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APPENDICES

ศูนย์วิทยทรัพยากร
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APPENDIX A

Turk Island Salt Solution + modified BG11 medium contained the following components:

1. Preparation of Turk Island Salt Solution

Stock solution A: KCl	33.3	g
MgCl ₂ .6H ₂ O	275.0	g
CaCl ₂ .2H ₂ O	73.3	g

and made up to 5 litres with distilled water .

Stock solution B: MgSO ₄ .7 H ₂ O	347.0	g
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and then made up to 5 litres with distilled water .

To make Turk Island Salt Solution, 500 ml of Stock solution A was added to 500 ml of Stock solution B. To this mixture 140.8 g NaCl was added and then final volume was made to 5 litres with distilled water.

2. Composition of modified BG₁₁ medium (BG₁₁ medium + NaNO₃ Solution)

NaNO ₃	(75g / 500 ml)	50	ml
KH ₂ PO ₄	(8g / 200 ml)	5	ml
MgSO ₄ .7H ₂ O	(15g / 200 ml)	5	ml
CaCl ₂ .2H ₂ O	(7.2g/ 200 ml)	5	ml
Na ₂ CO ₃	(4g/ 200 ml)	5	ml
Citric acid	(1.2g / 200 ml)	5	ml
EDTA.Na ₂	(0.2g / 200 ml)	5	ml
FeSO ₄ .7H ₂ O	(1.2g / 200 ml)	5	ml
*Trace element A ₅ solution + Co		5	ml

*Trace element A₅ solution + Co contained the following component in gram per litres H₃PO₄ : 2.86 ; ZnSO₄.7H₂O : 0.2 ; CuSO₄.5H₂O : 0.08 ; MnCl₂.4H₂O : 1.81 ; Na₂MnO₄.2H₂O : 0.39 ; Co(NO₃)₂.6H₂O : 0.049.

Culture medium of *Aphanothece halophytica* was prepared by adding all solution of item 2 at indicated volume to 5 litres of Turk Island Salt Solution and the pH was adjusted to 7.6 by slowly adding 2.0 M NaOH. The medium was sterilized by autoclaving at 15 lb/in² for 15 minutes.



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APPENDIX B

Preparation for denaturing polyacrylamide gel electrophoresis

1. Stock solutions

2.0 M Tris-HCl (pH 8.8)

Tris(hydroxymethyl)-aminomethane 24.2 g

Adjusted pH to 8.8 with 1.0 M HCl and adjusted volume to 100 ml with distilled water.

1.0 M Tris-HCl (pH 6.8)

Tris(hydroxymethyl)-aminomethane 12.1 g

Adjusted pH to 8.8 with 1.0 M HCl and adjusted volume to 100 ml with distilled water.

10 % SDS (w/v)

Sodium dodecyl sulfate (SDS) 10 g

Added distilled water to a total volume of 100 ml.

50 % Glycerol (w/v)

100 % glycerol 50 ml

Added 50 ml of distilled water.

1 % Bromophenol blue (w/v)

Bromophenol blue 100 mg

Brought to 10 ml with distilled water and stirred until dissolved.

Filtration will remove aggregate dye.

2. Working solutions

Solution A (30 % (w/v) acrylamide, 0.8 % (w/v) bis-acrylamide)

Acrylamide 29.2 g

N, N- methylene-bis-acrylamide 0.8 g

Adjusted volume to 100 ml with distilled water.

Solution B (1.5 M Tris-HCl pH 8.8, 0.4 % SDS)

2.0 M Tris-HCl (pH 8.8) 75 ml

10 % SDS 4 ml

Distilled water 21 ml

Solution C (0.5 M Tris-HCl pH 6.8, 0.4 % SDS)

1.0 M Tris-HCl (pH 6.8) 50 ml

10 % SDS 4 ml

Distilled Water 46 ml

10 % Ammonium persulfate

Ammonium sulfate 0.5 g

Distilled water 5 ml

Electrophoresis buffer (25 mM Tris, 192 mM glycine, 0.1 % SDS)

Tris (hydroxymethyl)-aminomethane 3 g

Glycine 14.4 g

SDS 1 g

Dissolved in distilled water to 1 litre without pH adjustment

(final pH should be approximately 8.3)

5X Sample buffer

(60 mM Tris-HCl pH 6.8, 25 % glycerol, 2% SDS, 0.1 % bromophenol blue,

14.4 mM 2- mercaptoethanol)

1.0 M Tris-HCl (pH 6.8)	0.6	ml
50 % Glycerol	5	ml
10 % SDS	2	ml
1 % Bromophenol blue	1	ml
2-mercaptoethanol	0.5	ml
Distilled water	0.9	ml

3. SDS-PAGE**12.5 % Separating gel**

Solution A	4.2	ml
Solution B	2.5	ml
Distilled water	3.3	ml
10 % Ammonium sulfate	50	μ l
TEMED	5	μ l

5.0 % Stacking gel

Solution A	0.67	ml
Solution C	1.0	ml
Distilled water	2.3	ml
10 % Ammonium sulfate	30	μ l
TEMED	5	μ l

APPENDIX C

Preparation for non-denaturing polyacrylamide gel electrophoresis (Native – PAGE)

1. Stock solutions

2.0 M Tris-HCl (pH 8.8)

Tris(hydroxymethyl)-aminomethane 24.2 g

Adjusted pH to 8.8 with 1.0 M HCl and adjusted volume to 100 ml with distilled water.

1.0 M Tris-HCl (pH 6.8)

Tris(hydroxymethyl)-aminomethane 12.1 g

Adjusted pH to 8.8 with 1.0 M HCl and adjusted volume to 100 ml with distilled water.

1% Bromophenol blue (w/v)

Bromophenol blue 100 mg

Brought to 10 ml with distilled water and stirred until dissolved.

Filtration will remove aggregated dye.

2. Working solutions

Solution A (30 %(w/v) acrylamide, 0.8 %(w/v) bis-acrylamide)

Acrylamide 29.2 g

N, N'- methylene-bis-acrylamide 0.8 g

Adjusted volume to 100 ml with distilled water.

Solution B (1.5 M Tris – HCl pH 8.8)

2 M Tris – HCl (pH 8.8) 75 ml

Distilled water	25	ml
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Solution C (1.5 M Tris – HCl pH 6.8)

1 M Tris – HCl (pH 6.8)	50	ml
Distilled water	50	ml

10 % Ammonium persulfate

Ammonium persulfate	0.5	g
Distilled water	5.0	ml

Electrophoresis buffer (25 mM Tris, 192 mM glycine)

Tris(hydroxymethyl)-aminomethane	3.0	g
Glycine	14.4	g

Dissolved in distilled water to 1 litres without pH adjustment
(final pH should be approximately 8.3)

5X Sample buffer

(312.5 mM Tris-HCl pH 6.8 , 50 % glycerol , 1 % bromophenol blue)

1.0 M Tris-HCl (pH 6.8)	0.6	ml
50 % Glycerol	5	ml
1 % Bromophenol blue	1	ml
Distilled water	1.4	ml

3. Native-PAGE

7.7 % Separating gel

Solution A	2.6	ml
Solution B	2.5	ml
Distilled water	4.9	ml
10 % Ammonium persulfate	50	μ l
TEMED	5.0	μ l

5.0 % Stacking gel

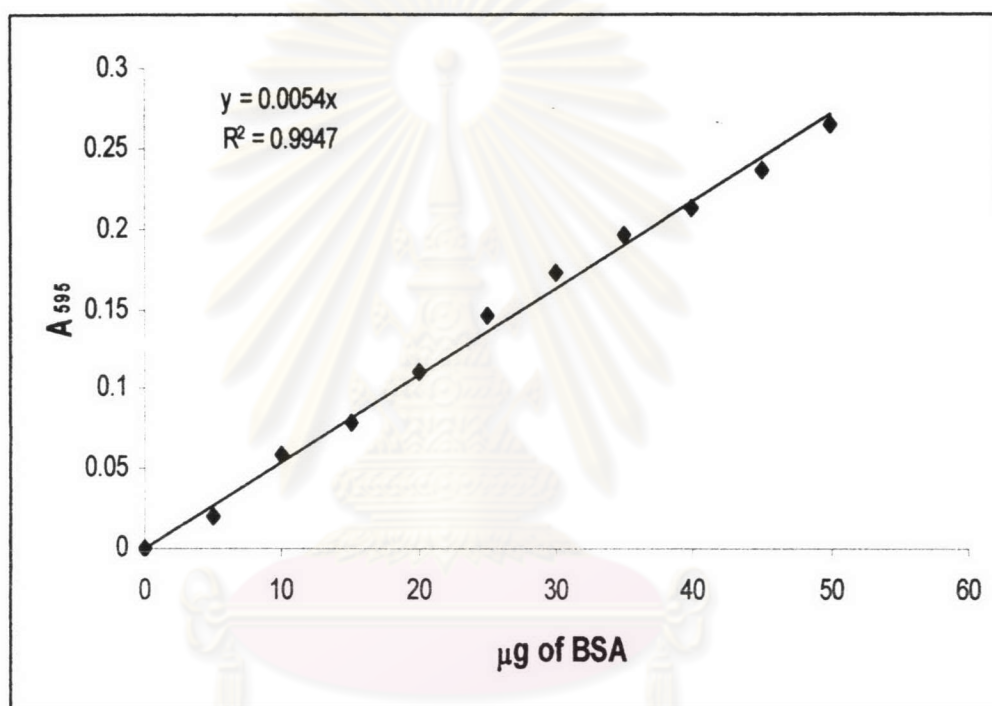
Solution A	2.6	ml
Solution B	2.5	ml
Distilled water	4.9	ml
10 % Ammonium persulfate	50	μ l
TEMED	5.0	μ l



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APPENDIX D

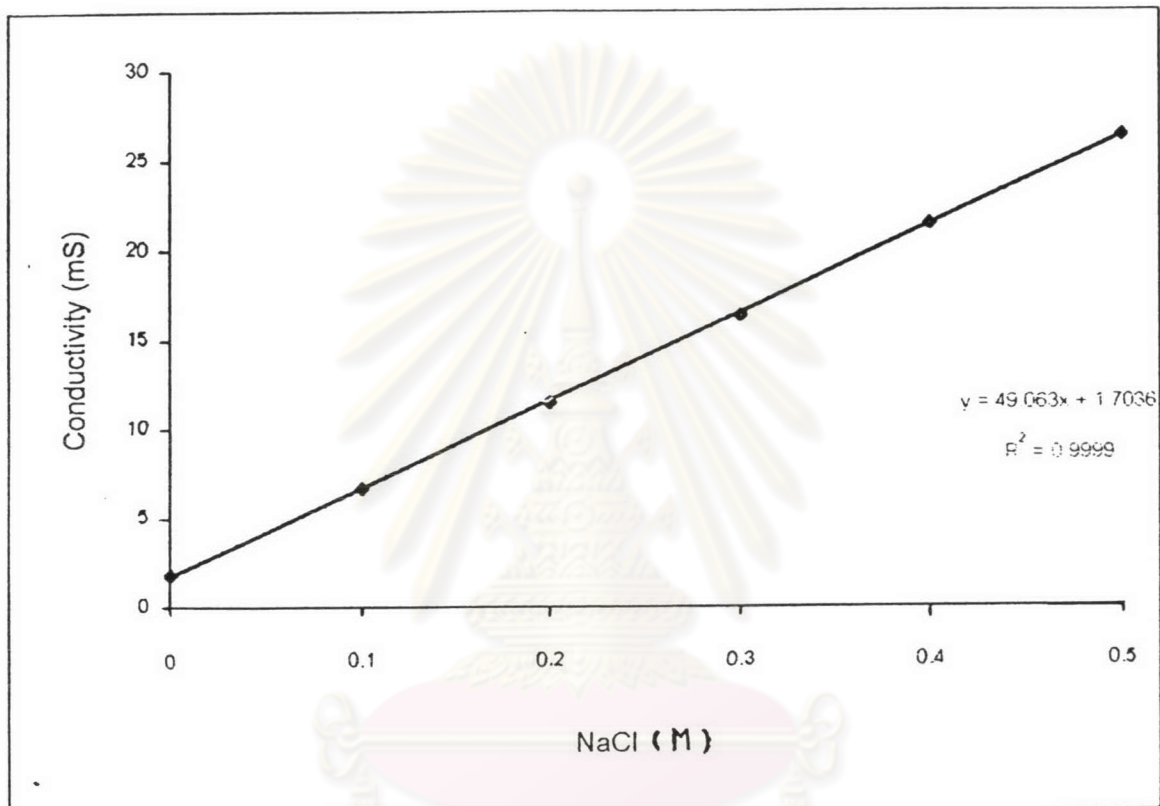
Calibration curve for protein determination by Bradford's method



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APPENDIX E

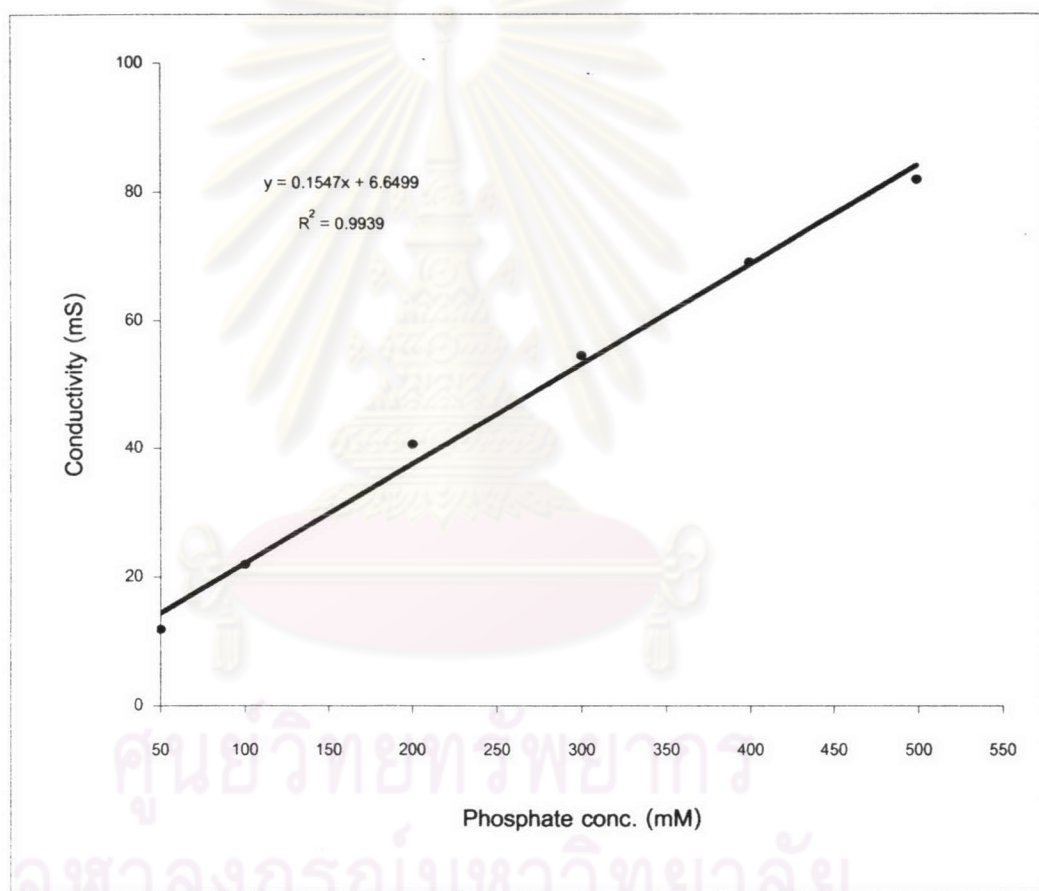
Calibration curve for conductivity of sodium chloride



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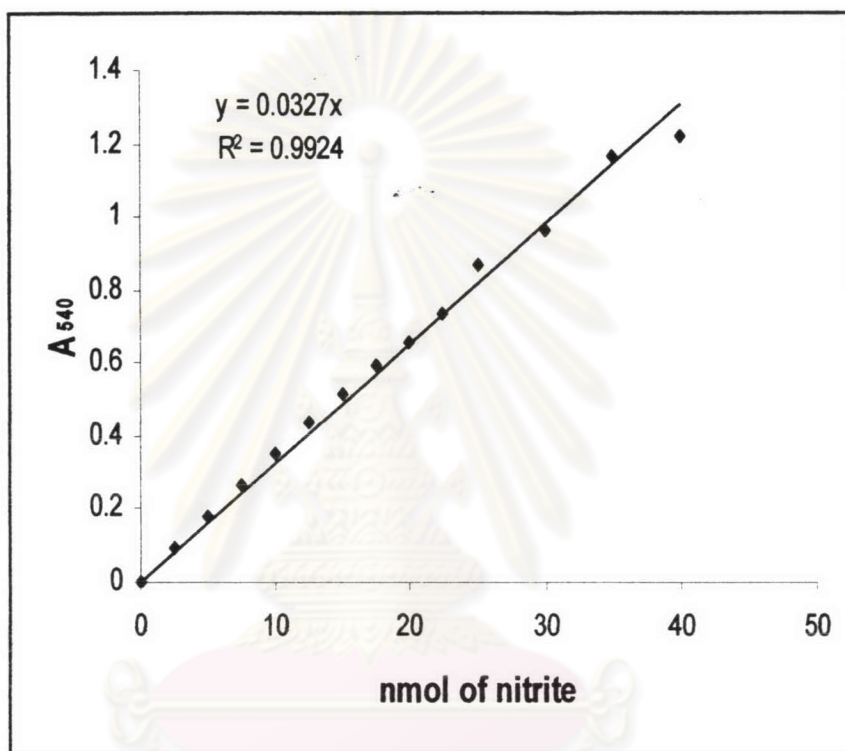
APPENDIX F

Calibration curve for conductivity of potassium phosphate



APPENDIX G

Standard curve for sodium nitrite



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BIOGRAPHY

Miss Soraya Thaivanich was born on December 2, 1978. She graduated with Bachelor degree of Science in Biochemistry from KhonKaen University in 2000. She has studies her Master's degree at the department of Biochemistry, Faculty of Science, Chulalongkorn University.



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