

เอนไซม์และยีนโพลีดีไทด์ซินเทสในเจตมูลเพลิงแดง



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**POLYKETIDE SYNTHASE ENZYMES AND GENES
IN *PLUMBAGO INDICA***

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การศึกษาในครั้งนี้เกี่ยวกับการค้นหาเอนไซม์ และยีนของเอนไซม์โพลีคีโตนซินเทสในเจตมูลเพลิงแดงเพื่อความเข้าใจเกี่ยวกับชีวสังเคราะห์ระดับเอนไซม์ของสารพลัมบาจิน การเพาะเลี้ยงเนื้อเยื่อของเจตมูลเพลิงแดงในครั้งนี้สามารถชักนำให้เกิดเป็นเนื้อเยื่อแคลลัส รากเพาะเลี้ยง และต้นพืชขนาดเล็กในหลอดทดลอง โดยแคลลัสที่ได้เกิดจากการใช้ส่วนต้นอ่อนของพืช ภายใต้สภาวะของอาหารสูตร MS ที่ประกอบด้วย 2,4-D 1 มิลลิกรัมต่อลิตร และ BA 0.1 มิลลิกรัมต่อลิตร ส่วนรากเพาะเลี้ยงเกิดจากการใช้ใบอ่อน ภายใต้สภาวะของอาหารสูตร B5 ที่ประกอบด้วย NAA 1 มิลลิกรัมต่อลิตร และ kinetin 0.1 มิลลิกรัมต่อลิตร จากนั้นทำการเพิ่มปริมาณรากเพาะเลี้ยง ภายใต้สภาวะของอาหารเหลวสูตร MS ในขณะที่ต้นพืชขนาดเล็กในหลอดทดลองเกิดจากการใช้ส่วนข้อของพืช ภายใต้สภาวะของอาหารสูตร LS เมื่อทำการวิเคราะห์หาปริมาณสารพลัมบาจินด้วยเทคนิค HPLC พบว่าส่วนต้นและรากของต้นพืชที่เกิดจากการเพาะเลี้ยงเนื้อเยื่อพืช มีการสะสมของสารมากกว่าแคลลัสและรากเพาะเลี้ยง อย่างไรก็ตาม เมื่อใช้สารกัมมันตรังสี malonyl-CoA เพื่อตรวจหากิจกรรมของเอนไซม์โพลีคีโตนซินเทสในสารสกัดเอนไซม์จากเนื้อเยื่อเพาะเลี้ยงต่างๆ ไม่พบว่ามีกิจกรรมของเอนไซม์โพลีคีโตนซินเทสในการสร้างพลัมบาจิน ดังนั้นจึงมีการนำเทคนิคชีววิทยาโมเลกุล มาใช้ในการหา ยีนของโพลีคีโตนซินเทส โดยการทำให้ cDNA library ที่ได้จากการใช้อาร์เอ็นเอ (RNA) จากชิ้นส่วนราก วิธีการนี้ทำให้ค้นพบ cDNA จำนวน 1 ชิ้นที่สมบูรณ์ ซึ่งเมื่อนำยีนนี้ไปแสดงออกและชักนำให้สร้างโปรตีนใน *Escherichia coli* แล้ว พบว่าเอนไซม์บริสุทธิ์ที่ได้สามารถใช้ acetyl-CoA ทำปฏิกิริยากับ malonyl-CoA ผลิตภัณฑ์ที่เกิดจากปฏิกิริยานี้พบว่าเป็นสารกลุ่มไพโรน (pyrone) หลายชนิด ซึ่งได้แก่ triketide, tetraketide, pentaketide อย่างละ 1 ชนิด และ hexaketide pyrone อีก 3 ชนิด นอกจากนี้การใช้เอนไซม์บริสุทธิ์ร่วมกับการเติมสารสกัดเอนไซม์จากเนื้อเยื่อเพาะเลี้ยงต่างๆ พบว่ากิจกรรมของเอนไซม์สามารถเปลี่ยนสาร hexaketide pyrone ตัวหนึ่งไปเป็นสารอีกชนิดหนึ่งที่ยังไม่รู้โครงสร้างทางเคมี อย่างไรก็ตามการทดลองที่ผ่านมายังไม่สามารถค้นพบการสร้างแนพโทควิโนนพลัมบาจินในสภาวะต่างๆที่มีการศึกษานี้ ปัจจุบันต่างๆที่เกี่ยวข้องกับการสร้างสารพลัมบาจินในหลอดทดลองยังไม่ถูกค้นพบ และต้องดำเนินการวิจัยต่อไป

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APHACHA JINDAPRASERT: POLYKETIDE SYNTHASE ENZYMES AND
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Studies on the enzyme and gene of a polyketide synthase in *Plumbagin indica* have been performed in order to understand the enzymatic formation of plumbagin in plant. Tissue cultures of *P. indica* were successfully established in forms of callus, root culture and *in vitro* plantlets. Callus cultures derived from young stem explants were generated on MS medium supplemented with 1.0 g/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.1 mg/l 6-benzylaminopurine (BA). Root cultures were established from young leaf segments on Gamborg's B5 (B5) medium supplemented with 1.0 mg/l α -naphthaleneacetic acid (NAA) and 0.1 mg/l kinetin. Induced roots were cultured in Murashige and Skoog (MS) liquid medium without growth regulators for root proliferation. Plantlets were regenerated from nodal segments and maintained on Linsmaier and Skoog (LS) medium also without growth regulators. Plumbagin content present in these *P. indica* tissues were determined using high performance liquid chromatography (HPLC). The analysis revealed that the content of plumbagin in the aerial parts and roots of the micropropagated plantlets was significantly higher than that in callus and root cultures. By using a radiolabelled compound of malonyl-CoA as substrate, a standard enzyme assay was established to detect plant polyketide synthase activities in crude protein extracts prepared from the various tissue cultures of *P. indica*. The formation of plumbagin was, however, not detected in the established enzyme assay conditions. The technique of molecular cloning was, therefore, introduced to express and characterize the enzyme. A cDNA encoding a polyketide synthase, PinPKS, was isolated from a cDNA library prepared from the RNA of *P. indica* roots. The recombinant PinPKS was expressed in *Escherichia coli* and assayed for its polyketide synthase activity with acetyl-CoA as a starter molecule and malonyl-CoA as a co-substrate. Analysis of the resulting reaction mixture revealed that there was enzymatic formation of various sizes of pyrone products. These included the pyrones of triketide, tetraketide, pentaketide (one each) and three hexaketides. Addition of a crude protein extract of *P. indica* tissues into the reaction mixture could lead to an unknown compound which has not been identified. With many attempts, no structures of naphthoquinones formed from the hexaketide level have been found in the enzyme assay. Required factors for plumbagin formation *in vitro* remain to be discovered.

Program Pharmaceutical Chemistry
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ABBREVIATIONS

Abs	absorbance
ACS	acridone synthase
ALS	aloesone synthase
APS	ammonium persulfate
B5	Gamborg medium
BA	6-benzylaminopurine
BAS	benzalacetone synthase
BBS	bibenzyl synthase
bp	base pair
BPS	benzophenone synthase
cDNA	complementary DNA
CHS	chalcone synthase
Ci	Curie
cm	centimeter (s)
CoA	coenzyme-A
cpm	count per minute
CTAS	Coumaroyl triacetic acid lactone synthase
dNTP	2'-deoxynucleoside 5'-triphosphate
dpm	disintegrations per minute
DNA	deoxyribonucleic acid
DTT	dithiothreitol
eV	electron volt
h	hour
HEDS	homoeriodictyol/eriodictyol synthase

HPLC	high performance liquid chromatography
IAA	indole-3-acetic acid
IPTG	isopropylthio- β -D-galactoside
g	gram (s)
kinetin	kinetin-6-furfurylaminopurine
kDa	kilodalton ($\times 10^3$ Da)
kg	kilogram
L	liter (s)
LC-ESI-MS	liquid chromatography electrospray ionization mass spectrometry
LC-MS	liquid chromatography mass spectrometry
LS	Linsmaier and Skoog medium
m	meter (s)
M	molar
min	minute (s)
mg	milligram
ml	milliliter
MMLV-RT	moloney murine leukemia virus-reverse transcriptase
MS	Murashige and Skoog medium
mRNA	messenger RNA
M^+	molecular ion
m/z	mass to charge ratio
nm	nanometer (s)
NAA	α -naphthaleneacetic acid
NADPH	nicotinamide adenine dinucleotide phosphate
OD	optical density
OKS	octaketide synthase

PCS	polyketide chromone synthase
PCR	polymerase chain reaction
Pfu	plaque forming unit
PinPKS	<i>Plumbago indica</i> polyketide synthase
PKS	polyketide synthase
RNA	ribonucleic acid
rpm	rotation per minute
RT-PCR	reverse transcriptase PCR
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
sec	second
STS	stilbene synthase
TEMED	<i>N,N,N',N'</i> -tetramethylethylenediamine
TLC	thin layer chromatography
Tris	tris (hydroxymethyl) aminomethane
U	unit
V	voltage
VPS	phlorisovalerophenone synthase
v/v	volume per volume (concentration)
w/v	weight per volume (concentration)
w/w	weight per weight (concentration)
X-gal	5-bromo-4-chloro-3-indolyl- β -D-galactoside
2,4-D	2,4-dichlorophenoxyacetic acid
2-PS	2-pyrone synthase
°C	degree Celsius
λ_{\max}	wavelength at maximum absorption

μg	microgram (s) (10^{-6} g)
μl	microliter (s) (10^{-6} l)
μM	micromolar (s)