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APPENDICES

APPENDIX A

BACTERIAL STRAINS

Table A.1 *Rhizobium* sp. CU-A1 transposon mutants used in this study and its ability to grow on carbon sources (Kriengkripipat, 2001)

Strain of *Rhizobium*	Growth on carbon sources					
	AE	AQ	NDA	N	SAL	GEN
CU-A1	+	+	+	+	+	+
A53	-	+	+	-	-	-
B1	-	-	-	+	+	+
E11	-	-	-	+	+	+

+ Growth, - No growth, AE; acenaphthylene, AQ; acenaphthenequinone, NDA; naph thalene-1,8-dicarboxylic acid, N; naphthalene, SAL; salicylic acid, GEN; gentisic acid (2,5-dihydroxybenzoic acid)

APPENDIX B

MEDIA, BUFFER AND CHEMICAL SOLUTIONS

1. Mineral salt medium (MM)

NH_4NO_3	3.0	g
$Na_2HPO_4 \bullet 12H_2O$	5.5	g
KH_2PO_4	0.8	g
$MgSO_4 \bullet 7H_2O$	0.01	g
$FeCl_3 \bullet 6H_2O$	0.005	g
$CaCl_2 \bullet 2H_2O$	0.005	g

Dissolve NH_4NO_3, $Na_2HPO_4 \bullet 12H_2O$ and KH_2PO_4 in 1,000 ml Distilled water, adjust pH to 7.5 by adding 1 N NaOH. Then, the solution was sterilized at 121°C for 20 min in an autoclave. Heat sensitive compounds, $MgSO_4 \bullet 7H_2O$, $FeCl_3 \bullet 6H_2O$ and $CaCl_2 \bullet 2H_2O$, were sterilized by filtration using 0.45 μm cellulose acetate filter, then, added to the sterilized solution. Solid media were prepared by the addition of 15 g Bacto agar to 1 L of the mineral medium solution before sterilization.

2. Luria –Bertani (LB)

Tryptone	10.0	g
Yeast extract	5.0	g
NaCl	5.0	g

Dissolve the three components in 1,000 ml Distilled water, adjust pH to 7.5 by adding 1 N NaOH. Then, the solution was sterilized at 121°C for 20 mins in an autoclave. Solid media were prepared by the addition of 15 g Bacto agar to 1 L of the mineral medium solution before sterilization.

3. Acenaphthylene in DMSO solution

Dissolve 0.1 g of acenaphthylene in 3.0 ml of dimethylsulfoxide. The solution was then vigorously mixed until all acenaphthylene pellet was dissolved. The acenaphthylene solution was then filtrated through 0.2 μm PTFE filter unit and kept in bottle at -20°C for further use.

4. Protocatechuic acid in ethanol

Dissolve 1.0 g of protocatechuic acid in 4.0 ml of ethanol. The mixture was vigorously mixed until all pellets were dissolved. The solution was then filtrated through 0.2 μm PTFE filter unit and kept in brown color bottle at -20°C for further use.

5. Standard PAHs and PAHs derivatives in methanol solution

Dissolve 0.1 g of acenaphthylene, acenaphthenequinone, naphthalene-1,8-dicarboxylic acid, 1-naphthoic acid, 1,2-dihydroxynaphthalene, salicylic acid, gentisic acid, naphthalene, acenaphthene, phenanthrene, fluorene, anthracene, fluoranthene and pyrene in 10 ml of methanol. The solution was vigorously mixed and then filtrated through 0.2 μm PTFE filter unit and kept in brown color bottle at -20°C for using as the standard for TLC and HPLC analysis. 1 M Acenaphthylene was prepared using this procedure.

6. Standard PAHs in DMSO solution

Dissolve 25 mg of acenaphthylene, acenaphthenequinone, naphthalene-1,8-dicarboxylic acid, 1-naphthoic acid, 1,2-dihydroxynaphthalene, salicylic acid, gentisic acid, naphthalene, acenaphthene, phenanthrene, fluorene, anthracene, fluoranthene and pyrene in 1.5 ml of dimethylsulfoxide. The solution was vigorously mixed and then filtrated through 0.2 μm PTFE filter unit and kept in brown color bottle at -20°C for furthers.

7. Kanamycin solution

Dissolve 30 mg of kanamycin in 1.0 ml of distilled water. Then, the solution was sterilized by filtrate through 0.2 μm cellulose acetate filter unit and kept in brown color bottle at -20°C for further use. After thawing, the solution could be kept at 4°C for 1 month.

8. 0.85% NaCl solution

Dissolve 0.85 g of NaCl in 100 ml of distilled water. Then, the solution was sterilized at 121°C for 20 min in a sterilizer.

9. 1 N NaOH solution

Dissolve 4.0 g of NaOH in 90 ml of distilled water in 100 ml volumetric flask and then adjust the volume to 100 ml.

10. Water-saturated ethyl acetate

Mix 150 ml of ethyl acetate with 150 ml of distilled water in 500 ml-Erlanmayer flask. The flask was tightly closed with rubber cock and sealed with parafilm and shaken at room temperature at 200 rpm for 24 h.

11. Glycerol solution

Sterilize glycerol solution at 121°C for 20 min in a sterilizer and kept at room temperature for 24 h. Then, the glycerol solution was sterilized in the same manner for two times.

12. Sodium phosphate buffer pH 7.5

Na_2HPO_4	133.16	g
NaH_2PO_4	7.44	g

1 M Sodium phosphate buffer stock solution was prepared by dissolve Na_2HPO_4 and NaH_2PO_4 in 900 ml distilled water and adjusted pH to 7.5. The volume was adjusted to 1,000 ml in 1,000 ml volumetric flask. The buffer was stored at 4°C for further uses.

13. Acetate buffer pH 4

1 M Acetate buffer stock solution was prepared by dissolve 12.15 g of CH_3COONa in 900 ml distilled water and adjusted pH to 4 by glacial acetic acid. The volume was adjusted to 1,000 ml in 1,000 ml volumetric flask. The buffer was stored at 4°C for further uses.

14. Tris-HCl buffer

1 M Tris-HCl buffer stock solution was prepared by dissolve 120 g of Tris in 900 ml distilled water and adjusted pH to desired pH. The volume was adjusted to 1,000 ml in 1,000 ml volumetric flask. The buffer was stored at 4°C for further uses.

15. Lowry solution

- Lowry A

Na_2CO_3	60.0	g
NaOH	12.0	g
NaKtartrate	0.6	g

  Dissolve all components in 3,000 ml of distilled water.

- Lowry B

  Dissolve 5.0 g of $CuSO_4$ in 1,000 ml of distilled water.

- Lowry C

  Mix Lowry A solution with Lowry B solution in a ratio 50:1.

- Lowry D

  Mix Folin phenol reagent with distilled water in a ratio 1:1.

16. Bradford dye solution

Dissolve 5.0 g of Coomassie blue dye in 100 ml of distilled water and then stored in brown color bottle for further uses.

17. Electrophoresis

- 1.5 M Tris HCl pH 8.8

Tris	18.15	g
Distilled water	100	ml
pH	8.8	

- 0.5 M Tris HCl pH 6.8

Tris	6.0	g
Distilled water	100	ml
pH	6.8	

- 10% APS

Ammonium persulphate	0.1	g
Distilled water	1.0	ml

- Staining Solution

Coomassi Brilliant Blue dye	0.175	% (w/v)
96% Ethanol	45.5	% (v/v)
Acetic acid	9.2	% (v/v)

- Destaining Solution

96% Ethanol	5.0	% (v/v)
Acetic acid	7.5	% (v/v)

Native-PAGE

- Electrophoresis buffer, Tris glycine electrophoresis buffer

Tris base	3.0	g
Glycine	14.4	g
Distilled water	1000	ml
pH	8.3	

- Loading buffer

0.5 M Tris HCl pH 6.8	1.2	ml
Glycerol	1.0	ml
1% Bromophenol Blue	0.5	ml
Distilled water	4.8	ml

Table B.1 Scheme for the preparation of 12% native gel

	12% Resolving gel	5% Stacking gel
Distilled water	3.28 ml	2.99 ml
40% Acrylamide	2.25 ml	485 µl
1.5 M Tris HCl pH 8.8	1.9 ml	-
0.5 M Tris HCl pH 6.8	-	500 µl
10% APS	75 µl	40 µl
TEMED	3 µl	3 µl

SDS-PAGE

- 10% SDS

Sodium dodecyl sulphate	1.0	g
Distilled water	10	ml

- SDS electrophoresis buffer

Tris base	3.0	g
Glycine	14.4	g
SDS	10.0	g
Distilled water	1000	ml
pH	8.3	

- SDS loading buffer

0.5 M Tris HCl pH 6.8	1.2	ml
Glycerol	1.0	ml
10% SDS	2.0	ml
1% Bromophenol Blue	0.5	ml
Distilled water	4.8	ml

Table B.2 Scheme for the preparation of 12% SDS gel

	12% Resolving gel	5% Stacking gel
Distilled water	3.2 ml	2.95 ml
40% Acrylamide	2.25 ml	485 µl
1.5 M Tris HCl pH 8.8	1.9 ml	-
0.5 M Tris HCl pH 6.8	-	500 µl
10% APS	75 µl	40 µl
10% SDS	75 µl	40 µl
TEMED	3 µl	3 µl

18. NADH Solution

Dissolve 0.71 g of NADH in 1 ml of distilled water. The solution was mixed and then filtrated through 0.2 µm cellulose acetate filter unit and kept at -20°C for further use.

19. Dithiothreitol (DTT) solution

Dissolve 0.154 g of DTT in 5 ml of distilled water. The solution was mixed and then filtrated through 0.2 µm cellulose acetate filter unit and kept at -20°C for further use.

20. N-ethylmaleimide (NEM) solution

Dissolve 0.125 g of NEM in 5 ml of distilled water. The solution was mixed and then filtrated through 0.2 μm cellulose acetate filter unit and kept at -20°C for further use.

21. $(NH_4)_2Fe(SO_4)_2$ solution

Dissolve 3.92 mg of $(NH_4)_2Fe(SO_4)_2 \bullet 6H_2O$ in 2 ml of distilled water. The solution was mixed and then filtrated through 0.2 μm cellulose acetate filter unit and kept at -20°C for further use.

APPENDIX C

STANDARD CURVES

1. Standard curve of acenaphthylene (mg/L)

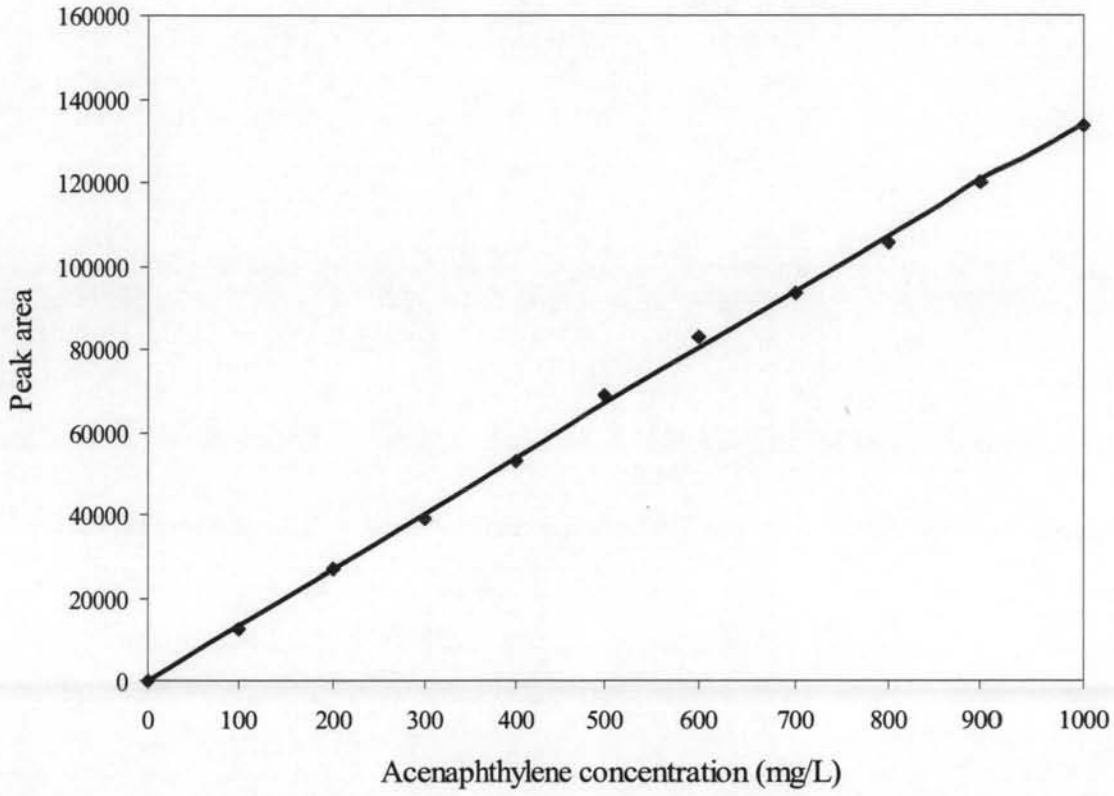

Fig. C.1 Standard curve of acenaphthylene generated by plotting of acenaphthylene concentration (mg/L) and peak area analyzed by HPLC

The concentration of acenaphthylene can be determined using following equation:

Peak area = (Slope of standard curve × Acenaphthylene concentration (mg/L)) + Y intercept

while slope of standard curve = 133.78

2. Standard curve of acenaphthylene (nmole)

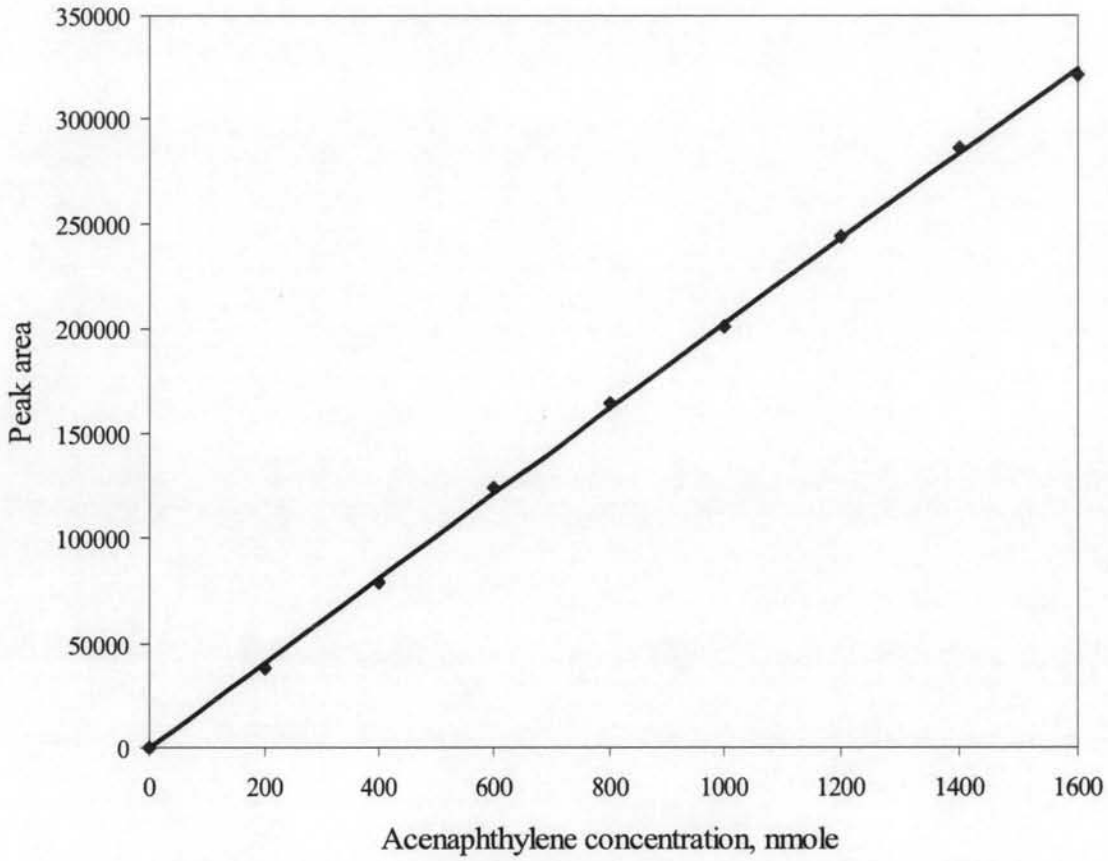

Fig. C.2 Standard curve of acenaphthylene generated by plotting of acenaphthylene concentration (nmole) and peak area analyzed by HPLC

The concentration of acenaphthylene can be determined using following equation:

Peak area = (Slope of standard curve × Acenaphthylene concentration (nmole)) + Y intercept

while slope of standard curve = 202.6

3. Standard curve of Bovine Serum Albumin (BSA)

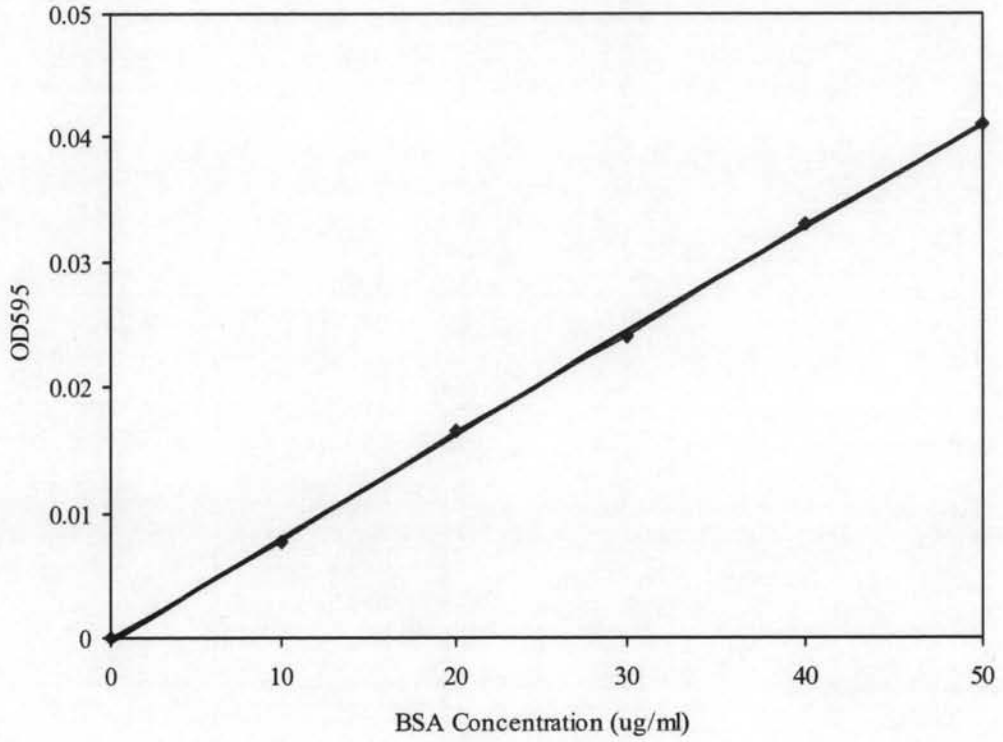

Fig. C.3 Standard curve of Bovine serum albumin for determination of protein concentration

The concentration of protein can be determined using following equation:

OD_{595} = (Slope of standard curve × Protein concentration (μg/ml)) + Y intercept

while slope of standard curve = 0.0083

Y intercept = -0.0085

4. Standard curve for determination of protein molecular mass by gel filtration using Superdex G200 column

Table C.1 Standard proteins and their K_{av} values

Sample	MW (kDa)	Log MW	V_e (ml)	K_{av}
Dextran	2000.0	3.30	128	0
Ferritin	440.0	2.64	208	0.387
BSA	66.0	1.82	245	0.565
Cytochrome *c*	13.2	1.12	288	0.773
Riboflavin	1.2	0.08	335	1

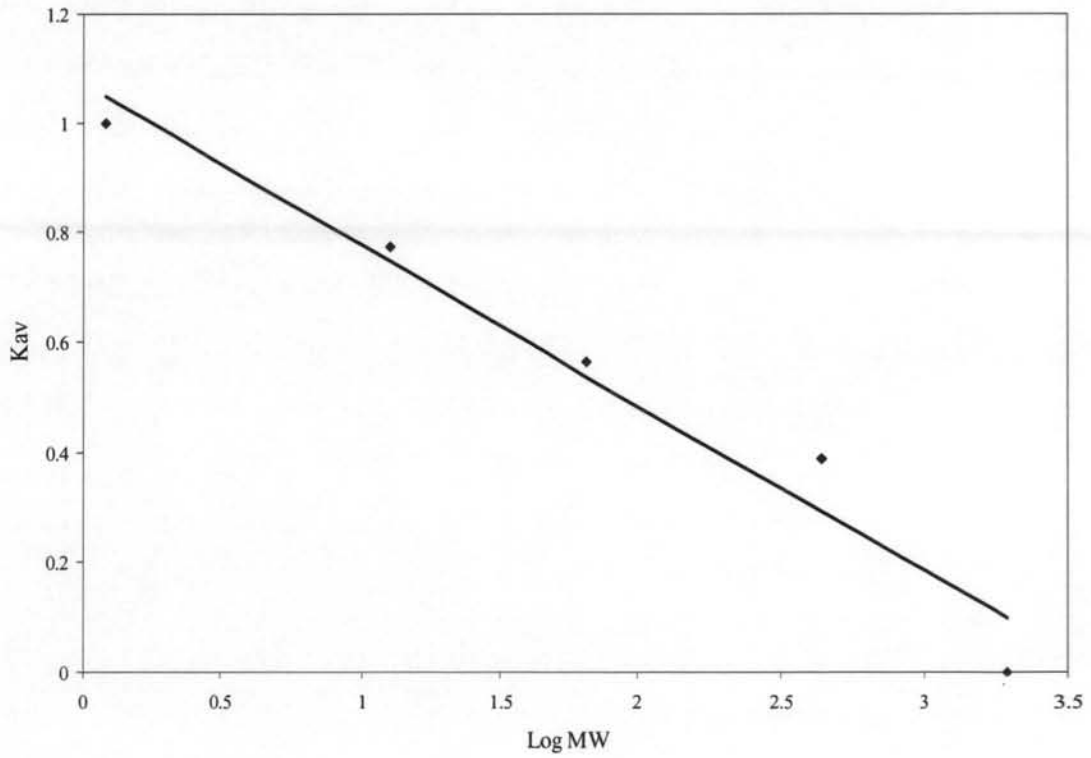

Fig. C.4 Calibration curve for determination of molecular mass of proteins by gel filtration chromatography

5. Standard curve for determination of protein molecular mass by SDS-PAGE

Table C.2 Standard proteins and their mobility in SDS-PAGE

Sample	MW (kDa)	Log MW	Mobility (cm)
Phosphorylase *b*	97.0	1.99	0.4
Albumin	66.0	1.82	1.2
Ovalbumin	45.0	1.65	2.2
Carbonic anhydrase	30.2	1.48	3.6
Trypsin inhibitor	20.1	1.30	5.3
α Lactalbumin	14.4	1.16	6.5

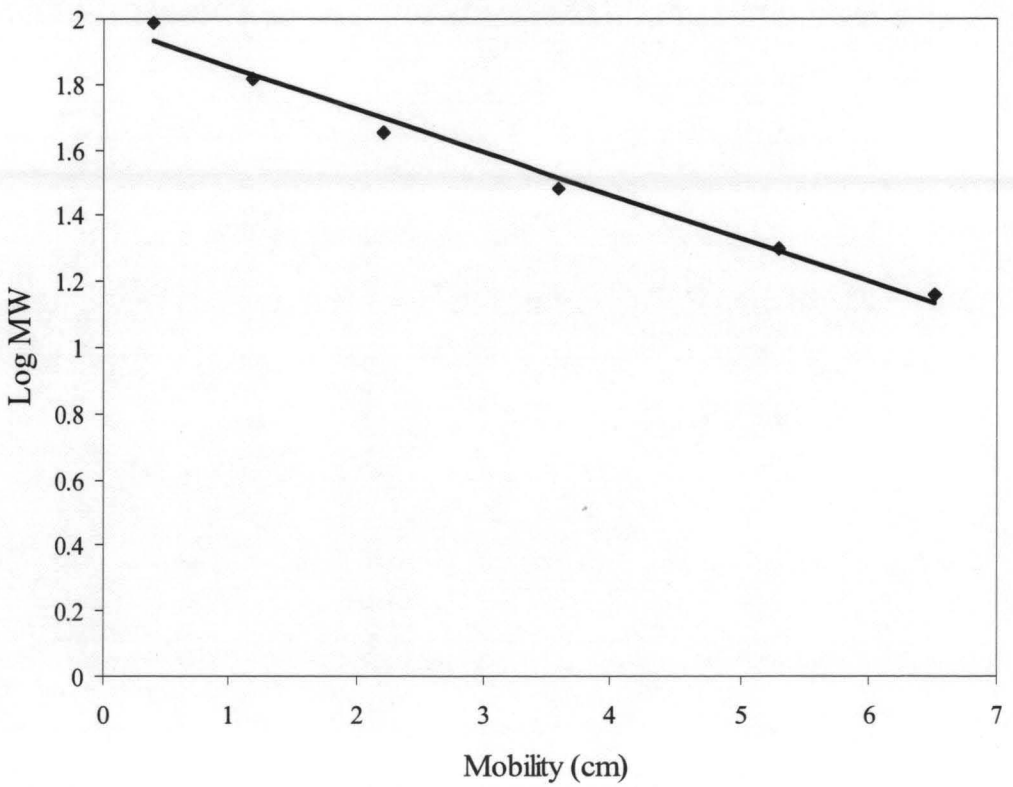

Fig. C.5 Calibration curve for determination of molecular mass of peptide by SDS-PAGE

BIOGRAPHY

Mr. Siriwat Poonthrigpun was born on September 28, 1978 in Bangkok, Thailand. He graduated Bachelor's Degree of Science in Microbiology from Department of Microbiology, Faculty of Science, King Mongkut's University of Technology Thonburi in 1998. He received the Degree of Master of Science in Industrial Microbiology from Department of Microbiology, Faculty of Science, Chulalongkorn University in 2002. He pursued his Doctor's Degree in the Inter-Disciplinary of Environmental Management (Environmental and Hazardous Waste Management), Graduate school, Chulalongkorn University in 2002.