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APPENDICES

APPENDIX A

MEDIA

1) Carbon free mineral medium (CFMM, pH 7)

Per liter

A)

NH_4NO_3	3 g
Na_2HPO_4	2.2 g
KH_2PO_4	0.8 g

Deionized water to 1 liter

Part A was sterilized by autoclaving at 15 psi for 20 min.

B)

$MgSO_4.7H_2O$	0.1 g
$FeCl_3.6H_2O$	0.05 g
$CaCl_2.2H_2O$	0.05 g

Part B was prepared as stock solution by dissolving each chemical in 1 ml distilled water and sterilized by filtering through a 0.22 µm pore size filter.

After sterilization, mixed both A and B thoroughly.

2) CFMM supplemented with 0.1% (w/v) phenanthrene (CFMM/phe)

Sterilized CFMM	1 l
Phenanthrene powder	1 g

3) CFMM supplemented with 0.1% (w/v) phenanthrene and 0.1% (w/v) pyrene (CFMM/phe/pyr)

Sterilized CFMM	1 l
Phenanthrene powder	1 g
Pyrene powder	1 g

4) CFMM supplemented with 0.1% (w/v) phenanthrene and 0.1% (w/v) fluoranthene (CFMM/phe/flu)

Sterilized CFMM	1 l
Phenanthrene powder	1 g
Fluoranthene powder	1 g

5) CFMM supplementd with 0.33 % (w/v) succinate (CFMM/Suc)

CFMM part A	1 l
Succinate	3.3 g

Sterilized by autoclaving at15 psi for 20 min.

After sterilization, added the sterilized CFMM part B and mixed thoroughly.

6) Luria Bertani medium (LB)*

Tryptone	10 g
Yeast extract	5 g
NaCl	10 g
Deionized water	800 ml

Adjusted pH to 7 with 5 N NaOH.

Brought up with deionized water to 1 l.

For solid medium, 1.5% agar was added.

Sterilized by autoclaving at 15 psi for 20 min.

7) 2×Yeast tryptone medium (2×YT)*

Tryptone	16 g
Yeast extract	10 g
NaCl	5 g
Deionized water	800 ml

Adjusted pH to 7 with 5 N NaOH.

Brought up with deionized water to 1 l.

For solid medium, 1.5% agar was added.

Sterilized by autoclaving at 15 psi for 20 min.

8) ψb medium

Bacto yeast extract	5 g
Bacto tryptone	20 g
$MgSO_4.7H_2O$	5 g

Adjusted pH to 7 with 5 N NaOH.

Brought up with deionized water to 1 l.

For solid medium, 1.5% agar was added.

Sterilized by autoclaving at 15 psi for 20 min.

*For supporting growth of *Escherichia coli* carrying plasmids pUC18, pUC19, pUC119 or pDI1, ampicilin (100 μg/ml) was added to the sterilized medium. For identification of recombinant plasmid containing insert DNA in *lacZ*, IPTG (0.1 mM) and X-Gal (20 μg/ml) were added.

APPENDIX B

REAGENTS

1) Tris-EDTA buffer (TE buffer, pH 8)

1 M Tris-HCl pH 8	1 ml
0.5 M EDTA pH 8	0.2 ml

Distilled water to 100 ml

Sterilized by autoclaving at 15 psi for 20 min.

2) Tris-EDTA/glucose buffer (TEG buffer, pH 8)

A)

1 M Tris-HCl pH 8	2.5 ml
0.5 M EDTA pH 8	2.0 ml

Distilled water to 90.5 ml

B)

1 M glucose solution	5 ml

Part A was sterilized by autoclaving at 15 psi for 20 min.

Part B was sterilized by filtering through a 0.22 µm pore size filter.

After sterilization, mixed both A and B thoroughly.

3) 50× Tris-acetate/EDTA buffer (50× TAE buffer, pH8)

Tris-base	242 g
Glacial acetic acid	57.1 ml
0.5 M EDTA	100 ml

Distilled water to 1 liter

4) 10% sodium dodecyl sulphate (10% SDS)

SDS	10 g
Distilled water	70 ml

Dissolved at 70°C and brought up with distilled water to100 ml.

5) Sodium hydroxy-sodium dodecyl sulphate solution (NaOH-SDS solution)

1M NaCl:10%SDS:distilled water = 2:1:7

6) Hexadeyl trimethyl ammoniumbromide/sodium chloride solution (CTAB/Nacl solution) (10% CTAB in 0.7M NaCl)

NaCl	4.1 g
Distilled water	80 ml
CTAB	10 g

Slowly added with stirring, while heating at 65°C.

Brought up with distilled water to 100 ml.

7) 5 M NaCl

NaCl	14.61 g

Distilled water to 50 ml

8) 3 M Sodium acetate

Sodium acetate.$3H_2O$	4.081 g

Distilled water 6 ml

Adjusted pH to 5.2 with glacial acetic acid.

Distilled water to 10 ml.

Sterilized by autoclaving at 15 psi for 20 min.

9) 5 M Potassium acetate (KOAc)

Potassium acetate	29.4 g
Glacial acetic acid	11.5 ml

Distilled water to 100 ml

Sterilized by autoclaving at 15 psi for 20 min.

10) Tfb1

KOAc	0.295 g
RbCl	1.21 g
$CaCl_2$	0.148 g
$MnCl_2$	0.99 g
Glycerol	15 ml
Distilled water	70 ml

Adjusted pH to 5.8 with 0.2 M acetic acid.

Distilled water to 100 ml.

Sterilized by filtering through 0.22 µm pore size filter.

11) Tfb11

2-[*N*-morpholino]ethanesulfonic acid	0.290 g
$CaCl_2$	1.103 g
RbCl	0.121 g
glycerol	15 ml
Distilled water	70 ml

Adjusted pH to 6.5 with 1 M KOH.

Distilled water to 100 ml.

Sterilized by filtering through a 0.22 µm pore size filter.

12) Phenol:chloroform

Phenol	25 ml
Chloroform	25 ml

Equilibrated by extraction several times with 0.1 M Tris-HCl (pH 7.6).

Stored the equilibrated mixture under equal volume of 0.01 M Tris-HCl (pH 7.6) in dark glass bottle.

13) Stock ampicilin (0.1g/ml)

Ampicilin	0.1g
Distilled water	1 ml

Sterilized by filtering through a 0.22 µm pore size filter.

Stored at –20°C.

14) Stock Isopropylthio-β-D-galactoside (IPTG, 1 M)

IPTG	238 mg
Distilled water	1 ml

Sterilized by filtering through a 0.22 µm pore size filter.

Store at –20°C.

15) 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal, 2% w/v)

X-Gal	20 mg
Dimethyformamide	1 ml

Sterilized by filtering through a 0.22 µm pore size filter.

Tube of the solution should be wrap with aluminum foil to prevent damage by light and store at –20°C.

16) Loading buffer

A)

Bromophenol blue	0.05 g
Absolute ethanol	1 ml

B)

Sucrose	12 g
Distilled water	17 ml

C)

1M EDTA pH 8	2 ml

Autoclaved part A and B at 15 psi for 20 min.

Mixed part A, B and C after autoclaving and kept at –20°C.

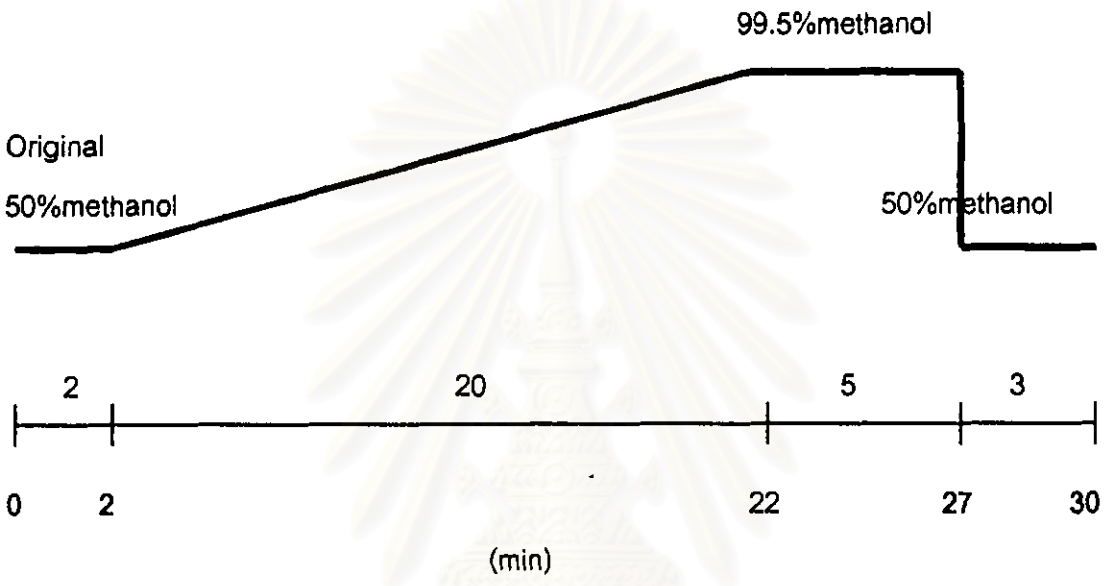
17) Diazomethane

Diazomethane was prepared by dissolving approximately 5 g of *p*-toluenesulfonyl-*N*-methyl-*N*-nitrosoamide in 5 ml of diethyl ether, chilled in ice for 5 min. Added 4-5 drops of potassium hydroxide-saturated ethanol to the solution. Reaction gas product was trapped in chilled diethyl ether, this reagent was kept in dark glass bottle and stored at –20°C (not more than 1 week).

APPENDIX C

Linear gradient solvent system used in reverse phase HPLC system

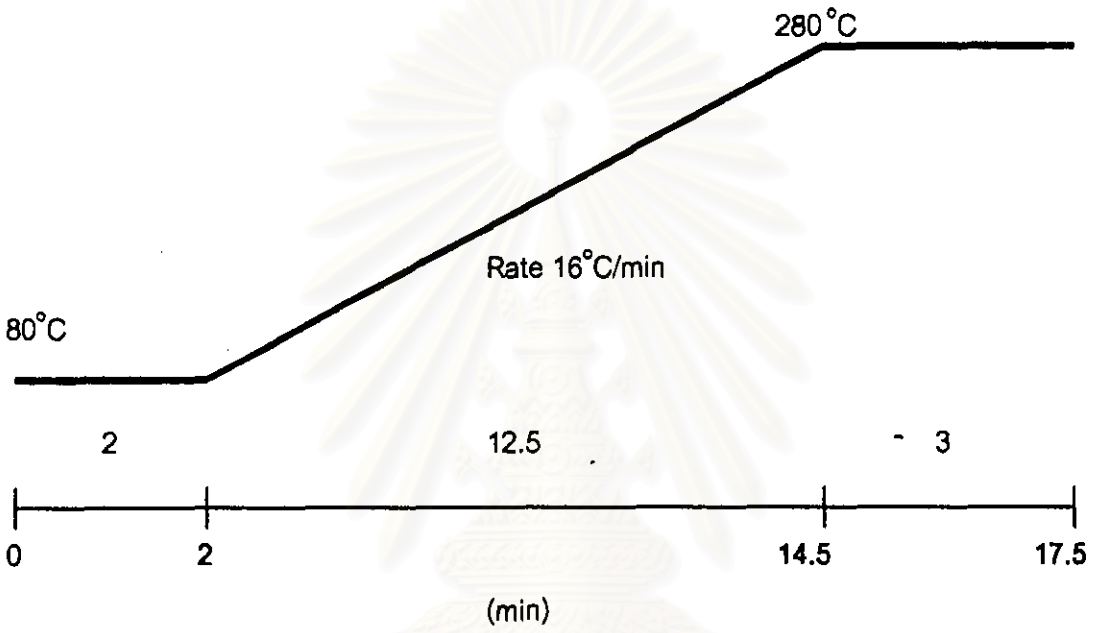
Time scale of mobile phase (50 to 100% by volume of methanol in water with 1% acetic acid [linear gradient within 30 min])



APPENDIX D

Temperature program of GC-MS spectroscopy

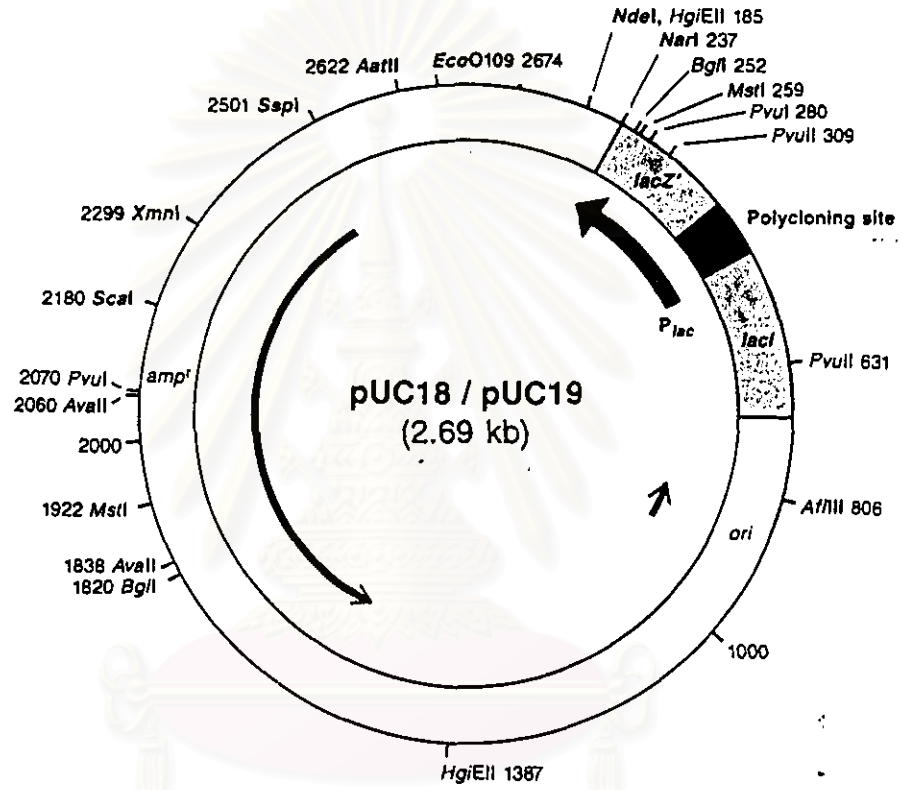
The column temperature of GC-MS spectroscopy was held at 80°C for 2 min then increased at 16 °C/min to 280 °C and held at 280 °C for 3 min.



APPENDIX E

Plasmids used in the study on dioxygenase gene

1) Plasmid pUC18 and pUC19 (Sambrook *et al.*, 1989)



Polycloning Sites

pUC18

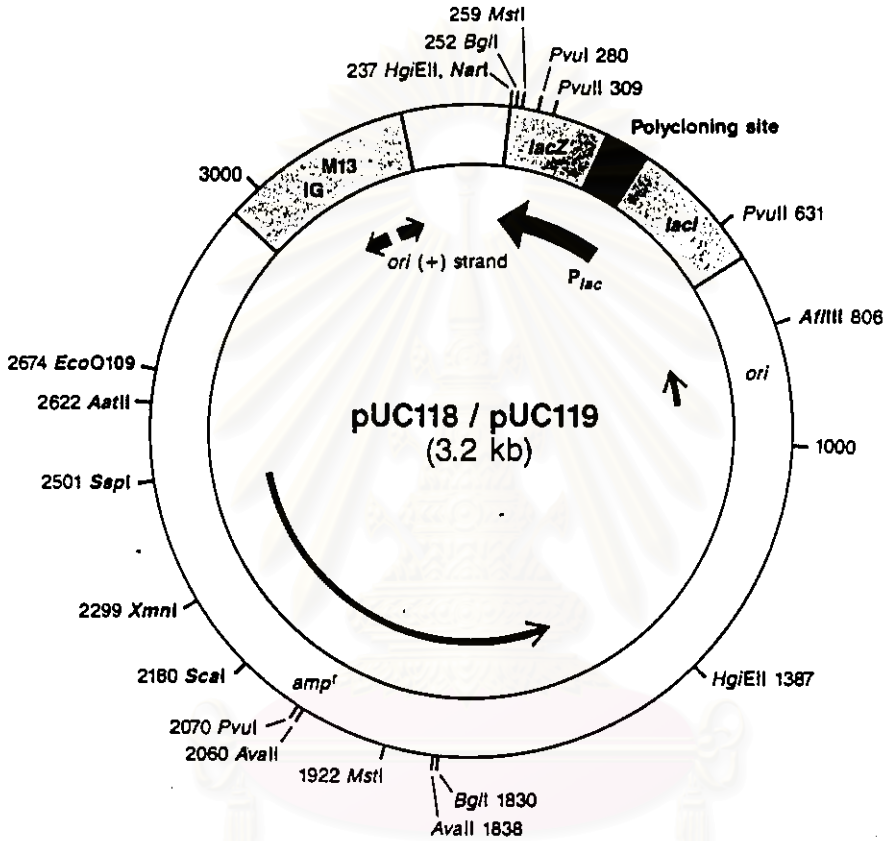
	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	7	8
	Thr	Met	Ile	Thr	Asn	Ser	Ser	Ser	Val	Pro	Gly	Asp	Pro	Leu	Glu	Ser	Thr	Cys	Arg	His	Ala	Ser	Leu	Ala	Leu	Ala
ATG	ACC	ATG	ATT	ACG	AAT	TCG	AGC	TCG	GTA	CCC	GGG	GAT	CCT	CTA	GAG	TCG	ACC	TGC	AGG	CAT	GCA	AGC	TTG	GCA	CTG	GCC
					EcoRI		*SacI*		*KpnI*	*SmaI* *XmaI*		*BamHI*		*XbaI*		*SalI* *AccI* *HincII*		*PstI*		*SphI*		*HindIII*				

pUC19

	1	2	3	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	5	6	7	8
	Thr	Met	Ile	Thr	Pro	Ser	Leu	His	Ala	Cys	Arg	Ser	Thr	Leu	Glu	Asp	Pro	Arg	Val	Pro	Ser	Ser	Asn	Ser	Leu	Ala
ATG	ACC	ATG	ATT	ACG	CCA	AGC	TTG	CAT	GCC	TGC	AGG	TCG	ACT	CTA	GAG	GAT	CCC	CGG	GTA	CCG	AGC	TCG	AAT	TCA	CTG	GCC
					HindIII		*SphI*		*PstI*		*SalI* *AccI* *HincII*		*XbaI*		*BamHI*		*SmaI* *XmaI*		*KpnI*		*SacI*		*EcoRI*			

In pUC18, the *EcoRI* site lies immediately downstream from P_{lac}.
In pUC19, the *HindIII* site lies immediately downstream from P_{lac}.

2) Plasmid pUC118 and pUC119 (Sambrook *et al.*, 1989)



Polycloning Sites

pUC118

	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	7	8
	Thr	Met	Ile	Thr	Asn	Ser	Ser	Ser	Val	Pro	Gly	Asp	Pro	Leu	Glu	Ser	Thr	Cys	Arg	His	Ala	Ser	Leu	Ala	Leu	Ala
ATG	ACC	ATG	ATT	ACG	AAT	TCG	AGC	TCG	GTA	CCC	GGG	GAT	CCT	CTA	GAG	TCG	ACC	TGC	AGG	CAT	GCA	AGC	TTG	GCA	CTG	GCC
					EcoRI		*SacI*		*KpnI*	*SmaI* *XmaI*		*BamHI*		*XbaI*		*SalI* *AccI* *HincII*		*PstI*		*SphI*		*HindIII*				

pUC119

	1	2	3	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	16	5	6	7	8
	Thr	Met	Ile	Thr	Pro	Ser	Leu	His	Ala	Cys	Arg	Ser	Thr	Leu	Glu	Asp	Pro	Arg	Val	Pro	Ser	Ser	Asn	Ser	Leu	Ala
ATG	ACC	ATG	ATT	ACG	CCA	AGC	TTG	CAT	GCC	TGC	AGG	TCG	ACT	CTA	GAG	GAT	CCC	CGG	GTA	CCG	AGC	TCG	AAT	TCA	CTG	GCC
					HindIII		*SphI*		*PstI*		*SalI* *AccI* *HincII*		*XbaI*		*BamHI*		*SmaI* *XmaI*		*KpnI*		*SacI*		*EcoRI*			

In pUC118, the *Eco*RI site lies immediately downstream from P_{lac}. In pUC119, the *Hin*dIII site lies immediately downstream from P_{lac}.

APPENDIX F

Co-metabolism of pyrene and fluoranthene with phenanthrene by *Sphingomonas* sp. P2

Nuttapun Supaka (personal communication) found that when cultivating Sphingomonas sp. P2 in CFMM supplemented with pyrene in the presence of phenanthrene, colour changing of culture medium (from colourless to green) was observed (Figure 1). Moreover, addition of fluoranthene to CFMM in the presence of phenanthrene, colour of culture medium was changed from colourless to red (Figure 2). This phenomenon suggested the possibility that *Sphingomonas* sp. P2 can cometabolized pyrene and fluoranthene by using phenanthrene as growth substrate.

Figure 1 Colour changing of *Sphingomonas* sp. P2 culture medium (phenanthrene plus pyrene)

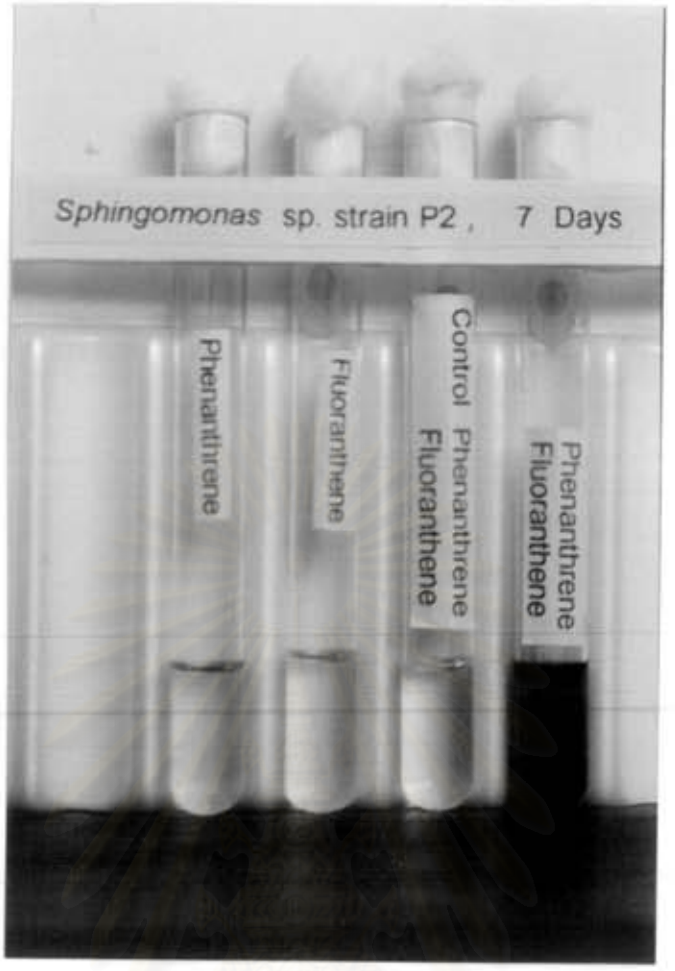


Figure 2 Colour changing of *Sphingomonas* sp. P2 culture medium (phenanthrene plus fluoranthene)

BIOGRAPHY

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