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ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



Appendices

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Appendix A

Bacterial Growth Media and Plant Nutrient Solutions

Preparation of all bacterial growth media and plant nutrient solutions are as described by Somasegaran and Hoben (1994) unless otherwise stated.

Yeast Extract Mannitol Broth (YMB)

Mannitol	10.0 g
K ₂ HPO ₄	0.5 g
MgSO ₄ .7H ₂ O	0.2 g
NaCl	0.1 g
Yeast extract	0.5 g
Deionized water	1.0 g

pH of medium was adjusted to 6.8 with 0.1 N NaOH. The medium was autoclaved at 121°C for 15 min.

Yeast Extract Mannitol Agar (YMA)

YMB	1 liter
Agar	15 g

Agar was added to 1 liter of YMB. The solution was shaken to suspend the agar then autoclaved at 121°C for 15 min. After autoclaving, the medium was shaken to ensure even mixing of melted agar with medium before pouring onto petridishes and left to solidify.

YMA with Congo Red

Congo Red stock solution: 250 mg of Congo Red dissolved in 100 ml of deionized water. 10 ml of Congo Red stock solution were added to 1 liter of YMA. The final Congo Red concentration was 25 µg.ml⁻¹. The medium was autoclaved at 121°C for 15 min.

N-free Nutrient Solutions

Stock Solutions	Chemicals	g/liter
1	CaCl ₂ .2H ₂ O	294.1
2	KH ₂ PO ₄	136.1
3	FeC ₆ H ₅ O ₇ .3H ₂ O	6.7
	MgSO ₄ .7H ₂ O	123.3
	K ₂ SO ₄	87.0
	MnSO ₄ .H ₂ O	0.338
4	H ₃ BO ₃	0.247
	ZnSO ₄ .7H ₂ O	0.288
	CuSO ₄ .5H ₂ O	0.100
	CoSO ₄ .7H ₂ O	0.056
	Na ₂ MoO ₄ .7 H ₂ O	0.048

Warm water was used to prepare stock solutions to get the ferric-citrate into solution. Ten liters of full-strength plant culture solution were prepared as follows:

- To 5 liters of water, add 5 ml of each stock solution and mix,
- Dilute to 10 liters by adding another 5 liters of water,
- Adjust pH to either 5.0 or 6.8 with 1 N HCl
- For positive control treatment, 0.05% KNO₃ was added to give final N concentration of 70 ppm.

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Appendix B

Chemicals and Solutions

1. Solutions for DNA extraction (Gibco BRL)

Saline-EDTA solution

15 mM NaCl, 10 mM EDTA, pH 8.0

0.9 g NaCl, 0.29 g EDTA were added to distilled water. The final volume was made to 100 ml. 0.1 N NaOH was used to adjust pH to 8.0 before autoclaving at 121°C for 15 min.

DNAzol

DNAzol solution (Gibco BRL) was used according to manufacturer's instruction.

2. Solutions for SDS-PAGE (Bio-rad)

Stock solutions

A. Acrylamide/bis (30% T, 2.67%C)

87.6 g acrylamide (29.2 g/100 ml)

2.4 g N'N'-bis-methylene-acrylamide (0.8 g/100 ml)

Make to 300 ml with deionized water. Filter and store at 4°C in the dark (30 days maximum).

B. 1.5 M Tris-HCl, pH 8.8

27.23 g Tris base (18.15 g/100 ml)

80 ml deionized water

Adjust to pH 8.8 with 6N HCl. Make to 150 ml with deionized water and store at 4°C

C. 0.5 M Tris-HCl, pH 6.8

6 g Tris base

60 ml deionized water

Adjust to pH 6.8 with 6N HCl. Make to 100 ml with deionized water and store at 4°C

D. 10% SDS

Dissolve 10 g SDS in 90 ml water with gentle stirring and bring to 100 ml with ddH₂O

E. Sample buffer (SDS reducing buffer) (store at room temperature)

Deionized water	3.8 ml
0.5 M Tris-HCl, pH 6.8	1.0 ml
Glycerol	0.8 ml
10% (w/v) SDS	1.6 ml
2-mercaptoethanol	0.4 ml
1 % (w/v) bromophenol blue	0.4 ml

Dilute the sample at least 1:4 with sample buffer, and heat at 95°C for 4 minutes

F. 5X electrode (running buffer), pH 8.3

Tris base	9.0 g	(15 g/l)
Glycine	43.2 g	(72 g/l)
SDS	3.0 g	(5 g/l)

Make to 600 ml with deionized water.

Store at 4°C. Warm to room temperature before use if precipitation occurs. Dilute 60 ml 5X stock with 240 ml deionized water for one electrophoretic run.

G. 10% Ammonium persulphate

One milliliter of aqueous 10% (w/v) Ammonium persulphate stock solution was prepared and stored at 4 C. Ammonium persulphate decomposes slowly, and fresh solutions were prepared weekly.

H. Buffers

The following buffers were chosen for controlling *B. japonicum* media pHs :

pH 4.0 - 5.0 : cis-endo-bicyclo(2.2.1)hept-5-ene-2,3-dicarboxylic anhydride

(NEDA)

6.0 : 2-(N-Morpholino)ethanesulfonic acid (MES)

7.0-8.0 : N-(2-Hydroxyethyl)piperazine-N'(2- ethanesulfonic acid) (HEPES)

9.0 : 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (AMPSO)



Appendix C

Duncan's Multiple Range Test

Duncan's Multiple Range Test has been used to determine multiple groupings. Means which do not differ significantly are grouped into one homogenous range (Steel & Torrie, 1980). In the following tables of results on average plant dry weights or average nodule dry weights, the means are arranged by magnitude from the smallest value to the largest value. The Duncan's Multiple Range Test (SPSS Manual, Chapter 8) is then used to carry out the multiple range groupings. The results are indicated in the following tables.

Table A.1 Duncan's Multiple Range Test for average plant dry weight for *Bradyrhizobium japonicum* strains S76, S78, S162 and *Glycine max* cv CM 2, CM 60, ST 2 in Leonard jars with nitrogen-free medium pH 5.0 (Level of probability, $\alpha = 0.05$)

Oneway

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.774	14	.412		
Within Groups	4.681	30	.156		
Total	10.455	44		2.643	.012

Duncan^a

S_AND_C	N	Subset for alpha = .05	
		1	2
NC,CM2	3	.3700	
S76,CM2	3	.4367	
S78,CM2	3	.4433	
S162,CM2	3	.4667	
S76,ST2	3	.6333	
NC,ST2	3	.6367	
S162,ST2	3	.6600	
NC,CM60	3	.8567	.8567
PC,CM2	3	.9000	.9000
S78,ST2	3	.9467	.9467
S76,CM60	3	1.0333	1.0333
S162,CM60	3	1.0567	1.0567
PC,ST2	3	1.1167	1.1167
S78,CM60	3		1.4433
PC,CM60	3		1.6067
Sig.		.060	.052

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A.2 Duncan's Multiple Range Test for average nodule dry weight for *Bradyrhizobium japonicum* strains S76, S78, S162 and *Glycine max* cv CM 2, CM 60, ST 2 in Leonard jars with nitrogen-free medium pH 5.0 (Level of probability, $\alpha = 0.05$)

Oneway

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.965E-02	8	4.956E-03	3.924	.008
Within Groups	2.273E-02	18	1.263E-03		
Total	6.239E-02	26			

Duncan^a

S_AND_C	N	Subset for alpha = .05		
		1	2	3
S162,CM2	3	1.000E-02		
S76,CM2	3	2.667E-02	2.667E-02	
S78,CM2	3	3.667E-02	3.667E-02	
S76,ST2	3	4.333E-02	4.333E-02	
S162,ST2	3	4.667E-02	4.667E-02	
S76,CM60	3	6.333E-02	6.333E-02	
S162,CM60	3		8.333E-02	8.333E-02
S78,ST2	3		9.333E-02	9.333E-02
S78,CM60	3			.1433
Sig.		.119	.057	.065

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A.3 Duncan's Multiple Range Test for average plant dry weight for *Bradyrhizobium japonicum* strains S76, S78, S162 and *Glycine max* cv CM 2, CM 60, ST 2 in Leonard jars with nitrogen-free medium pH 6.8 (Level of probability, $\alpha = 0.05$)

Oneway

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.677	14	.405	2.289	.028
Within Groups	5.314	30	.177		
Total	10.991	44			

Duncan^a

S_AND_C	N	Subset for alpha = .05		
		1	2	3
NC,ST2	3	.3967		
NC,CM2	3	.6100	.6100	
PC,ST2	3	.6433	.6433	
S162,ST2	3	.6700	.6700	
NC,CM60	3	.7700	.7700	
S76,CM2	3	.9600	.9600	
S78,ST2	3	.9800	.9800	
PC,CM60	3	1.0067	1.0067	
S162,CM2	3	1.0467	1.0467	
S78,CM60	3	1.0600	1.0600	
S76,ST2	3	1.1367	1.1367	
PC,CM2	3	1.1767	1.1767	
S78,CM2	3	1.1867	1.1867	
S76,CM60	3		1.2833	1.2833
S162,CM60	3			1.9567
Sig.		.062	.109	.059

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A.4 Duncan's Multiple Range Test for average nodule dry weight for *Bradyrhizobium japonicum* strains S76, S78, S162 and *Glycine max* cv CM 2, CM 60, ST 2 in Leonard jars with nitrogen-free medium pH 6.8 (Level of probability, $\alpha = 0.05$)

Oneway

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.305E-02	8	2.881E-03	.970	.489
Within Groups	5.348E-02	18	2.971E-03		
Total	7.654E-02	26			

Duncan

S_AND_C	N	Subset for alpha = .05
		1
S162,CM2	3	9.667E-02
S78,ST2	3	.1033
S76,CM2	3	.1033
S78,CM2	3	.1167
S162,ST2	3	.1167
S76,CM60	3	.1433
S76,ST2	3	.1433
S162,CM60	3	.1700
S78,CM60	3	.1833
Sig.		.106

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A.6 Values of F (Steel & Torrie, 1980)

Denominator d.f.	Probability of a larger F	Numerator d.f.									Numerator d.f.										
		1	2	3	4	5	6	7	8	9	10	12	15	20	24	30	40	60	120	∞	
1	.100	39.86	49.50	53.59	55.83	57.24	58.20	58.91	59.44	59.85	60.19	60.71	61.22	61.74	62.00	62.53	63.06	63.33	.100	1	
	.050	16.14	19.85	21.57	22.96	24.02	24.88	25.68	26.35	26.95	27.19	27.97	28.59	29.11	29.61	29.92	29.97	29.99	.050		
	.025	6.05	6.47	6.84	7.16	7.42	7.67	7.92	8.16	8.37	8.56	8.76	8.97	9.17	9.37	9.57	9.77	9.97	.025		
	.010	4.93	5.35	5.70	6.03	6.25	6.46	6.67	6.87	7.06	7.25	7.44	7.63	7.82	8.01	8.20	8.39	8.58	.010		
	.005	4.03	4.42	4.71	5.00	5.26	5.50	5.71	5.90	6.08	6.26	6.44	6.62	6.80	6.97	7.14	7.30	7.45	.005		
	.001	2.00	2.11	2.15	2.18	2.20	2.22	2.24	2.26	2.28	2.29	2.31	2.32	2.33	2.34	2.35	2.36	2.37	.001		
2	.100	8.53	9.00	9.16	9.24	9.29	9.33	9.37	9.38	9.39	9.39	9.39	9.41	9.42	9.44	9.45	9.46	9.47	9.48	.100	2
	.050	8.51	19.00	19.16	19.23	19.30	19.33	19.35	19.37	19.38	19.39	19.40	19.43	19.43	19.45	19.45	19.46	19.47	19.48	.050	
	.025	38.51	39.60	39.91	39.97	39.99	39.99	39.99	39.99	39.99	39.99	39.99	39.99	39.99	39.99	39.99	39.99	39.99	39.99	.025	
	.010	98.50	99.00	99.17	99.22	99.25	99.30	99.33	99.36	99.37	99.37	99.40	99.42	99.43	99.45	99.45	99.46	99.47	99.48	.010	
	.005	198.5	199.0	199.2	199.3	199.3	199.3	199.3	199.4	199.4	199.4	199.4	199.4	199.5	199.5	199.5	199.5	199.5	199.5	.005	
	.001	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	.001	
3	.100	5.54	5.56	5.59	5.61	5.63	5.64	5.67	5.67	5.68	5.68	5.70	5.72	5.74	5.74	5.75	5.76	5.76	5.76	.100	4
	.050	10.13	10.13	10.13	10.13	10.13	10.13	10.13	10.13	10.13	10.13	10.13	10.13	10.13	10.13	10.13	10.13	10.13	10.13	.050	
	.025	17.44	16.09	15.44	15.10	14.88	14.73	14.57	14.54	14.52	14.50	14.47	14.42	14.37	14.32	14.25	14.17	14.12	14.08	.025	
	.010	34.22	30.82	29.46	28.71	28.24	27.91	27.67	27.49	27.33	27.25	27.05	26.87	26.69	26.50	26.32	26.13	26.13	26.13	.010	
	.005	55.55	49.60	47.47	46.19	45.39	44.84	44.43	44.13	43.88	43.69	43.39	43.08	42.78	42.62	42.47	42.31	41.83	41.83	.005	
	.001	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	.001	
4	.100	4.54	4.52	4.51	4.50	4.48	4.46	4.45	4.44	4.43	4.42	4.41	4.40	4.38	4.37	4.36	4.35	4.34	4.34	.100	5
	.050	12.22	10.65	9.58	8.95	8.39	7.83	7.39	6.98	6.60	6.26	5.96	5.66	5.36	5.06	4.76	4.46	4.16	4.06	.050	
	.025	22.78	18.60	16.68	15.58	14.75	13.95	13.39	12.95	12.50	12.06	11.61	11.17	10.72	10.30	9.87	9.47	9.07	8.67	.025	
	.010	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	.010	
	.005	31.33	26.28	24.25	23.15	21.95	21.62	21.35	21.14	20.97	20.70	20.44	20.17	20.03	19.89	19.75	19.61	19.47	19.32	.005	
	.001	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	.001	
5	.100	4.06	3.78	3.62	3.52	3.45	3.40	3.37	3.34	3.32	3.30	3.27	3.24	3.21	3.19	3.17	3.15	3.14	3.13	.100	6
	.050	6.61	5.79	5.41	5.19	4.85	4.55	4.28	4.02	3.77	3.52	3.27	3.02	2.77	2.52	2.27	2.02	1.77	1.52	.050	
	.025	10.01	8.43	7.76	7.16	6.65	6.26	5.85	5.45	5.05	4.65	4.26	3.86	3.47	3.07	2.67	2.27	1.87	1.47	.025	
	.010	16.26	13.27	12.06	11.39	10.72	10.17	9.67	9.17	8.67	8.17	7.67	7.17	6.67	6.17	5.67	5.17	4.67	4.17	.010	
	.005	22.78	18.31	16.53	15.36	14.21	13.02	11.77	10.57	9.39	8.10	7.03	6.03	5.03	4.03	3.03	2.03	1.03	1.03	.005	
	.001	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	.001	
7	.100	3.59	3.74	3.76	3.77	3.78	3.78	3.78	3.78	3.78	3.78	3.78	3.78	3.78	3.78	3.78	3.78	3.78	3.78	.100	8
	.050	5.59	6.54	7.54	8.07	8.42	8.88	9.37	9.87	10.37	10.87	11.37	11.87	12.37	12.87	13.37	13.87	14.37	14.87	.050	
	.025	12.25	9.55	8.45	7.81	7.46	7.12	6.77	6.42	6.07	5.72	5.37	5.02	4.67	4.32	3.97	3.62	3.27	2.92	.025	
	.010	22.78	18.31	16.53	15.36	14.21	13.02	11.77	10.57	9.39	8.10	7.03	6.03	5.03	4.03	3.03	2.03	1.03	1.03	.010	
	.005	31.33	26.28	24.25	23.15	21.95	21.62	21.35	21.14	20.97	20.70	20.44	20.17	20.03	19.89	19.75	19.61	19.47	19.32	.005	
	.001	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	.001	
8	.100	3.46	3.11	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	.100	9
	.050	5.46	5.12	4.81	4.52	4.24	3.95	3.67	3.39	3.11	2.83	2.55	2.27	1.99	1.71	1.43	1.15	0.87	0.59	.050	
	.025	12.25	9.55	8.45	7.32	6.26	5.21	4.26	3.39	2.55	1.77	1.03	0.43	0.19	-0.14	-0.43	-0.93	-1.43	-1.93	.025	
	.010	22.78	18.31	16.53	15.36	14.21	13.02	11.77	10.57	9.39	8.10	7.03	6.03	5.03	4.03	3.03	2.03	1.03	1.03	.010	
	.005	31.33	26.28	24.25	23.15	21.95	21.62	21.35	21.14	20.97	20.70	20.44	20.17	20.03	19.89	19.75	19.61	19.47	19.32	.005	
	.001	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	.001	
10	.100	3.01	3.14	3.47	3.71	3.93	4.07	3.95	3.85	3.75	3.64	3.54	3.44	3.34	3.24	3.14	3.04	2.94	2.84	.100	11
	.050	5.96	4.71	3.86	3.12	2.48	1.84	1.23	0.68	0.13	-0.30	-0.85	-1.39	-1.93	-2.47	-2.91	-3.35	-3.79	-4.23	.050	
	.025	12.25	9.55	8.45	7.32	6.26	5.21	4.26	3.39	2.55	1.77	1.03	0.43	0.19	-0.14	-0.43	-0.93	-1.43	-1.93	.025	
	.010	22.78	18.31	16.53	15.36	14.21	13.02	11.77	10.57	9.39	8.10	7.03	6.03	5.03	4.03	3.03	2.03	1.03	1.03	.010	
	.005	31.33	26.28	24.25	23.15	21.95	21.62	21.35	21.14	20.97	20.70	20.44	20.17	20.03	19.89	19.75	19.61	19.47	19.32	.005	
	.001	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	.001	
12	.100	3.18	3.18	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	.100	13
	.050	4.75	3.89	3.46	3.12	2.86	2.39	1.91	1.31	0.71	0.11	-0.50	-1.09	-1.68	-2.27	-2.86	-3.45	-4.04	-4.64	.050	
	.025	12.25	9.55	8.45	7.32	6.26	5.21	4.26	3.39	2.55	1.77	1.03	0.43	0.19	-0.14	-0.43	-0.93	-1.43	-1.93	.025	
	.010	22.78	18.31	16.53	15.36	14.21	13.02	11.77	10.57	9.39	8.10	7.03	6.03	5.03	4.						

Table A.6 Values of F (Continued)

Denominator df	Probability of a larger F	Numerator df									Numerator df									P		df
		1	2	3	4	5	6	7	8	9	10	12	15	17	20	24	30	40	60	120	∞	P
15	.100	3.07	2.70	2.49	2.36	2.27	2.21	2.16	2.12	2.09	2.06	2.02	1.97	1.92	1.87	1.85	1.82	1.79	1.76	.100	.15	
	.025	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.48	2.43	2.39	2.32	2.29	2.20	2.16	2.11	2.07	.050	
	.010	6.20	4.77	4.15	3.80	3.58	3.41	3.29	3.20	3.12	3.06	2.95	2.86	2.76	2.64	2.59	2.52	2.46	2.40	2.37	.025	
	.005	10.80	6.36	5.12	4.89	4.36	4.14	4.00	3.89	3.72	3.62	3.49	3.37	3.29	3.19	3.09	3.03	2.95	2.87	.010		
16	.100	3.03	2.67	2.46	2.33	2.24	2.18	2.13	2.09	2.06	2.03	1.97	1.94	1.89	1.84	1.81	1.78	1.75	1.72	.100	.16	
	.025	4.19	3.63	3.24	3.01	2.85	2.74	2.67	2.59	2.54	2.49	2.42	2.35	2.28	2.24	2.19	2.15	2.11	2.07	2.01	.050	
	.010	6.12	5.63	5.08	4.82	4.37	4.05	3.85	3.73	3.62	3.54	3.40	3.29	3.19	3.09	3.02	2.98	2.94	2.88	2.82	.025	
	.005	10.58	6.53	5.23	4.92	4.47	4.14	3.92	3.73	3.52	3.40	3.20	3.02	2.89	2.79	2.68	2.57	2.51	2.45	2.38	.010	
17	.100	3.03	2.64	2.44	2.31	2.22	2.15	2.10	2.06	2.03	2.00	1.96	1.91	1.86	1.84	1.81	1.78	1.75	1.72	1.69	.100	
	.025	4.45	3.59	3.20	2.96	2.81	2.72	2.61	2.55	2.49	2.45	2.38	2.33	2.27	2.22	2.19	2.15	2.10	2.07	2.01	.17	
	.010	6.40	4.82	4.01	3.66	3.44	3.28	3.16	3.06	2.95	2.82	2.72	2.62	2.56	2.49	2.44	2.38	2.32	2.25	2.20	.025	
	.005	10.38	7.35	5.18	4.67	4.34	4.00	3.72	3.40	3.14	2.86	2.59	2.31	2.06	1.81	1.56	1.31	1.04	2.65	2.63	.010	
18	.100	3.01	2.62	2.42	2.29	2.20	2.13	2.08	2.04	2.00	1.98	1.93	1.89	1.84	1.78	1.75	1.72	1.69	1.66	.100	.18	
	.025	4.41	3.55	3.16	2.95	2.81	2.72	2.66	2.58	2.51	2.46	2.41	2.34	2.27	2.21	2.15	2.06	2.02	1.97	1.92	.025	
	.010	6.38	5.30	5.64	5.27	4.92	4.51	4.38	4.27	4.10	3.92	3.73	3.54	3.35	3.18	3.10	3.02	2.93	2.84	2.73	.010	
	.005	10.58	7.53	5.30	4.92	4.57	4.27	3.95	3.64	3.30	3.01	2.71	2.41	2.11	1.81	1.46	1.11	1.03	1.00	1.00	.005	
19	.100	3.03	2.64	2.44	2.31	2.22	2.15	2.10	2.06	2.03	2.00	1.96	1.91	1.86	1.81	1.79	1.75	1.72	1.69	1.66	.100	
	.025	4.18	3.32	3.13	2.90	2.74	2.63	2.54	2.47	2.42	2.38	2.31	2.23	2.16	2.11	2.07	2.03	1.98	1.93	1.90	.19	
	.010	6.18	5.12	4.59	4.30	4.05	3.77	3.56	3.33	3.17	2.95	2.72	2.42	2.16	1.86	1.56	1.31	1.04	1.00	1.00	.050	
	.005	10.07	7.09	5.92	5.27	4.85	4.56	4.34	4.18	4.04	3.93	3.76	3.52	3.30	3.15	3.00	2.82	2.73	2.67	2.61	.025	
20	.100	2.97	2.59	2.38	2.25	2.16	2.09	2.04	2.00	1.96	1.94	1.89	1.84	1.79	1.75	1.72	1.69	1.66	1.64	1.61	.100	
	.025	4.35	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39	2.35	2.27	2.23	2.17	2.12	2.08	2.04	1.99	1.94	1.90	.20	
	.010	6.22	5.03	4.57	4.26	3.95	3.57	3.29	3.01	2.71	2.41	2.04	1.74	1.43	1.13	1.00	1.00	1.00	1.00	1.00	.050	
	.005	10.22	7.21	5.03	4.57	4.26	3.95	3.57	3.29	3.01	2.71	2.41	2.04	1.74	1.43	1.13	1.00	1.00	1.00	1.00	.025	
21	.100	2.96	2.51	2.32	2.17	2.06	1.98	1.92	1.88	1.84	1.80	1.76	1.72	1.68	1.63	1.59	1.55	1.52	1.50	1.50	.21	
	.025	4.32	3.47	3.07	2.84	2.68	2.57	2.49	2.42	2.37	2.32	2.25	2.18	2.12	2.05	2.01	1.96	1.92	1.88	1.84	.21	
	.010	6.19	5.04	4.57	4.26	3.95	3.57	3.29	3.01	2.71	2.41	2.04	1.74	1.43	1.13	1.00	1.00	1.00	1.00	1.00	.050	
	.005	10.15	7.10	5.05	4.59	4.28	3.97	3.58	3.29	3.01	2.71	2.41	2.04	1.74	1.43	1.13	1.00	1.00	1.00	1.00	.025	
22	.100	2.95	2.56	2.35	2.22	2.13	2.06	2.01	1.97	1.93	1.89	1.86	1.81	1.76	1.73	1.70	1.67	1.64	1.60	1.57	.22	
	.025	4.29	3.44	3.09	2.84	2.66	2.55	2.46	2.39	2.34	2.29	2.23	2.15	2.09	2.03	1.98	1.94	1.90	1.86	1.82	.22	
	.010	6.09	5.00	4.53	4.22	3.91	3.56	3.22	3.05	2.89	2.64	2.40	2.16	1.86	1.56	1.26	1.06	1.00	1.00	1.00	.050	
	.005	10.05	7.05	5.00	4.53	4.22	3.91	3.56	3.22	3.05	2.89	2.64	2.40	2.16	1.86	1.56	1.26	1.06	1.00	1.00	.025	
23	.100	2.94	2.54	2.34	2.21	2.11	2.05	1.99	1.95	1.92	1.89	1.84	1.79	1.74	1.70	1.67	1.62	1.58	1.55	1.50	.23	
	.025	4.28	3.42	3.03	2.78	2.53	2.37	2.21	2.06	1.91	1.86	1.80	1.74	1.68	1.63	1.58	1.53	1.49	1.45	1.42	.23	
	.010	6.04	5.00	4.53	4.22	3.91	3.56	3.22	3.05	2.89	2.64	2.40	2.16	1.86	1.56	1.26	1.06	1.00	1.00	1.00	.050	
	.005	10.01	6.98	5.00	4.53	4.22	3.91	3.56	3.22	3.05	2.89	2.64	2.40	2.16	1.86	1.56	1.26	1.06	1.00	1.00	.025	
24	.100	2.93	2.54	2.33	2.19	2.10	2.04	1.98	1.94	1.91	1.88	1.83	1.78	1.73	1.69	1.65	1.62	1.58	1.55	1.50	.24	
	.025	4.26	3.40	3.01	2.78	2.53	2.37	2.21	2.06	1.91	1.86	1.80	1.74	1.68	1.63	1.58	1.53	1.49	1.45	1.42	.24	
	.010	6.00	5.00	4.53	4.22	3.91	3.56	3.22	3.05	2.89	2.64	2.40	2.16	1.86	1.56	1.26	1.06	1.00	1.00	1.00	.050	
	.005	9.98	6.91	5.00	4.53	4.22	3.91	3.56	3.22	3.05	2.89	2.64	2.40	2.16	1.86	1.56	1.26	1.06	1.00	1.00	.025	
25	.100	2.92	2.53	2.32	2.18	2.09	2.02	1.97	1.93	1.89	1.85	1.80	1.75	1.70	1.66	1.62	1.58	1.55	1.52	1.50	.25	
	.025	4.24	3.44	3.09	2.84	2.60	2.45	2.30	2.24	2.19	2.14	2.09	2.03	1.98	1.93	1.88	1.83	1.79	1.75	1.72	.25	
	.010	6.04	5.00	4.53	4.22	3.91	3.56	3.22	3.05	2.89	2.64	2.40	2.16	1.86	1.56	1.26	1.06	1.00	1.00	1.00	.050	
	.005	9.91	6.94	5.00	4.53	4.22	3.91	3.56	3.22	3.05	2.89	2.64	2.40	2.16	1.86	1.56	1.26	1.06	1.00	1.00	.025	
26	.100	2.91	2.52	2.31	2.17	2.08	2.01	1.96	1.92	1.88	1.84	1.80	1.76	1.71	1.67	1.62	1.58	1.54	1.50	1.48	.26	
	.025	4.23	3.45	3.09	2.84	2.59	2.47	2.32	2.27	2.21	2.16	2.11	2.06	1.99	1.95	1.90	1.85	1.80	1.76	1.72	.26	
	.010	6.06	5.02	4.54	4.23	3.92	3.57	3.23	3.08	2.93	2.78	2.63	2.48	2.34	2.29	2.24	2.19	2.07	1.93	1.88	.050	
	.005	9.84	6.93	5.00	4.53	4.22	3.91	3.56	3.22	3.05	2.89	2.64	2.40	2.16	1.86	1.56	1.26	1.06	1.00	1.00	.025	
27	.100	2.90	2.51	2.30	2.17	2.07	2.00	1.95	1.91	1.87	1.85	1.80	1.75	1.70	1.67	1.64	1.60	1.57	1.54	1.50	.27	
	.025	4.21	3.43	3.08	2.83	2.58	2.44	2.30	2.24	2.19	2.14	2.09	2.03	1.98	1.93	1.88	1.84	1.79	1.75	1.72	.27	
	.010	6.01	5.01	4.53	4.22	3.91	3.56	3.22	3.05	2.89	2.64	2.40	2.16	1.86	1.56	1.26	1.06	1.00	1.00	1.00	.050	
	.005	9.79	6.92	5.00	4.53	4.22	3.91	3.56	3.22	3.05	2.89	2.64	2.40	2.16	1.86	1.56	1.26	1.06	1.00	1.00	.025	
28	.100	2.89	2.49	2.29	2.18	2.09	2.02	1.97	1.93	1.89	1.85	1.80	1.75	1.70	1.67	1.64	1.60	1.57	1.54	1.50	.28	
	.025	4.20	3.44	3.09	2.84	2.59	2.45															

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Table A.6 Values of F (Continued)

Denominator df	Probability of a larger F	Numerator df									Numerator df									P	df
		1	2	3	4	5	6	7	8	9	10	12	15	20	24	30	40	60	120	∞	
29	.100	2.89	2.50	2.28	2.15	2.05	1.99	1.93	1.89	1.86	1.78	1.73	1.68	1.65	1.62	1.58	1.55	1.51	1.47	.100	29
	.050	4.18	3.33	2.93	2.70	2.43	2.27	2.05	1.89	1.86	2.10	2.03	1.94	1.89	1.85	1.80	1.73	1.69	1.64	.050	
	.025	5.39	4.20	3.61	3.27	3.04	2.88	2.76	2.67	2.59	2.43	2.32	2.22	2.12	2.09	2.03	1.96	1.89	1.81	.025	
	.010	7.60	5.32	4.54	4.04	3.50	3.23	3.09	3.00	2.93	2.73	2.57	2.45	2.34	2.23	2.13	2.03	1.94	1.83	.010	
	.005	9.23	6.40	5.28	4.66	4.26	3.88	3.57	3.61	3.48	3.09	3.38	3.00	2.87	2.76	2.66	2.56	2.45	2.33	.005	
	.001	10.0	7.32	5.88	4.99	4.17	3.42	2.92	2.28	2.14	2.05	1.93	1.88	1.85	1.72	1.67	1.57	1.54	1.50	.001	30
30	.100	2.88	2.49	2.32	2.21	2.05	1.98	1.93	1.88	1.85	1.77	1.72	1.67	1.64	1.61	1.57	1.54	1.50	1.46	.100	30
	.050	4.17	3.32	2.92	2.69	2.33	2.12	2.01	1.93	1.88	2.11	2.01	1.93	1.89	1.84	1.79	1.74	1.68	1.62	.050	
	.025	5.37	4.18	3.59	3.25	2.90	2.65	2.37	2.25	2.17	2.04	2.01	1.94	1.88	1.82	1.76	1.70	1.64	1.59	.025	
	.010	7.56	5.39	4.51	4.02	3.70	3.47	3.20	3.17	3.07	2.80	2.63	2.43	2.23	2.03	1.88	1.71	1.61	1.51	.010	
	.005	9.18	6.33	5.24	4.62	4.23	3.95	3.58	3.74	3.45	3.01	2.81	2.52	2.32	2.12	1.93	1.76	1.66	1.57	.005	
	.001	10.0	2.84	2.44	2.23	2.09	2.00	1.93	1.87	1.83	1.79	1.71	1.66	1.61	1.54	1.51	1.47	1.42	1.38	.001	40
40	.100	2.84	2.44	2.23	2.09	2.00	1.93	1.87	1.83	1.79	1.73	1.66	1.61	1.54	1.51	1.47	1.42	1.38	1.33	.100	40
	.050	4.08	3.32	2.84	2.45	2.16	1.94	1.74	1.64	1.54	2.05	1.96	1.88	1.80	1.74	1.69	1.64	1.58	1.53	.050	
	.025	5.42	4.15	3.45	3.03	2.61	2.24	2.05	1.93	1.87	2.08	1.98	1.90	1.82	1.75	1.69	1.63	1.57	1.51	.025	
	.010	7.31	5.18	4.31	3.83	3.51	3.29	3.01	2.91	2.85	2.80	2.75	2.65	2.55	2.45	2.35	2.29	2.21	2.15	.010	
	.005	8.83	6.07	4.98	4.37	3.99	3.71	3.49	3.31	3.12	3.22	3.01	2.89	2.75	2.61	2.49	2.35	2.20	2.11	.005	
	.001	10.0	2.79	2.39	2.18	2.04	1.95	1.87	1.82	1.77	1.74	1.70	1.66	1.61	1.57	1.51	1.48	1.44	1.40	.001	60
60	.100	2.79	2.39	2.18	2.04	1.95	1.87	1.82	1.77	1.74	1.70	1.66	1.61	1.57	1.51	1.48	1.44	1.40	1.35	.100	60
	.050	4.00	3.15	2.76	2.33	2.07	1.87	1.75	1.67	1.61	2.04	1.92	1.82	1.72	1.62	1.53	1.49	1.45	1.41	.050	
	.025	5.29	3.93	3.34	3.01	2.79	2.43	2.15	2.03	1.93	2.07	1.92	1.82	1.72	1.62	1.53	1.48	1.44	1.40	.025	
	.010	7.08	4.98	4.38	4.13	3.65	3.34	3.02	2.85	2.72	2.63	2.50	2.35	2.20	2.12	2.03	1.94	1.87	1.80	.010	
	.005	8.49	5.79	4.73	4.36	3.76	3.49	3.29	3.01	2.91	2.80	2.74	2.57	2.39	2.29	2.19	2.08	1.96	1.88	.005	
	.001	10.0	2.75	2.35	2.13	1.99	1.90	1.82	1.77	1.72	1.68	1.60	1.55	1.50	1.45	1.41	1.37	1.32	1.26	.001	120
120	.100	2.75	2.35	2.13	1.99	1.90	1.82	1.77	1.72	1.68	1.60	1.51	1.46	1.41	1.37	1.32	1.26	1.21	1.19	.100	120
	.050	3.92	3.07	2.68	2.45	2.29	2.17	2.07	1.96	1.86	2.05	1.91	1.81	1.71	1.62	1.56	1.50	1.43	1.35	.050	
	.025	5.15	3.90	3.23	2.95	2.67	2.39	2.20	2.07	1.96	2.05	1.91	1.81	1.71	1.62	1.54	1.49	1.43	1.37	.025	
	.010	7.25	5.15	4.79	4.35	3.95	3.48	3.17	2.96	2.79	2.66	2.56	2.45	2.31	2.17	2.06	1.96	1.86	1.76	.010	
	.005	8.18	5.54	4.50	4.05	3.55	3.28	3.02	2.81	2.63	2.50	2.37	2.21	2.09	1.96	1.87	1.77	1.67	1.53	.005	
	.001	10.0	2.71	2.30	2.06	1.94	1.85	1.77	1.72	1.67	1.63	1.60	1.55	1.49	1.42	1.38	1.32	1.24	1.17	.001	100
∞	.100	2.74	2.30	2.06	1.94	1.85	1.77	1.72	1.67	1.63	1.60	1.55	1.49	1.42	1.38	1.32	1.24	1.17	1.00	.100	∞
	.050	3.74	3.00	2.60	2.29	2.06	1.94	1.85	1.77	1.72	1.67	1.63	1.55	1.49	1.42	1.38	1.32	1.24	1.17	.050	
	.025	5.02	3.63	3.12	2.79	2.47	2.19	2.01	1.89	1.84	1.80	1.75	1.67	1.57	1.48	1.42	1.36	1.27	1.19	.025	
	.010	6.63	4.61	3.78	3.23	2.80	2.48	2.18	2.02	1.90	1.86	1.80	1.72	1.62	1.52	1.44	1.37	1.28	1.20	.010	
	.005	7.88	5.30	4.28	3.72	3.15	2.80	2.42	2.32	2.14	2.09	2.02	1.92	1.82	1.72	1.62	1.52	1.42	1.32	.005	
	.001	10.0	2.74	2.32	2.08	1.96	1.85	1.77	1.72	1.67	1.63	1.60	1.55	1.49	1.42	1.38	1.32	1.24	1.17	.001	120

Sources: A portion of the tables of percentage points of the inverted beta ($F_{\text{di-tilde}}^{\text{inverted}}$, "Biometrika," vol. 33, 1943) by M. Merrington and G. M. Thompson, and from Table 18 of Biometrika Tables for Statisticians, vol. I, Cambridge University Press, 1934, edited by E. S. Pearson and H. O. Hartley. Reproduced with permission of the authors, editors, and Biometrika trustees.

Appendix D

Preliminary results on N-terminal amino acid sequencing of the 53 kDa polypeptide of mid-log phase cells of *B. japonicum* S76 grown in yeast extract mannitol medium, pH 5.5, at 200 rpm, 30°C.

S76

SAMPLE: PS0043 9/4/2004
 [Friday, April 9, 2004, 13:10]

Sample Amount: 75.0 picomoles

AACID #	AACID ID	R. TIME (min)	C. TIME (min)	PMOL (raw)	PMOL (-back)	PMOL (+lag)	AACID ID
1	N	7.58	7.67	0.46	0.12	0.12	Asn
2	S	8.72	8.55	0.08	0.02	0.03	Ser
3	V	19.58	19.31	0.04	0.02	0.02	Val
4	T	9.18	9.18	0.10	0.04	0.04	Thr

REPETITIVE YIELD ANALYSIS:

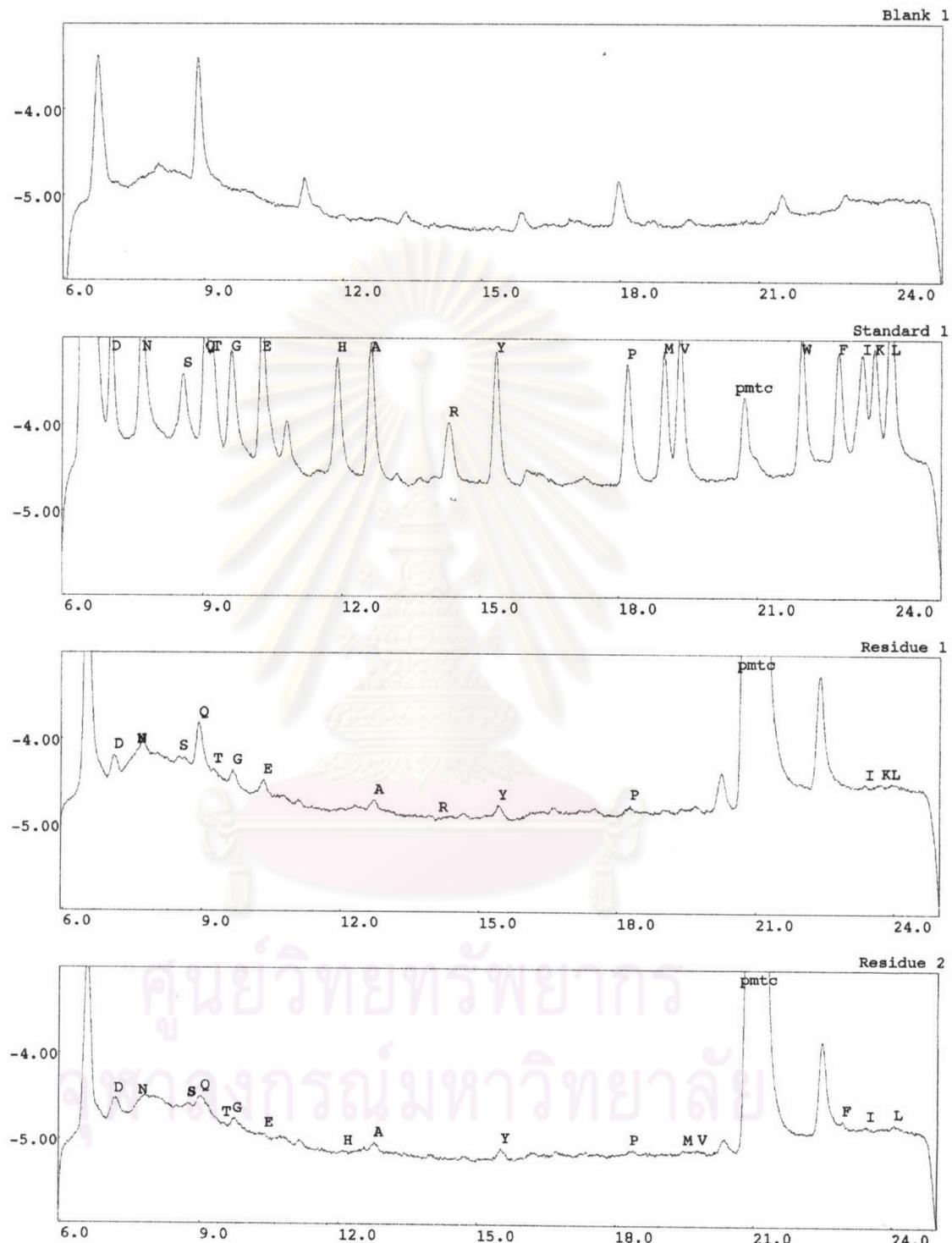
Rep. Yield Variance

Combined Repetitive Yield	71.78%	0.302
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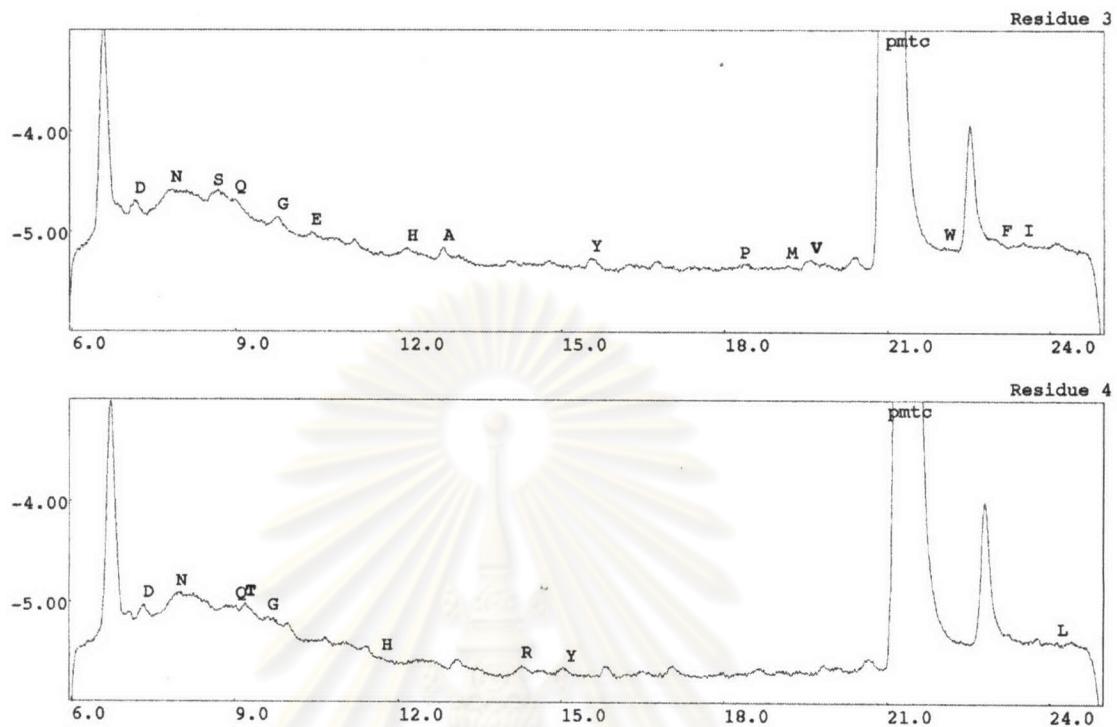
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

S76



Applied Biosystems Procise - PROCISE-CLC

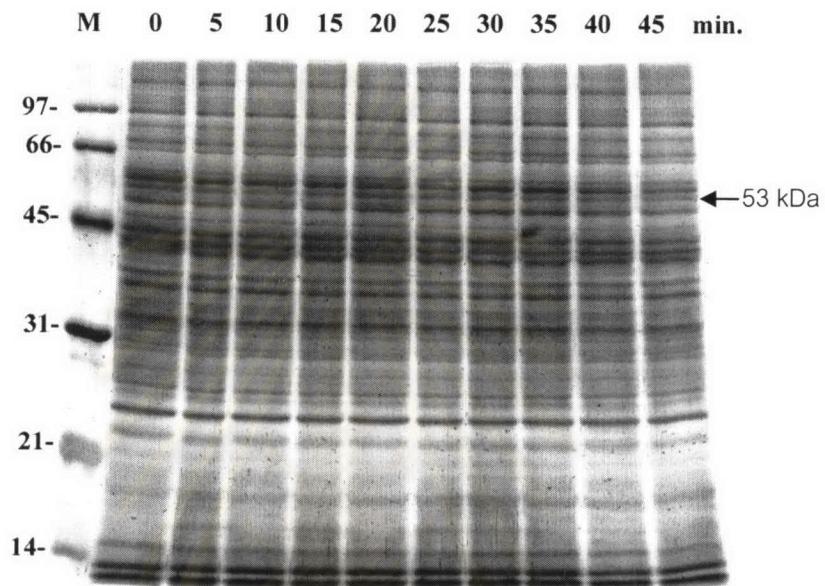
S76



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Appendix E

S76 pH 5.5 → 6.0



SDS-PAGE of intracellular protein profiles of mid-log phase cells of *B. japonicum* S76 when cultured in buffered yeast extract mannitol medium pH 5.5 then transferred to pH 6.0 at 200 rpm, 30°C for 45 minutes at 5 minute intervals.

คุณยุวทัยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Biography

Miss Salisa Jumpa was born on March 15, 1980. She obtained a Bachelor of Science Degree in Microbiology from Kasetsart University, Bangkok, Thailand, in 2001.

Publications

- 1) Salisa Jumpa, Suwat Saengkerdsu, Kanjana Chansa-ngavej. 2002. RAPD-PCR fingerprints as indicators of nitrogen-fixing potential in pH-tolerant *Bradyrhizobium japonicum*. Proceedings of the 14th Annual Meeting of the Thai Society for Biotechnology. 5 pages. CD-ROM format.
- 2) Patima Permpoonpattana, Siroj Srisarakorn, Salisa Jumpa and Kanjana Chansa-ngavej. 2002. Heat, pH and nodulation responses of fast-growing *Sinorhizobium fredii* S174 and slow-growing *Bradyrhizobium japonicum* S178. Proceedings of the 14th Annual Meeting of the Thai Society for Biotechnology. 4 pages. CD-ROM format.

Presentation at Scientific Conferences

- 1) ศลิษา จำปา และ กานุจนา ชาญส่ง่าเวช. 2546. ผลของแอลค่าไลน์พีเอชต่อโปรดีนโพร์ไฟล์ ของ *Burkholderia* sp. S172, *Sinorhizobium fredii* S174 และ *Bradyrhizobium japonicum* S162. หนังสือรวมบทคัดย่อการประชุมวิชาการ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย. ครั้งที่ 11 : หน้า 7.
- 2) ศลิษา จำปา สุภกิตร์ พัฒนาพักรสกุล สมโชค กາลา และ กานุจนา ชาญส่ง่าเวช. 2546. ลายพิมพ์ดีอินເອງ *Bradyrhizobium japonicum* 6 สายพันธุ์แยกจากดินกรด หนังสือรวมบทคัดย่อการประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย. ครั้งที่ 29 : หน้า 229