Appendixes

Appendix A

Media

1) Medium D stock (20x concentrate)

Distilled water	3500 ml
NTA (nitrilotriacetic acid)	8.0 g
Nitsch's Solution	40.0 ml
$FeCl_3$ Solution	80.0 ml
$CaSO_4 \bullet 2H_2O$	4.8 g
$MgSO_4 \bullet 7H_2O$	8.0 g
NaCl	0.64 g
KNO_3	8.24 g
$NaNO_3$	55.12 g
Na_2HPO_4	8.88 g
(or $Na_2HPO_4 \bullet 7H_2O$	16.75 g)

Bring volume to 4 L after adding all ingredients

To make D Medium add 50 ml of above stock solution to 950 ml of distilled water. Adjust the pH to 8.2 with 2N NaOH, before autoclaving. Final pH after autoclaving is about 7.5

2) D Medium

Double distilled water	1000 ml
NTA (nitrilotriacetic acid)	0.1 g
Nitsch's Solution or micronutrient solution	0.5 ml
$FeCl_3$ Solution (0.29 g/l)	1.0 ml
$CaSO_4 \bullet 2H_2O$	0.06 g
$MgSO_4 \bullet 7H_2O$	0.10 g
NaCl	0.008 g

KNO_3	0.10 g
$NaNO_3$	0.70 g
Na_2HPO_4	0.11 g

For agar plates use 15 g/l Bacto-agar

3) Medium ND Stock (20x concentrate)

Distilled water	3500 ml
NTA	8 g
Nitsch's micronutrients solution	40.0 ml
$FeCl_3$	80.0 ml
$CaSO_4 \cdot 2H_2O$	4.8 g
$MgSO_4 \cdot 7H_2O$	8.0 g
NaCl	0.64 g
Na_2HPO_4	5.6 g
(or $Na_2HPO_4 \cdot 7H_2O$	10.56 g)
KH_2PO_4	2.88 g

Make up to 4 liters

Add 50 ml of the above stock solution to 950 ml. of distilled water. Adjust the pH to 8.2 with 2 N NaOH before autoclaving.

4) $FeCl_3$ Solution

H_2O	1 liter
$FeCl_3$	o.2905 g

1 ml. equals ca. 0.1 mg Fe. Store refrigerated.

5) Nitsch's Trace element Solution

Distilled H_2O	1 liter
H_2SO_4 (concentrated)	0.5 ml
$MnSO_4 \cdot H_2O$	2.28 g

$ZnSO_4 \bullet 7H_2O$	0.50 g
H_3BO_3	0.50 g
$CuSO_4 \bullet 5H_2O$	0.025 g
$Na_2MoO_4 \bullet 2H_2O$	0.025 g
$CoCl_2 \bullet 6H_2O$	0.045 g

Store refrigerated

6) LB medium (Luria-Bertani Medium)

Deionized water	950 ml
Tryptone	10 g
Yeast extract	5 g
NaCl	10 g

Shake until the solutes have dissolved. Adjust the pH to 7.0 with 5.0 N NaOH. Adjust the volume of the solution to 1 liter with deionized water before autoclaving.

Buffer

1) Tris EDTA (TE) buffer, pH 8.0

Tris.HCl 10 mM [1 ml of 1M stock, pH 8.0]

EDTA 1 mM [200 ul of 0.5 stock, pH 8.0]

Adjust the volume of the solution to 100 ml with deionized water before filtering and sterile by autoclaving.

2) 10X TBE Buffer (Tris-Borate-EDTA)

Tris Base	108 g
Boric Acid	55g
0.5M EDTA	20 mL
Water to	1.0 liter

First, dissolve everything in slightly less than 1 liter of water, and adjust the volume to 1 liter by adding water. The solution can be stored at room temperature. To use as a buffer, dilute the 10X stock 10-fold.

3) 1X TBE Buffer

10X TBE	100 mL
Water	900 mL

DGGE solutions

1) 40% Acrylamide (37.5:1)

Acrylamide	38.93 g
Bis-acrylamide	1.07 g
Deionized water (sterile) to	100 ml
Filtrate with filter mesh size	0.45 μm

Use a dark bottle and store at 4°C.

2) 50x TAE buffer

		final concentration
Tris base	242.2 g	2 M
Acetic acid or garcial	57.1 ml	1 M
0.5 M EDTA (ph 8.0)	100 ml	50 mM
Deionized water to	1,000 ml	

3) 1x TAE running buffer

50x TAE	20 ml
Deionized water	980 ml
Total volume	1, 000 ml

4) 10% Ammonium Persulfate

Ammonium Persulfate	0.1 g
Deionized water (sterile)	1.0 ml

Stored at -20°C

5) D code dye solution

Bromophenol Blue		0.05 g
50x TAE buffer		0.2 ml
Deionized water	to	10 ml

Stored at room temperature

6) 2x gel loading dye

2% Bromophenol Blue	0.25 ml
2% Xylene Cyanol	0.25 ml
100% Glycerol	7.0 ml
Deionized water	2.5 ml

Autoclave, keep at room temperature or 4°C

Appendix B

Fifty-five cyanobacterial enrichment cultures from all six hot springs

cyanobacterial enrichment cultures	Morphotypes (main characteristics)
Chroococcidiopsis sp. PR45*	Green colony,1.5-2.0 μm diameter
Chroococcidiopsis sp. PD60*	Green colony,1.5-2.0 μm
Chroococcidiopsis sp. KC45*	Green colony,1.5-2.0 μm
Chroococcidiopsis sp. RS40*	Green colony,1.5-2.0 μm
Chroococcidiopsis sp. RS40-1	Green colony,1.5-2.0 μm
Chroococcidiopsis sp. RS40-2	Green colony,1.5-2.0 μm
Chroococcidiopsis sp. RS55	Green colony,1.5-2.0 μm
Cyanosarcina sp. RS50	Brown colony, 3.9-4μm
Cyanosarcina sp. SK40*	Brown colony, 3.9-4μm
Leptolyngbya sp. PR40*	Green filament, 3.5 μm broad
Leptolyngbya sp. PR45	Green filament, 1-1.5x2.5 μm
Leptolyngbya sp. PD40*	Green filament, 2x2-2.8μm, cross walls slightly constricted
Leptolyngbya sp. PD40-1	Green filament, 2x2-2.5μm
Leptolyngbya sp. PD45*	Green filament, straight, 2x 2μm
Leptolyngbya sp. PD45-1	Green filament, straight, 2x 2.5μm
Leptolyngbya sp. PD45-2	Green filament, straight, 2x 2μm
Leptolyngbya sp. KC45*	Brown-red filament, curve filament (sometime), 2.5x3-3.2μm
Leptolyngbya sp. RS40	Green filament, 2x1.8-2μm
Leptolyngbya sp. RS45*	Green filament, 2x1.8-2μm
Leptolyngbya sp. RS45-1	Green filament, 2x1.8-2μm
Leptolyngbya sp. RS50	Green filament, straight, 2-2.5x 2μm
Leptolyngbya sp. RS55	Green filament, straight or curve, 1.8-2x2 μm
Leptolyngbya sp. SK40*	Green filament, straight, 3-3.2x1μm
Leptolyngbya sp. SK40-1	Green filament, straight, 2-2.5x 2μm
Leptolyngbya sp. SK40-2	Green filament, straight, 2x 2-2.2μm
Mastigocladus sp. SK40	Green filament, straight, true branching, 3x5μm
Mastigocladus sp. TP50	Green filament, straight, true branching, 2.5x2-2.5μm
Oscillatoria sp. KC45	Green filament, cross walls slightly constricted or none, 1.2x1μm

Oscillatoria sp. KC50*	Green filament, cross walls slightly constricted, 1.2x2.8-3μm
Oscillatoria sp. TP65*	Green filament, straight, 1 μm broad
Phormidium sp. PR40*	Brown filament, cross walls slightly constricted, 1.2-1.3x2μm
Phormidium sp. PD40	Brown filament, cross walls slightly constricted, 2x2μm
Phormidium sp. PD45*	Brown filament, cross walls slightly constricted, 2x2μm
Phormidium sp. PD45-1	Brown filament, cross walls slightly constricted, 1.2x1.8-2μm
Phormidium sp. RS40*	Brown filament, cross walls slightly constricted, 1.5x1.8-2μm
Phormidium sp. SK40	Green filament, straight, 3x5μm
Phormidium sp. SK40-2	Brown filament, cross walls slightly constricted, 1.5x1.8-2μm
Phormidium sp. SK45	Brown filament, cross walls slightly constricted, 1.5x1.6-1.7μm
Scytonema sp. PD45	Green filament, false branch, 3x3μm
Scytonema sp. PD50	Green filament, false branch, 2x2μm, heterocyst 2x2.5μm
Scytonema sp. TP40	Green filament, straight, 2.2x2.5μm
Scytonema sp. TP45	Green filament, false branch, 2.5x2μm
Scytonema sp. TP50	Green filament, false branch, 3x2μm, heterocyst 3x3.5-4μm
Synechoccoccus sp. PD55*	Unicellular, Rod shape, 1.4-1.5μm broad
Synechoccoccus sp. PD65	Unicellular, Rod shape, 1.1-1.2μm broad
Synechoccoccus sp. SK40*	Unicellular, Rod shape, 1.2-1.5μm broad
Synechoccoccus sp. SK40-1	Unicellular, Rod shape, 1.2-1.5μm broad
Synechoccoccus sp. SK45	Unicellular, Rod shape, 2μm broad
Synechoccoccus sp. SK50	Unicellular, Rod shape, 1.2-1.5μm broad
Synechoccoccus sp. SK55*	Unicellular, Rod shape, 1.2-1.5μm broad
Synechoccoccus sp. SK60	Unicellular, Rod shape, 1.2-1.5μm broad
Synechoccoccus sp. SK65	Unicellular, Rod shape, 0.9-1μm broad
Synechoccoccus sp. SK70	Unicellular, Rod shape, 1.1-1.2μm broad
Synechoccoccus sp. TP65*	Unicellular, Rod shape, 1.2-1.5μm broad
Synechoccoccus sp. TP65-1	Unicellular, Rod shape, 1.5-1.8μm broad

* Twenty cloning cultures were selected to plot in phylogenetic trees.

Appendix C

Physical and chemical properties data of the hot spring waters data

Hot spring	Season	Code	Air temp. (^oC)	Max. temp. (^oC)	pH	Conductivity (Us/cm)	Total alkalinity (mg/l)	Hardness (mg/l $CaCO_3$)	Ca (mg/l)	Mg (mg/l)
San Kamphaeng (KP)	Rainy	SK-r	32.5	67.5	8	940	260	206	34.47	29.28
	Cold dry	SK-w	32	64	8	752	260	180	31.26	24.89
	Summer	SK-s	34	67	8.5	1,120	255	186	33.67	24.89
Pong Deud (PD)	Rainy	PD-r	28	67	8.96	528	154	180	48.1	14.64
	Cold dry	PD-w	28	68	8.5	520	143	160	64.13	34.16
	Summer	PD-s	30	68	8	530	148	200	56.11	14.64
Theppanom (TP)	Rainy	TP-r	30	68	8.42	645	222	200	25.65	33.18
	Cold dry	TP-w	31	67	8	546	188.5	160	22.5	25.62
	Summer	TP-s	32	72	8	625	189	195.5	24.05	33.06
Pra Rueang (PR)	Rainy	PR-r	31	50	7.72	788	264	34	8.42	3.17
	Cold dry	PR-w	27	46.5	7.5	539	249.5	28	6	3.2
	Summer	PR-s	34	49	8.3	620	246.5	30	6.41	3.42
Raksawarin Public Park (RS)	Rainy	RS-r	30.5	57	7.38	490	142	65	21.64	2.68
	Cold dry	RS-w	32	56	7	2,190	135	63	20.4	3
	Summer	RS-s	32	58	7	1,450	132	61	21.24	1.95
Khaochaison (KC)	Rainy	KC-r	36	51.5	7.57	575	222	168	47.29	12.2
	Cold dry	KC-w	26	51	7	1,740	189	170	48.9	11.7
	Summer	KC-s	34	51	7.8	474	197.5	172	52.1	10.25

Physical and chemical properties data of the hot spring waters (continue)

Hot spring	Season	Code	Sulfides (mg/l)	Sulfate (mg/l)	SRP (mg/l)	NO_3^- (mg/l)	NH_4^+ (mg/l)	Sodium (mg/l)	K (mg/l)	Chloride (mg/l)	Iron (mg/l)
San Kamphaeng (KP)	Rainy	SK-r	5.33	220	0.12	6.3	0.72	132.142	11.296	10	<0.005
	Cold dry	SK-w	0.321	24	0.26	25.9	2.38	181.25	9.94	10	<0.005
	Summer	SK-s	6.02	16	0.24	19.5	2.04	125.69	10.86	10	<0.005
Pong Deud (PD)	Rainy	PD-r	0.003	27	0.64	0.8	0.01	76.429	4.444	10.4	<0.005
	Cold dry	PD-w	0.036	21	0.37	1.6	0.01	91.89	4.03	10	<0.005
	Summer	PD-s	0.01	27	0.28	0.7	0	80.5	4	10.2	<0.005
Theppanom (TP)	Rainy	TP-r	0.013	10	1.03	13.6	0	92.143	5.167	12.4	<0.005
	Cold dry	TP-w	0.065	8	0.44	1.3	0.03	128.75	3.67	11.6	<0.005
	Summer	TP-s	0.09	34	1.68	1	0.01	88.46	5.24	11.8	<0.005
Pra Rueang (PR)	Rainy	PR-r	0	18	0.25	1	0.08	114.07	3.85	22.8	<0.005
	Cold dry	PR-w	0	16	0.14	0.7	0.01	147.25	5.11	20.1	<0.005
	Summer	PR-s	0	17	0.06	0.7	0	135.27	5.01	22.4	<0.005
Raksawarin Public Park (RN)	Rainy	RS-r	0.001	24	0.16	1.1	0.15	47.04	2.57	8.4	<0.005
	Cold dry	RS-w	0.001	20	0.14	1.1	0.15	65.05	3.07	8.8	<0.005
	Summer	RS-s	0.001	28	0.16	1.3	0.16	55.01	3.01	8.8	<0.005
Khaochaison (KC)	Rainy	KC-r	0	1	0.34	1.4	0.21	25.93	2.29	6.8	<0.005
	Cold dry	KC-w	0	10	0.25	1.3	0.15	28.13	1.71	10.1	<0.005
	Summer	KC-s	0.18	14	0.18	1.3	0.13	35.2	2.3	9.4	<0.005

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1998-2001 M. Sc. (Biology), Chiang Mai University, Chiang Mai, Thailand

- Biodiversity of Algae in Some Hot Spring Areas in the Upper Part of Northern Thailand

1995-1998 B. Sc. (Microbiology), Chiang Mai University, Chiang Mai, Thailand

- Water Quality, Phytoplankton and Coliform Bacteria Distribution in the Reservoir of Ratchamangkla Park, Chiang Mai Province

Field of Specialization

- Taxonomy of cyanobacteria or blue-green algae by using morphology and molecular biology
- Assessment of water quality by using freshwater phytoplankton and macroalgae as biological indicator
- Biotechnology of algae; algal collection, algal cultivation

Scholarship and the prize

2001-2004	Ph.D. Thesis funded by The Royal Golden Jubilee Ph.D. Program and The Thailand Research Fund
2000	The first prize of APSAP Young Scientist Poster Awards by the Asian-Pacific Society for Applied Phycology in "Diversity of Blue-green Algae in the Hot Spring Areas of Northern Thailand"
1999-2000	M.Sc. Thesis funded by TRF/BIOTEC Special Program for Biodiversity Research and Training grant

Workshop in field specialization

April 2005	Workshop on freshwater algal research techniques at Phuket Rajabhat University, Phuket, THAILAND
Nov. 2003	Workshop on construction of dendrograms and phylogenetic trees for marine cyanobacteria, microalgae, and fungi at Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok, THAILAND
Sep. 2003	Workshop on the application of spectroscopy to ecological research at the Suranaree University of Technology, Nakhon Ratchasima, THAILAND
March 2003	Instruction of workshop on small industrial scale of *Spirulina* cultivation at Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, THAILAND
Nov. 2002	Workshop on molecular characterizing cyanobacterial diversity and community structure in hot springs at Department of Ecology & Biodiversity, The University of Hong Kong, HONG KONG, CHINA
Feb. 2001	Workshop on eutrophication and water quality management in some water resources in Thailand by Water Research Center (WRC) at Biology Department, Faculty of Science, Chiang Mai University, Chiang Mai, THAILAND

Nov. 1999 Workshop on cyanoprocaryotes for taxonomy and environmental monitoring in lotic ecosystem at the Institut fur Botanik, Universtat Innsbruck, Innsbruck, AUSTRIA

Attended Conference

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30 Oct – 4 Nov 2005 Poster presentation in "Genetical distribution of cyanobacterial communities at some hot springs of Thailand" in The Fourth Asian-Pacific Phycological Forum at Rama Gardena Hotel, Bangkok, THAILAND

24 August 2005 Oral presentation in "Diversity of Cyanobacteria in Some Hot Springs of Thailand" in The RGJ Seminar Series XXXVI: Biodiversity III at Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, THAILAND

13-19 August 2005 Oral presentation in "Morphological and Phylogenetic Criteria in Taxonomic Studies of Cyanobacteria at Some Hot Spring Areas of Thailand" in The 8th International Phycological Congress at Durban, SOUTH AFRICA

28-30 April 2005 Oral presentation in The 6th Annual RGJ Ph.D. Congress at Jomtein Palm Beach Resort, Chonburi, THAILAND

23-25 March 2005 Poster presentation in "Morphological and Genetic Criteria in Taxonomic Studies of Cyanobacteria at Some Hot Spring Areas of Thailand" in The 2nd National Conference on Algae and Plankton at Holiday Garden Hotel, Chiang Mai, THAILAND

3-7 Feb. 2003 Attended in The 3rd World Congress on Medicinal and Aromatic Plant for Human Welfare (WOCMAP) at Pang Suan Kaew Hotel, Chiang Mai, THAILAND

Jan. 2003 Poster presentation in "Diatoms and Blue-green Algae in the Thermal Springs of Northern Thailand" in The International Conference of Electron Microscopy at Pang Suan Kaew Hotel, Chiang Mai, THAILAND

Sep. 2001 Poster presentation in "Cyanophytes in Hot Spring Areas of Northern Thailand" in The 4th International Phycological Congress at Thessaloniki University, Thessaloniki, GREECE

9-12 Oct. 2000 Poster presentation in "Biodiversity of Algae in Some Hot Spring Areas in the Upper Part of Northern Thailand" in The 4th Annual Conference of Biodiversity Research and Training Program (BRT). Ammarin Lagoon Hotel, Pitsanuloke, THAILAND

3-6 July 2000 Poster presentation in "Diversity of Blue Green Algae in the Hot Spring Areas of Northern Thailand" in The 4th Asia-Pacific Conference on Algal Biotechnology. Hong Kong Convention and Exibition Centre, HONG KONG, CHINA

Publication

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