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## **APPENDICES**

**APPENDIX A****MEDIA, REAGENTS, MATERIALS, AND INSTRUMENTS****A. MEDIA AND REAGENTS**

Absolute ethanol	(Scharlau, Spain)
Agarose	(GIBCO BRL, USA)
Boric acid	(Bio-Rad, Canada)
Caffeic acid	(Sigma, USA)
Ethidium bromide	(USB, UK)
Ethylenediaminetetraacetic acid	(Bio-Rad, Canada)
L-canavanine	(Sigma, USA)
Potato dextrose agar	(Difco, USA)
Sabouraud dextrose agar	(Sanofi, France)
Sabouraud dextrose broth	(Difco, USA)
Sodium bromthymol blue	(Sigma, USA)
Sodium chloride	(Merck, Germany)
Taq DNA polymerase	(Promega, USA)
Thiamine-HCl	(Sigma, USA)
Tris-Base	(Promega, USA)

**B. MATERIALS**

Eppendorf

Micropipette

Test tubes

Tips

**C. INSTRUMENTS**

Autoclave (model SS-325)	(Tomy Seiko, Japan)
Electrophoresis chamber (model SGE-014)	(CBS, USA)
Freezer (model FC-19)	(Sanyo, Japan)
Hybridization oven (model B71183)	(Thermo hybrid, USA)
Incubator (model 96097)	(Contherm, New Zealand)
Microcentrifuge	(Hanil, Korea)
Microwave (model R-3V16)	(Sharp, Japan)
pH meter (model 420A)	(Orion, USA)
Power supply (model E15250)	(CBS, USA)
Refrigerator centrifuge (model 3740)	(Kubota, Japan)
Refrigerator	(Sanyo, Japan)
Rotary shaker (model NR-1)	(Bellco, Glass, USA)
Vortex mixer (model G-560E)	(Scientific, USA)
Water bath	(Yamato, Japan)
UV transilluminator (model 75501582)	(Bio-Rad, Canada)

## APPENDIX B

### MEDIA AND REAGENT PREPARATION

#### A. MEDIA FOR YEAST CULTURE AND IDENTIFICATION

##### 1. Sabouraud Dextrose Agar

Sabouraud dextrose agar powder	65	g
Distilled water	1,000	ml

Dissolve the powder in 1,000 ml of distilled water, mix thoroughly and sterilize by autoclave at 121 °C, 15 pound/inch<sup>2</sup> pressure, for 15 min.

##### 2. Sabourand Dextrose Broth

Sabouraud dextrose broth powder	30	g
Distilled water	1,000	ml

Dissolve the powder in 1,000 ml of distilled water, mix thoroughly and sterilize by autoclave at 121 °C, 15 pound/inch<sup>2</sup> pressure, for 15 min.

##### 3. Caffeic Acid Agar

Sabouraud dextrose agar with chloramphenicol		
	1	g
Potato dextrose agar	1	g
Agar powder	1	g
Caffeic acid	0.05	g
Distilled water	100	ml

Dissolve 1 g of Sabouraud dextrose agar with chloramphenicol, 1 g of potato dextrose agar, and 1.5 g of agar powder in 80 ml of distilled water. Adjust to pH 6.0 with 1 N NaOH (solution A). Dissolve caffeic acid (0.05 g) in 20 ml of distilled water (solution B). Sterilize both solution A and B by autoclave at 121 °C, 15 pound/inch<sup>2</sup> pressure, for 15 min. While the agar was still hot, add solution B to solution A and stir thoroughly before dispensing aseptically in plates.

#### 4. Canavanine-glycine-bromothymol blue (CGB) Agar

##### Solution A

Glycine	10	g
KH <sub>2</sub> PO <sub>4</sub>	1	g
MgSO <sub>4</sub>	1	g
Thiarfame-HCl	1	mg
L-canavanine sulfate	30	mg
Distilled water	100	ml

Dissolve the ingredients in 100 ml of distilled water, adjust to pH 5.6 with conc. HCl and sterilize by filtration.

##### Solution B

Sodium bromothymol blue	0.4	g
Distilled water	100	ml

To prepare a liter of the medium, mix 880 ml of distilled water, 20 ml of solution B, and 20 g of agar and autoclave for 15 min at 121 °C. While the agar is still hot, add 100 ml of solution A and stir thoroughly before dispensing aseptically in tubes.

### 5. Urea Agar

Urea agar base (BBL)	29	g
Agar	15	g
Distilled water	1,000	ml

Dissolve urea agar base in 100 ml of distilled water and sterilize by filtration. Suspend agar in water, heat with gentle mixing to boiling, and autoclave at 121 °C for 15 min. Add the sterilized urea agar base to the cooled (50 °C) agar and dispense in sterile tubes.

### 6. 0.85% Normal Saline

NaCl	0.85	g
Distilled water	100	ml

Dissolve NaCl in 100 ml of distilled water and sterilize by autoclave at 121 °C for 15 min. The reagent is kept at room temperature.

## VITAE

Mr. Damrongdej Piyabongkarn was born in May 17, 1976 in Bangkok, Thailand. He graduated with the Doctor of Veterinary Medicine from the Faculty of Veterinary science, Chulalongkorn University, in 1998.

