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APPENDICES

APPENDIX I

REAGENTS AND EQUIPMENTS

1. Media and reagents

Absolute ethanol	(Merck, Germany)
Agarose	(USB, UK)
Beef extract	(Difco, USA)
Boric acid	(Sigma, USA)
Bromcresol purple	(Fluka, Germany)
Ducitol	(Merck, Germany)
D-Cellobiose	(Sigma, USA)
D-Galactose	(Difco, USA)
D-Xylose	(Merck, Germany)
dNTPs	(Amersham, USA)
Ethylenediaminetetraacetic acid (EDTA)	(Bio-Rad, Canada)
Ethiumdium bromide	(Amresco, USA)
Glucose	(Merck, Germany)
Innositol	(Difco, USA)
Lactose	(Merck, Germany)
Maltose	(Sigma, USA)
Melibiose	(Sigma, USA)
NaCl	(Merk, Germany)
Noble agar	(Difco, USA)
ϕx 174/*Hae* III	(Amersham, USA)
Raffinose	(Difco, USA)
Sabouraud dextrose agar (SDA)	(Sanofi, France)
Sabouraud dextrose broth (SDB)	(Difco, USA)
Sucrose	(Merck, Germany)
Trehalose	(Difco, USA)
Taq DNA polymerase	(Promega, USA)
Tris base	(Promega, USA)
Vortex mixer	(Scientific, USA)

Yeast nitrogen base	(Difco, USA)

2 Equipments

ABI prism 310 automated sequencer	(Perkin Elmer, USA)
Autoclave (model SS-325)	(Tomy seiko, Japan)
Automatic pipette	(Gilson, Lyon, France)
Electrophoresis chamber	(CBS, USA)
Gel Doc	(BIORAD, USA)
Incubator	(Contherm, New Zealand)
Microcentrifuge	(Hanil, Korea)
Mixer-Vertex-Genic	(Scientific industries,USA)
Polymerase chain reaction machine	(Thermo Hybaid, USA)
Power supply	(CBS, USA)
pH meter	(Orion, USA)
Refrigerator centrifuge	(Kubota, Japan)
Spectrophotometer	(Bio-Rad, USA)

APPENDIX II

REAGENT PREPARATION

1 Media for *Candida* identification

1.1 Sabouruad dextrose agar (SDA), (Sanofi, France)

Formula :

SDA powder	65	g
Distilled water	1000	ml

Suspend 65 g of the powder in 1000 ml of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 min to completely dissolve the powder. Autoclave at 121 °C for 15 min.

1.2 Sabouruad dextrose broth (SDB), (Difco, USA)

Formula :

SB	30	g
Distilled water	1000	ml

Dissolve 30 g of the powder in 1000 ml purified water and autoclave at 121 °C 15 min.

1.3 Carbohydrate assimilation medium

Formula :

Noble agar	20	g
Yeast nitrogen base	0.67	g
Distilled water	1000	ml

Carbohydrate disk: glucose, maltose, sucrose, lactose, galactose, trehalose, inositol, melibiose, cellibiose, raffinose, ducitol, xylose.

1.4 Carbohydrate fermentation medium

Formula :

Beef extract	3	g
Peptone	10	g
NaCl	5	g
Distilled water	1000	ml
Bromcresol purple	1.6	g

Stock carbohydrate solution: 10 % aqueous solutions of dextrose, maltose, sucrose, lactose, galactose, and trehalose.

1.5 Chlamydospore agar

Formula :

Glutinious rice	2.5	g
Dextrose	10	%
Agar	2	%
Tween 80	8	drop

Boil glutinous rice with dextrose for 10 minutes. Wait for 2 hrs. and filtrate by cotton get light autoclave added 2 % agar with tween 80 about 8 drops autoclave and pour plate.

2 Reagents for agarose gel electrophoresis

2.1 1xTBE buffer (Promega, USA)

Formula :

Tris base	10.8	g
Boric acid	5.5	g
Na_2EDTA	0.744	g
Distilled water to	1000	ml

The solutions were dissolved by autoclave 121 °C for 5 min.

2.2 2 mM dNTP (Amersham, USA)

Formula :

100 mM dATP	2	µl
100 mM dCTP	2	µl
100 mM dGTP	2	µl
100 mM dTTP	2	µl
Distilled water	92	µl

Mix and centrifuge. Stores at –20 °C.

2.3 3% Agarose gel (USB, UK)

Formula :

Agarose	3	g
1x TBE buffer	100	ml

The solutions were melted in microwave, leave it to cool down to 50 °C before pouring and let the gel sit for at least 45 min to solidify.

2.4 10 mg/ml Ethidium bromide (Et-Br) (Stock)

Formula:

Ethidium bromide	1	g
DW	100	ml

Stir on a magnetic stirrer for several hours to ensure that dye has dissolved. Wrap the container in aluminum foil or transfer to a dark bottle and stores at 4 °C.

Reagents for PCR product purification

3 Buffer PB (Ready to use)

3.2 Buffer PE

Buffer PE is supplied as a concentrate. Before using for the first time, add the 55 ml of ethanol (96-100%) to buffer PE concentrate as indicated on the bottle.

4 Reagents for Sequencing

4.1 3 M Sodium acetate, pH 8.0

Formula :

Sodium acetate, 3 H2O	408.1	g
Distilled water	800	ml

Adjust pH 8.0 with glacial acetic acid, Adjust volume to 1,000 ml.

BIOGRAPHY

Miss Kamonrat Phopin was born on September 4, 1978 in Bangkok, Thailand. She graduated with the bachelor degree of science (medical technology) from Faculty of Medical Technology, Mahidol University in 1999.