopy
nm	nanometre
NMR	Nuclear magnetic resonance
nt	nucleotide
OD	optical density
PAHs	polycyclic aromatic hydrocarbons
PCR	Polymerase chain reaction

phe	phenanthrene
pmol	picomole
ppm	parts per million
psi	pound per square inch
pyr	pyrene
R_t	retention time in chromatography
rpm	revolution per minute
Suc	succinate
SDS	sodium dodecyl sulphate
sec	second
sp.	species
sq.	square
t	triplet
TAE	Tris-acetate/EDTA
TE	Tris-EDTA
TEG	Tris-EDTA/glucose
TLC	Thin layer chromatography
U	unit
UV	ultraviolet
V	volt
v	volume
w	weight
X-gal	5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside

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