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APPENDIX I

CULTURE MEDIA

1. Brain Heart Infusion Broth

Ingredients per liter

BHI (GIBCO)	37 g
distilled water to	1,000 ml

The medium was heated until dissolved completely and sterilized by autoclaving at 121°C for 15 min.

2. Semi-Solid Agar

Ingredients per liter

nutrient agar (GIBCO)	7 g
distilled water to	1,000 ml

The medium was heated until dissolved completely and sterilized by autoclaving at 121°C for 15 min.

3. Tetrazolium-Lactose Agar

Ingredients per liter

nutrient agar	23.0 g
lactose	10.0 g
sodium sulphite	2.5 g
distilled water to	1,000 ml



The medium was heated until dissolved completely and sterilized by autoclaving at 121°C for 15 min. Before pouring plates, held melted agar of 56°C and 5 ml of a sterilized solution (by membrane filtration) a 1% w/v 2,3,5-triphenyltetrazoliumchlorid (BDH) was added.

This medium differentiates lactose-fermenting and nonlactose - fermenting bacteria. Nonlactose - fermenting bacteria produced reddish colonies whereas lactose-fermenting bacteria produced colourless colonies

APPENDIX II

REAGENTS



1. Reagent for HA and HAI Test

1.1 Alsever's Solution

Ingredients per liter

dextrose	20.5 g
sodium citrate,	8.0 g
citric acid	0.6 g
sodium chloride	4.2 g
distilled water to	1,000 ml

Dissolve the ingredients and mix. The solution was sterilized by membrane filtration and stored at 4°C.

1.2 Guinea Pig Erythrocytes (GPE)

Guinea pig blood, collected aseptically, was treated with an equal volume of Alsever's solution and stored at 4°C.

1.3 Carbohydrates, 10%

The following carbohydrates were used for inhibition studies: D-mannose, methyl α -D-mannopyranoside,

D-glucose, D-galactose, D-altrose, L-rhamnose, lactose, and sucrose. 10% solution of the carbohydrate were made with a 1% suspension of GPE as diluent.

1.4 PBS-BSA, 0.1 M, pH 7.2

Ingredients per liter

0.2 M sodium phosphate, anhydrous dibasic (Na_2HPO_4)	360 ml
0.2 M sodium phosphate monobasic ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$)	140 ml
Bovine serum albumin	0.01 g
sodium azide	0.20 g
normal saline solution to	1,000 ml

2. Reagents for Fimbrial Preparation

According to the method of:

2.1 Dodd and Eisentein

2.1.1 Tris-HCl, 5 mM, pH 7.8

Ingredients per liter

Tris aminomethane (Merck)	0.06 g
distilled water to	1,000 ml

Adjust to pH 7.8 with HCl.

2.1.2 Urea, 5 M, Tris, 10 mM

Ingredients per 25ml

urea (SIGMA)	7.5 g
Tris aminomethane	0.3 g
distilled water to	25 ml

2.1.3 Sucrose, 1 M, Urea, 1 M, Tris, 5mM

Ingredients per 100ml

sucrose	34.23 g
urea	6.00 g
Tris aminomethane	0.06 g
distilled water to	100 ml

2.2 Knutton et al.2.2.1 PBS, 0.1 M, pH 7.2

Ingredierdient per liter

0.2 M Na_2HPO_4	360 ml
0.2 M $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	140 ml
normal saline solution to	1.000 ml

2.2.2 CsCl, 1.29 g/cm³

Ingredients per 5 ml

cesium chloride (SIGMA) 1.55 g
distilled water to 5 ml

(density of CsCl 1.29 g/cm³ = 31 weight%)

2.3 Salit and Gotschlich

2.3.1 Tris-HCl, 0.05 M, pH 7.8

Ingredients per liter

Tris aminomethane 6.06 g
distilled water to 1,000 ml

Adjust to pH 7.8 with HCl.

2.3.2 Sodium Acetate, 0.1 M, pH 3.9

Ingredients per liter

sodium acetate 0.27 g
glacial acetic acid 5.63 ml
distilled water to 1,000 ml

2.3.3 Saturated Ammonium Sulfate

Ingredients per liter

ammonium sulfate 700 g
distilled water to 1,000 ml

Heated until dissolved completely. After

cooled, removed excess ammonium sulfate and store at 4°C.

2.3.4 CsCl, 1.30 g/cm³

Ingredients per 5 ml

cesium chloride	1.6 g
distilled water to	5 ml

(density of CsCl 1.3 g/cm³ = 32 weight%)

3. Reagent for Protein Estimation

3.1 Na₂CO₃, 2% in NaOH, 0.1 M (solution A)

Ingredients per 200 ml

sodium carbonate	4 g
0.1 M sodium hydroxide	200 ml

3.2 CuSO₄.5H₂O, 0.5% in C₄H₄KNaO₆.4H₂O, 1% (solution B)

Ingredients per 200 ml

1% copper sulfate	100 ml
2% potassium sodium tartrate	100 ml

3.3 Solution C

Ingredients per 5l ml

solution A	50 ml
solution B	1 ml

Make up fresh each time.

3.4 Solution D

Ingredients per 100 ml

Folin Ciocalteus phenol	50 ml
distilled water to	100 ml

Stored at 4°C in dark bottles.

4. Reagent for Electron microscopy

4.1 Phosphotungstic acid, 2%

Ingredients per 10 ml

phosphotungstic acid	0.2 g
0.1 M PBS	10 ml

Adjust to pH 6.5.

5. Reagent for IEP

5.1 Sodium Barbital-HCl, 0.05 M, pH 8.2

Ingredients per 420 ml



barbital sodium	4.8 g
1 M HCl	6.9 ml
10% sodium azide	0.4 ml
distilled water to	420.00 ml

Dissolve the ingredients and mix. Adjust to pH 8.2

5.2 Noble Agar, 1.5%

Ingredients per 100 ml

noble agar	1.5 g
0.05 M sodium barbital-HCl pH 8.2	100 ml

The agar was heated until dissolved completely, stored at 4°C.

6. Reagent for SDS-PAGE

6.1 Stock Acrylamide, 30%

Ingredients per 100 ml

acrylamide (LKB)	30.0 g
N,N'-methylene bisacrylamide (SIGMA)	0.8 g
distilled water to	1,000 ml

Remove insoluble material by filtration, stored at 4°C in dark bottles.

6.2 Separating Gel, 12.5%

Ingredients per 10 ml

stock, acrylamide	4.1660 ml
1.5 M Tris pH 8.8	2.5000 ml
20% SDS (SIGMA)	0,1000 ml
0.2 M EDTA (Ethylenediaminetetra- acetic acid)	0.1000 ml
distilled water	2.9250 ml
TEMED (N,N,N'-tetramethylethylene diamine)	0.0050 ml
10% ammonium per sulfate	0.2000 ml

10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$ was prepared just before use.

6.3 Stacking Gel, 5%

Ingredients per 5 ml

stock, acrylamide	0.8300 ml
0.5 M Tris pH 6.8	0.0500 ml
20% SDS	0.0500 ml
0.2 M EDTA	0.0500 ml
distilled water	2.7100 ml
TEMED	0.0025 ml
10% ammonium per sulfate	0.1000 ml

10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$ was prepared just before use.

6.4 Sample Buffer, 62 mM Tris

Ingredients per 20 ml

SDS	0.60 g
bromphenol blue	0.03 μ g
0.5 M Tris-HCl, pH 6.8	3.75 ml
2-mercaptoethanol	1.50 ml
glycerol	3.00 ml
distilled water to	20.00 ml



Store in a tightly sealed bottle.

6.5 Electrode Buffer, 25 mM Tris, pH 8.3

Ingredients per liter

Tris aminomethane	3.0 g
glycine	14.4 g
SDS	1.0 g
distilled water to	1,000 ml

Adjust to pH 8.3.

6.6 Standard MW Markers

Individual proteins included in the MW-SDS-70 kit
(SIGMA)

lysozyme	14,300 K
B-lactoglobulin	18,400 K
trypsinogen	24,000 K
pepsin	34,700 K
ovalbumin	45,000 K
BSA	66,000 K

Reconstitute contents of MW-SDS-70 kit vial with volume of sample buffer. Aliquots may be frozen at -20°C for future use.

6.7 Fixing Solution

Ingredients per liter

methanol	400 ml
glacial acetic acid	70 ml
distilled water to	1,000 ml

6.8 Staining Solution

Ingredients per liter

Coomassie Brilliant Blue R (SIGMA)	2.5 g
fixing solution	1,000 ml

Filter the solution immediately before use and store tightly capped at room temperature.

6.9 Destaining Solution

Ingredients per liter

methanol	500 ml
glacial acetic acid	750 ml
distilled water	1,000 ml

7 Reagent for Oral Immunization

7.1 Saturated Sodium Bicarbonate, 50%

Ingredients per 100 ml

saturated NaHCO ₃	50 ml
distilled water to	100 ml

BIOGRAPHY

Mr. Somkiat Tritanipakul, was born on August 21, 1961 in Bangkok, Thailand. He graduated with the degree of Bachelor of Science in Microbiology from the Faculty of Science, Srinakharinwirot University in 1984.

