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APPENDIX I

Medias, Chemical agents, Materials, Instruments and Identification procedures.

A. Medias

A1. 5% sheep blood agar (Difco, USA)

Bacto Tryptose	10.0	g.
Bacto Beef extract	3.0	g.
Sodium Chloride	5.0	g.
Bacto Agar	15.0	g.
Distilled water	950.0	g.

A2. Mannitol Salt agar (Difco, USA)

Proteose peptone No. 3 , Difco	10.0	g.
Bacto Beef extract	1.0	g.
Bacto D-mannitol	10.0	g.
Sodium Chloride	75.0	g.
Bacto agar	15.0	g.
Phenol Red	0.025	g.
Distilled water	1000.0	g.

A3. Mueller Hinton agar Medium (Difco, USA)

Beef, Infusion form	300.0	g.
Bacto casamino acids, Technical	17.5	g.
Starch	1.5	g.
Bacto agar	17.0	g.
Distilled water	1000.0	g.

A4. O-F carbohydrate base (Difco, USA)

Peptone	2.0	g.
Sodium Chloride	5.0	g.

K_2HPO_4	0.3	g.
Agar	3.0	g.
Distilled water	1000.0	g.
Bromthymol Blue, 0.2% aqueous solution	15.0	ml.

A5. Tryptic soy agar (Merck, Germany)

Peptone from casein	15.0	g.
Peptone from soymeal	5.0	g.
Sodium Chloride	5.0	g.
Agar	15.0	g.
Distilled water	1000.0	g.

A6. Tryptic soy broth (Merck, Germany)

Peptone from casein	17.0	g.
Peptone from soymeal	3.0	g.
D (+) Glucose	2.5	g.
Sodium Chloride	5.0	g.
di-Potassium Hydrogen Phosphate	2.5	g.
Distilled water	1000.0	g.

Media preparation :

All of ingredients were dissolved in distilled water and then steriled by autoclaving at 121°C, 15 pounds/inch^2 pressure, for 15 minutes. The sterile medium was cooled to 45°C to 50°C, and dispensed into sterile plates or tubes. For sterile tryptose blood agar base medium, 50 ml of sterile defibrinated blood was added aseptically into sterile plates before dispension. For O-F carbohydrate base, the medium was sterile at 115°C, 10 pounds/inch^2 pressure, for 20 minutes, and a sterile solution of glucose were added aseptically to give a final concentration of 1%. The medium was mixed and then distributed aseptically in 10 ml volumes into sterile tubes of not more than 16 mm diameter.

B. Chemical agents

Low melting point agarose (Difco, USA)

Ultrapure high-melting temperature agrose (Gibco BRL, Spain)

Brij-58 (Sigma, USA)

Sodium deoxycholate (Sigma, USA)

Sodium lauroyl sarcosine (Sigma, USA)

Proteinase K (Amresco, USA)

Tris (Amresco, USA)

Sodium chloride (Merck, Germany)

EDTA (Amresco, USA)

Boric acid (Bio-Rad, USA)

C. Materials

15-ml snap-top tubes (Fisher, USA)

5-ml snap-top tubes (Fisher, USA)

15-ml round bottom tube, screw cap (Pyrex, USA)

Insert mold (Bio-Rad, USA)

Glass tray (20 by 30 cm)

Metal tray (16 by 25 cm)

D. Instruments

Incubator 37°C, 42°C (Memmert, Germany)

Shaking waterbath (United Instrument, USA)

Turbidity meter

Mixer Vortex (Scientifix, USA)

Digital sliding vernier caliper

Roller (Life Science, USA)

Refrigerator centrifuge (4°C) (Sigma, USA)

Refrigerator (-20°C) (Listed Household Freezer , USA)

Autometic pipette, p20/p200/p1000 (Gilson Medical Electronic, France)

PH meter (Beckman, USA)

Millipore filter

Pulsed-Field Gel Box (Bio-Rad, USA)

Pump, Gel Molds (Bio-Rad, USA)

Colling water bath (Bio-Rad, USA)

Power supply, Pulse wave switcher (Bio-Rad, USA)

Gel Doc 1000 (Bio-Rad, USA)

E. Enzyme and Molecular Marker

Lysozyme (Amresco , USA)

Lysostaphin (Sigma , USA)

*Sma*I (Boehringer, Germany)

λ ladder marker (Bio-Rad, USA)

F. Identification procedures

F1. Gram staining procedure

Gram crystal violet solution

Gram iodine solution

Gram safranin solution

95% ethanol

Staining procedure : The organisms were smeared on a clean slide and allowed to dry. The slide was heated with a flame to fix the smear. Gram crystal violet was dropped on the smear. After minute, the slide was then washed with water and drained. Next, gram iodine solution was dropped on the smear, and washed with water after 1 minute. The smear was decolorized with 95% ethanol and then washed with water. Gram safranin solution was next dropped on the smear in order to use as counterstain

for 30 seconds. The smear was allowed to dry and then examined by microscopy under 100x objective len over the entire smear.

F2. Catalase test

Several colonies of *S. aureus* were smeared on a clean slide. The 3% hydrogen peroxide was dropped and mixed with the organisms. The positive result was shown as bubbles formation.

F3. Coagulase test

F3.1. Slide coagulase teat

Several colonies of *S. aureus* were mixed generously with a drop of human plasma onto a clean slide

The positive result was shown as the white precipitate formation within 5 minutes.

F3.2. Tube coagulase test

The 0.5 ml of staphylococcal broth culture was mixed with 0.5 ml of human plasma in a clean tube. The tube was then incubated at 37°C for 3 to 4 hours.

The positive result was developed as a clot gel.

APPENDIX II

Reagents

1. **PIV buffer** :

- 10 mM Tris (pH 7.6)	0.6057 g.
- 1M NaCl	29.22 g.

All ingredients were dissolved in 500 ml of ultrapure water. The buffer was adjusted the pH to 7.6, and then sterile at 121 ^{o}C, 15 pounds/inches^{2} pressure. The PIV buffer was stored at 4 ^{o}C.

2. **Lysis buffer** :

- 6 mM Tris (pH 7.6)	0.0726 g.
- 1M NaCl	5.884 g.
- 100mM EDTA (pH 7.6)	3.7224 g.
- 0.5% Brij-58	0.5 g.
- 0.2% Sodium deoxycholate	0.2 g.
- 0.5% Sodium lauroylsarcosone	0.5 g.

All ingredients were dissolved in 500 ml of ultrapure water, and then adjusted the pH to 7.6. The buffer was sterile by millipore filter, and then stored at 4 ^{o} C.

- **Lysostaphin stock solution**

- Lysostaphin buffer : The 0.3028 g of Tris and 0.4383 g of NaCl were dissolved in 50 ml of ultrapure water. The buffer was then adjusted the pH to 7.6 and sterile at 121 ^{o}C, 15 pounds/inch^{2} pressure. The buffer was stored at room temperature.

- Lysostaphin

Five thousand units of lysostaphin were dissolved in 10 ml of lysostaphin buffer and then dispensed 200 µl in each microcentrifuge tube. The lysostaphin was freezed at -20 ^{o}C. (This aliquots could be thawed only once)

- **Lysozyme stock solution**

The 0.5 g of lysozyme was dissolved in 10 ml of sterile water (final concentration, 50 mg/ml or 10 mg/μl). The 200 μl of lysozyme was dispensed in each microcentrifuge tube, and then freezed at -20°C. (This aliquots could be refrozen once)

- **Lysis solution**

- 5 U of lysostaphin per ml
- 1 mg of lysozyme per ml
- Lysis buffer

The lysis solution was prepared by mixing the 400 μl of lysostaphin stock solution, 800 μl of lysozyme stock solution, and 40 ml of lysis buffer together.

3. **ES buffer**

- 0.5M EDTA (pH 8.0)	93.06 g.
- 10% Sodium laurylsarcosine	50.0 g.

All ingredients were dissolved in 500 ml of ultrapure water, and then adjusted the pH to 8.0. The buffer was sterile by millipore filter and stored at room temperature.

- **20x Proteinase K stock solution**

One hundred grams of proteinase K was dissolved in 50 ml of ES buffer and then incubated at 50°C for 1 h. The solution was stored at 4°C.

- **ESP solution**

- 100 μg of Proteinase K per ml of ES buffer
- ES buffer

Twenty five milliliters of 20x proteinase K was added to 475 ml of ES buffer, and then mixed thoroughly. The solution was stored at 4°C.

4. **1xTE buffer**

- 10 mM Tris (pH 7.6)	0.6057 g.
- 0.1 M EDTA (pH 7.6)	0.0186 g.

All ingredients were dissolved in 500 ml of ultrapure water, and then adjusted the pH to 7.6. The buffer was sterile at 121°C, 15 pounds/inch^2 pressure and stored at room temperature.

5. **10x TBE buffer**

- 0.1 M Tris (pH8.5)	108.0 g.
- 0.1 M Boric acid	55.0 g.
- 4 mM EDTA	7.44 g.

All ingredients were dissolved in 500 ml ultrapure water and then adjusted the pH to 8.5. The buffer was sterile at 121°C, 15 pounds/inch^2 pressure. Approximately 300 ml of sterile Ultrapure water was added and then mix thoroughly. The buffer was stored at room temperature.

6. **Ethidium bromide solution**

- 0.5 µg in water

One pellet of ethidium bromide was dissolved in 11 ml of ultrapure water.

Working solution : The 100 µl ethidium bromide stock solution was mixed with 200 ml water before used.

BIOGRAPHY

Miss Nutthaporn Ruchikachorn was born on January 26, 1976 in Bangkok, Thailand. She graduated with the Bachelor degree of Science in Microbiology from the Faculty of Science, Kasetsart University in 1996.