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APPENDICES

APPENDIX A

Bacterial Identification of the bacterial strain PP8

Characteristics	Reaction
Gram reaction	+ve
Fermentative production of acid from:	
- Gryceraol	+
- Erythritol	-
- D-arabinose	+
- L-arabinose	+
- Ribose	+
- D-xylose	+
- L-xylose	-
- Adonitol	-
- β -methyl-D-xyloside	+
- galactose	+
- D-glucose	+
- D-fructose	+
- D-mannose	+
- L-sorbose	-
- Rhamnose	+
- Dulcitol	-
- Inositol	+
- Mannitol	+
- Sorbitol	-
- α-methyl-D-mannoside	+
- α methyl-D-glucoside	+
- N-acetyl-D-glucosamine	+
- Amygdaline	+
- Arbutin	+
- Esculine	+

Remark : +ve = Gram positive bacteria

\+ = Positive reaction

\- = Negative reaction

Characteristics of the bacterial strain PP8 (continued)

Characteristics	Reaction
Fermentative production of acid from: (continued)	
- Salicine	+
- Cellobiose	+
- Maltose	+
- Lactose	+
- Melibiose	+
- Sucrose	+
- Trehalose	+
- Inuline	+
- Melezitose	+
- D-raffinose	+
- Starch	+
- Glycogen	+
- Xylitol	-
- β-gentiobiose	+
- D-turanose	+
- D-lyxose	-
- D-tagatose	-
- D-fucose	-
- L-fucose	+
- D-arabitol	-
- L-arabitol	+
- Gluconate	+
- 2-keto-gluconate	-
- 5- keto-gluconate	+

Remark : +ve = Gram positive bacteria

\+ = Positive reaction

\- = Negative reaction

APPENDIX B

Preparation for polyacrylamide gel electrophoresis

1.) **Stock reagent**

30% Acrylamide 0.8% bis stock solution

Acrylamide	30.00 g
N,N'-methylene-bis-acrylamide	0.80 g

Adjust volume to 100 ml with distilled water

Tris-SDS stock solution, pH 6.8

Tris (hydroxymethyl)-aminomethane	3.94 g
SDS	0.20 g

Adjust pH to 6.8 with 1 N HCl and adjust volume to 100 ml with distilled water

Tris-SDS stock solution, pH 8.9

Tris (hydroxymethyl)-aminomethane	11.82 g
SDS	0.20 g

Adjust pH to 8.9 with 1 N HCl and adjust volume to 100 ml with distilled water

Ammonium persulfate solution "Make up fresh each time"

Ammonium persulfate	1.00 g

Dissolve in 1 ml distilled water

Sample buffer (5x)

Tris-SDS stock, pH 6.8	5.0 ml
SDS	0.40 g
Glycerol	3.0 ml
β-mercaptoethanol	1.0 ml
1% Bromophenol blue	0.5 ml

Adjust volume to 10 ml with distilled water

Tris-glycine

Tris (hydroxymethyl)-aminomethane	3.03 g
Glycine	14.40 g
SDS	1.00 g

Adjust pH to 8.9 with 1 N HCl and adjust volume to 200 ml with distilled water

Staining solution

Dissolve 1.25 g of Coomassie Blue R250 in 500 ml of 95% methanol. Stir for one hour, add 500 ml of 15% acetic acid, and filter.

Destaining solution

7% acetic acid and 5% methanol

2) SDS-PAGE

12.5%Separating gel

30% Acrylamide solution	4.3 ml

Tris-SDS stock solution, pH 8.9	1.2 ml
TEMED	2.7 µl
10% Ammonium persulfate	70.0 µl
Distilled water	4.4 ml
Total volume	10.0 ml

3% Stacking gel

30% Acrylamide solution	0.7 ml
Tris-SDS stock solution, pH 8.9	2.0 ml
TEMED	2.0 µl
10%Ammonium persulfate	20.0 µl
Distilled water	1.3 ml
Total volume	4.0 ml

One part of sample buffer (5x) was added to four parts of sample. The mixture was heated 5 minutes in boiling water before loading to the gel.

APPENDIX C

Preparation of McIlvain buffer

McIlvain buffer is prepared from 0.1 M of citic acid solution (Solution A) and 0.2 M Na2HPO4 (Solution B). Table 7 shows how prepare McIlvain buffer in pH range 3-7. The data in table are the volume (X ml) of solution B. Solution B is mixed with solution A (100-X) ml. No water is added.

Table 7. Preparation of McIlvain buffer pH 3-7.

pH	.00	.05	.10	.15	.20	.25	.30	.35	.40	.45
3	20.6	21.6	22.6	23.6	24.7	25.6	26.6	27.5	28.5	29.4
4	38.6	39.3	40.0	40.7	41.4	42.1	42.75	43.4	44.05	44.8
5	51.5	52.05	52.6	53.1	53.6	54.2	54.7	55.2	55.8	56.4
6	63.2	63.9	64.6	65.4	66.1	66.9	67.7	68.5	69.3	70.2
7	82.4	-	-	-	86.9	-	-	-	90.7	-
pH	.50	.55	.60	.65	.70	.75	.80	.85	.90	.95
3	30.3	31.1	32.2	33.1	33.9	34.7	35.5	36.3	37.1	37.8
4	45.4	46.1	46.75	47.4	48.0	48.7	49.3	49.9	50.4	50.95
5	56.9	57.5	58.0	58.6	59.2	59.85	60.5	61.1	61.8	62.5
6	71.0	7.9	72.8	73.8	76.1	76.1	77.2	78.6	79.8	81.2
7	-	-	93.6	-	-	-	95.7	-	-	-

APPENDIX D

pBluescript SK⁻ Map

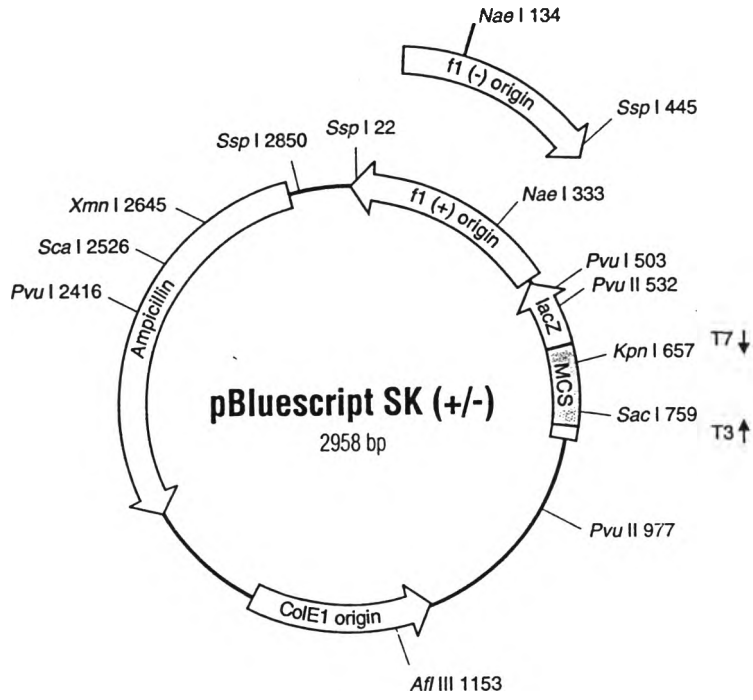

Figure 26. pBluescript SK⁻ map.

BIOGRAPHY

Miss. Supida Tubtimthep was born on March, 25th (1978) in Bangkok, Thailand. She graduated with a Bechelor Degree in Microbiology, faculty of Science, Chulalongkorn University in 1998. She was enrolled in the M.Sc.Biotechnolofy Program since 1999.