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APPENDIX

Appendix I

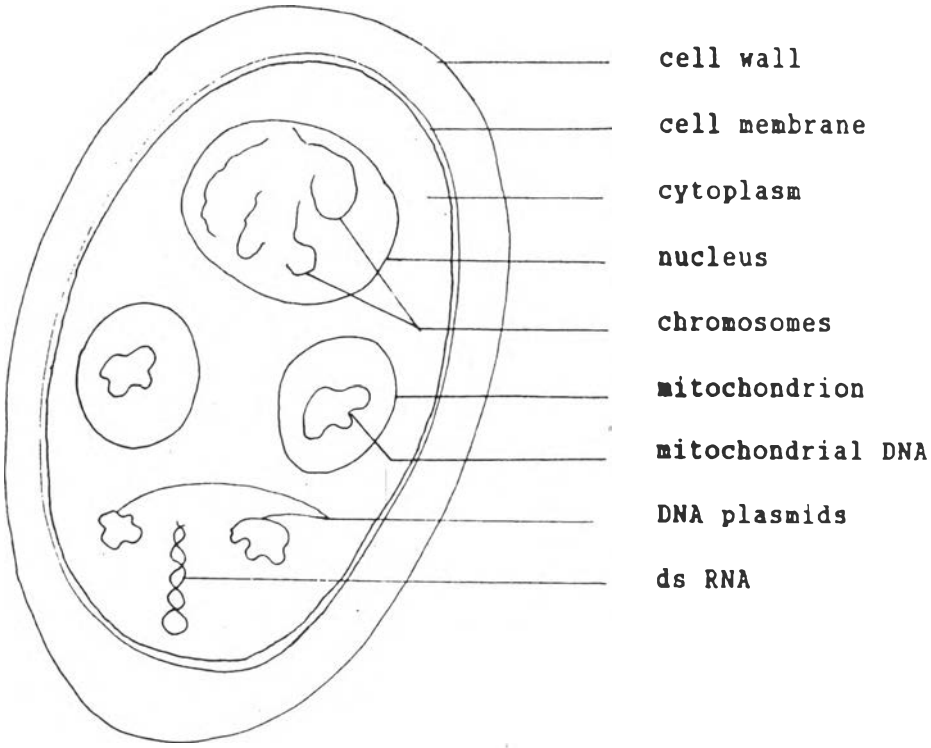

Figure A Location of genetic material in the yeast cell

Information of genetic material in yeasts (Figure A)

The major part of the genetic information of yeast, Saccharomyces cerevisiae, is carried on 17 long linear molecules of deoxyribonucleic acid (DNA) called chromosomes located in a special structure in the cell called the nucleus. Some of the yeast cell's genetic information which enables the cell to grow aerobically, different types of plasmids, which are small circular molecules of DNA, are found in the cytoplasm. Also found in the cytoplasm is another kind of nucleic acid, ribonucleic acid (RNA), which can also carry genetic information, and there are one type is responsible for the killer phenomenon in yeast.

Appendix II

Method for determining yeast viable numbers by haemacytometer,

The method aims to measure cell numbers and to be able to provide an estimate of percentage of viable cells present in a sample of yeast. It is the direct microscopic count in which a measured volume of a sample is spread over a given area of a slide. By making a yeast count in a known area and multiplying by the appropriate dilution, which can calculate the number of yeasts in the original sample. There is a special counting chamber, the Petroff-Hauser slide which had known depth and volume and marked off into squared areas. The number of yeasts in an area is counted and the total number of yeasts in the sample can be calculated from the total volume known to be contained in the area (Figure B). The methylene blue staining method was used for the assessment of yeast viability. When cells were immersed in 0.1 % methylene blue, those were indicated and measured directly, living or dead. Therefore, viable cells contain enzymes which are able to reduce methylene blue to a colourless compound. On the other hand, dead cells, in which the enzyme is inactive, do not do so and accordingly stain blue (Kirsop, Painting and Henry, 1984). The percentage of unstained cells is thus a measure of viability.

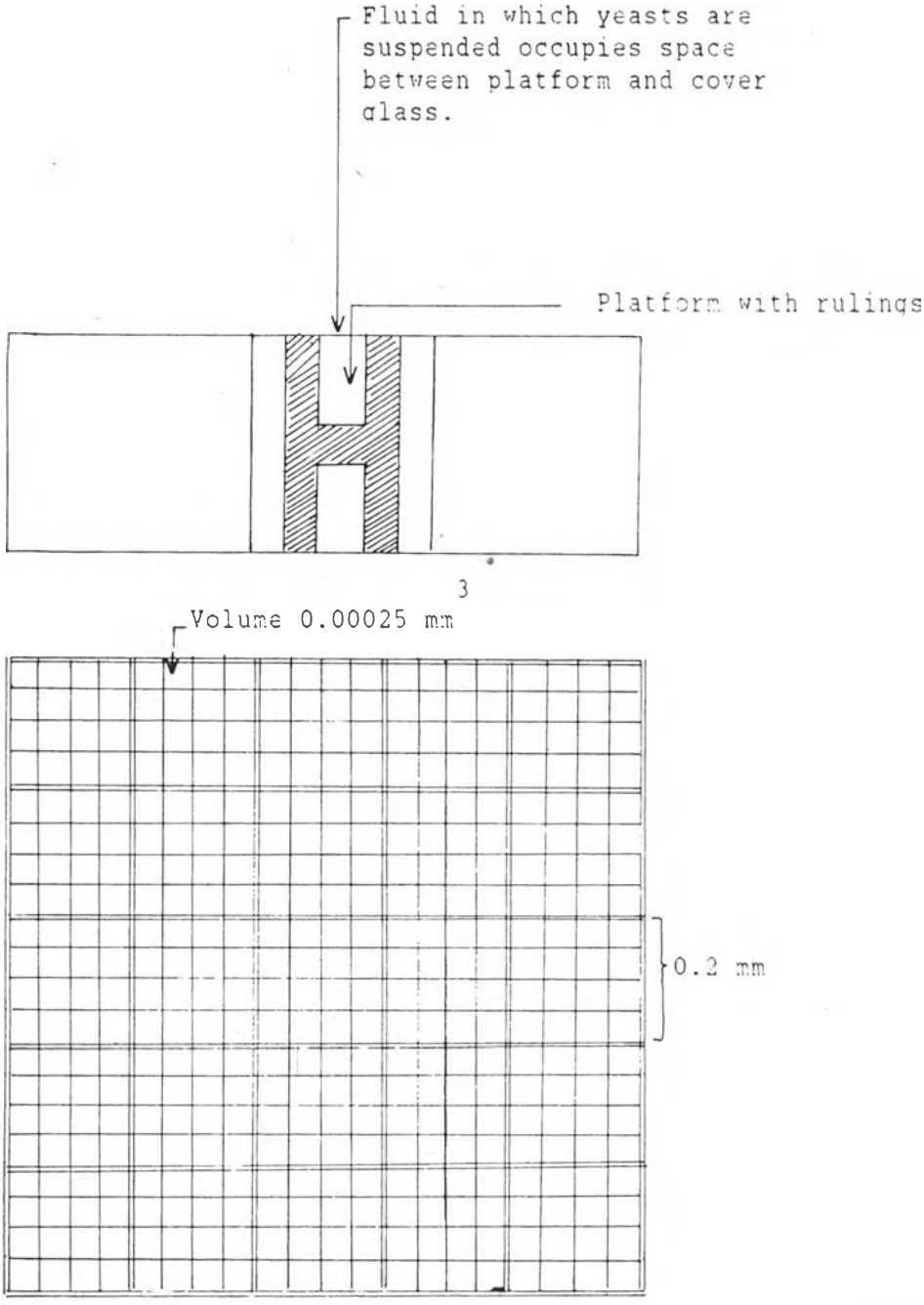

Figure B Pattern of haemacytometer and Magnified picture of ruling on platform.

Appendix III

Preparation of agarose gel electrophoresis.

Agarose gels are prepared by

1. Add 0.7 g of powdered agarose into 100 ml of electrophoresis buffer.

2. Heat the slurry in a microwave oven until the agarose dissolved.

3. Cool the solution to 50 ^{o}c and seal the edges of the mold with small quantity of the agarose solution by using a Pasteur pipette (see Figure C).

4. When the seal is set, pour the rest of the warm agarose solution into the mold and immediately clamp the comb, the teeth of which will form the sample well, into position near one end of the gel. Check and see that there is 0.5-1.0 mm of agarose between the bottom of the teeth and base of the gel, so that the sample wells are completely sealed (see Figure D).

5. After the gel is completely set (30-45 minutes at room temperature), carefully remove the comb and mount the gel in the electrophoresis tank.

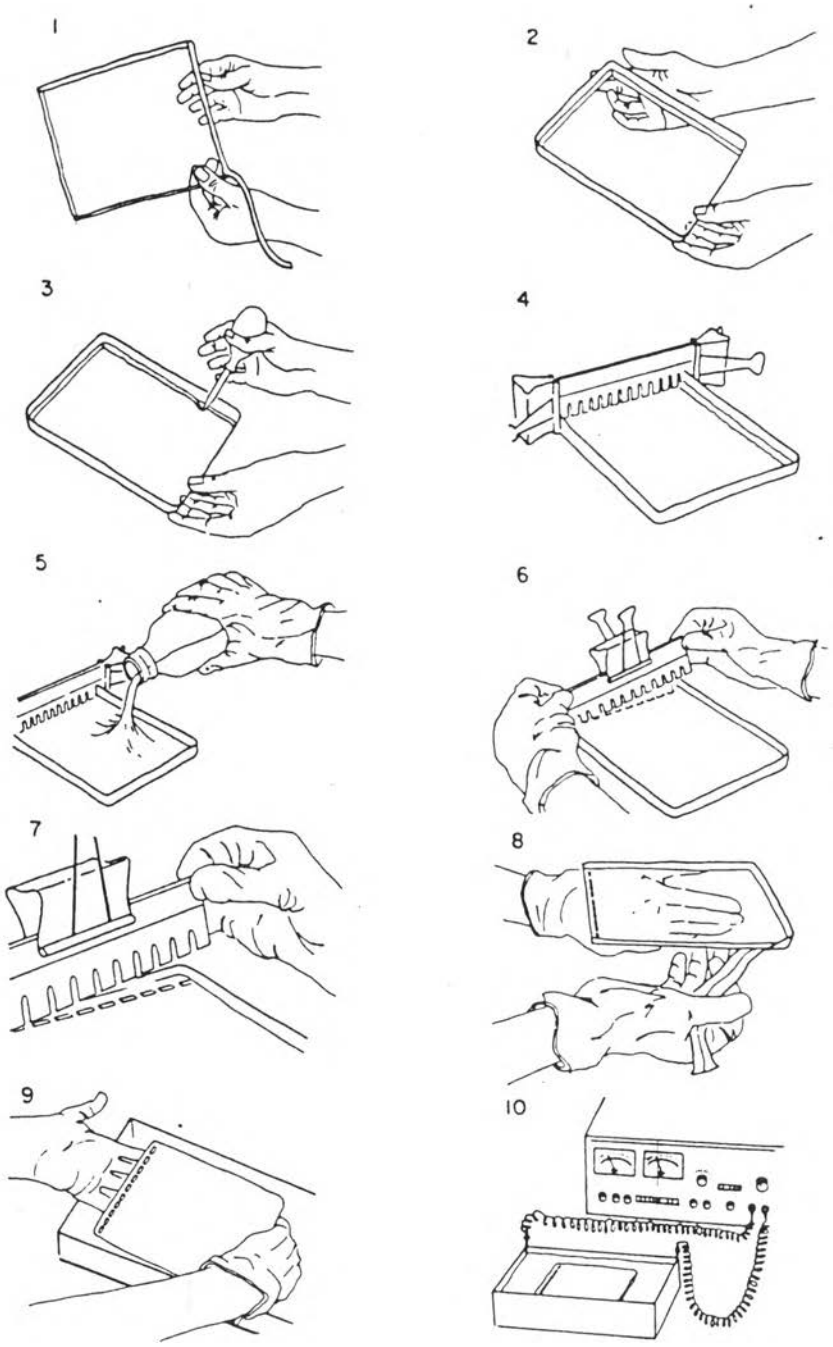

Figure C The method of pouring gels.

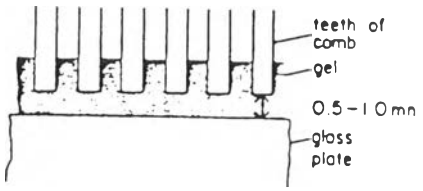

Figure D There is 0.5-1.0 mm of agarose between the bottom of the teeth and the base of the gel.

Appendix IV

Preparation of the agar slab

Place a sterile glass slide in an empty petridish and pour plate with a thin layer agar on the slide. After agar set up, cut out a rectangular agar slab using a sterile scalpel. Thus the agar slab will be on the glass slide.

Appendix V

1. Media

1.1 YM

Yeast extract	0.3	g
Malt extract	0.3	g
Bacto-peptone	0.5	g
D-glucose	1.0	g
Distilled water	100	ml

(for solid medium added Bacto-agar 2 g)

all components are autoclaved together at 15 pounds for 15 minutes.

1.2 YEPD (OR Complete medium)

Yeast extract	1	g
Bacto-peptone	1	g
D-glucose	2	g
Distilled water	100	ml

(for solid medium add Bacto-agar 2 g or screening medium add 0.01 % chloramphenicol) all components are autoclaved together at 15 pounds for 15 minutes.

1.3 Potassium acetate agar (Sporulation medium)

Potassium chloride	0.82	g
Sodium acetate	0.18	g
D-glucose	0.05	g
Yeast extract	0.1	g
Bacto-agar	1.5	g
Distilled water	100	ml

Dissolve and dispense, in 10 ml amounts in test tubes. Autoclave 15 pounds/15 minutes and allow to set at an angle.

1.4 Gorodkowa agar

D-glucose	0.1	g
Bacto-peptone	1	g
Sodium chloride	0.5	g
Bacto-agar	2	g
Distilled water	100	ml

Dissolve and dispense in 10 ml amounts in test tubes. Autoclave 15 pounds/15 minutes and allow to set at an angle.

1.5 Fermentation medium

Yeast extract	0.5	g
Distilled water	100	ml

Dissolve and dispense in 4.5 ml amount in screw capped test tubes containing durham tubes. Autoclave at 10 pounds/15 minutes. Add 0.5 ml of each of the 20 % filter sterilised sugar solutions*, except for raffinose for which 1 ml was added.

* Sugar used in fermentation tests

Glucose

Galactose

Sucrose

Maltose

Melibiose

Raffinose

1.6 Starch fermentation medium

Yeast extract	0.5	g
Starch	2	g
Distilled water	100	ml

Dissolve and dispense in 5 ml amounts in screw capped test tubes containing durham tubes. Autoclave at 10 pounds/15 minutes.

1.7 Nitrogen assimilation medium

Yeast carbon base	11.7	g
Ethylamine hydrogen chloride	0.64	g
Distilled water	100	ml

Filter sterilised, then add 1 ml to test tubes containing 9 ml of sterile water. The test tubes shall be covered with loose fitting caps to keep the conditions as aerobic as possible.

1.8 Carbon assimilation medium

Yeast nitrogen base	6.7	g
carbon compound *	5	g
Distilled water	100	ml

Filter sterilised, then added 1 ml to test tubes containing 9 ml of sterile water. The test tubes shall be covered with loose fitting caps to keep the condition as aerobic as possible.

* Compounds used in carbon assimilation

Glucose, Galactose, Sucrose, Maltose, Melibiose and Raffinose

For other carbon sources the glucose is replaced by the weight of carbon compound containing the same amount of carbon as 5 g glucose. An exception is raffinose, which need to be twice as concentrated as glucose.

1.9 Vitamin-free medium

Vitamin-free yeast base 'Difco"	16.7	g
Distilled water	100	ml

Dissolve, filter sterilise and store in 10 ml amounts in a sterile bottle. To use, add 1 ml to 9 ml sterile distilled water.

1.10 Cycloheximide medium

D-glucose	0.5	g
Yeast nitrogen base	0.67	g
Cycloheximide	100	ppm
Distilled water	100	ml

Dissolve, filter sterilise and dispense in 10 ml amounts in a sterile bottle. Gloves should be worn in the preparation of this medium.

1.11 Glucose chalk agar

Yeast extract	0.5	g
D-glucose	5	g
Cacium carbonate	0.5	g
Bacto-agar	1.5	g
Distilled water	100	ml

Dissolve, autoclave 15 pounds/15 minutes and pound plates.

1.12 PDA

Potato Dextrose Agar "Difco"	3.9	g
Distilled water	100	ml

Dissolve, autoclave 15 pounds/15 minutes and pour plates.

1.13 Killer detect assay medium (YEPD-MB)

Yeast extract	1	g
Bacto-peptone	1	g
D-glucose	2	g
Bacto-agar	2	g
Distilled water	90	ml

Autoclave the above ingredient and add, the following sterile solutions Phosphate-citrate buffer 10 ml and 1 % Methylene blue 0.4 ml.

Phosphate-citrate buffer

0.1 M Citric acid

0.2 M Disodium hydrogen phosphate

Adjust to pH 2.0-7.6 with either citric acid and disodium hydrogen phospate solution; sterilise by autoclaving.

1.14 Synthetic (minimum or complete) medium.

Bacto-yeast nitrogen base without amino acid 0.67 g

D-glucose	2	g
Bacto-agar	2	g

Add distilled water 100 ml and autoclave for minimum medium or add with various constituents for synthetic complete medium. It is convenient to prepare sterile stock solutions. All stock solutions can be autoclaved at 15 pounds for 15 minutes. The appropriate volume of the stock solutions is added to the ingredients of minimal medium and sufficient distilled water is added so that the total volume was 600 ml. The threonine and aspartic acid solution should be added seperately after autoclaving. Some stock solution should be stored at room temperature in order to prevent precipitation while the other solutions are refrigerated.

Constituent	Final concentration mg/l	Stock solution per 100 ml	ml for 600 ml
adenine sulfate	20	120 mg o	10
L-arginine-HCI	20	240 mg	5
L-aspartic acid	20	400 mg o *	15
L-glutamic acid	100	600 mg o	10
L-histidine-HCI	20	240 mg	5
L-isoleucine	30	360 mg	5
L-leucine	30	360 mg o	5
L-lysine-HCI	30	360 mg	5
L-methionine	20	240 mg	5
L-phenylalanine	50	300 mg o	10
L-serine	375	4.5 g	5
L-threonine	200	2.4 g *	5
L-tryptophan	20	240 mg	5
L-tyrosine	30	90 mg o	20
uracil	20	240 mg o	5
L-valine	150	1.8 g	5

o Store at room temperature

* Added after autoclaving the media

1.15 Pre-sporulation medium

Bacto-yeast extract	0.8	g
Bacto-peptone	0.3	g
D-glucose	10	g
Bacto-agar	2	g
Distilled water	100	ml

Dissolve and dispense in 10 ml amounts in test tubes. Autoclave 15 pounds/15 minutes and allow to set at an angle.

1.16 GY medium

glucose	2.5	%
yeast extract	0.25	%
Ammonium sulfate	0.25	%
Bacto-agar	1.0	%
0.1 M Citrate-phosphate buffer	10	% (pH 4.5)
Distilled water	100	ml

all components are autoclaved together at 15 pounds for 15 minutes.

2. Reagents

2.1 50 mM EDTA

EDTA	5.58	g
Distilled water	300	ml

Adjust pH 7.0 by 10 N sodium hydroxide, and autoclave

2.2 50 mM Tris-H_2SO_4

Tris	1.82	g
Distilled water	300	ml

Adjust pH 9.0 by 1 N sulfuric acid, and autoclave

2.3 SDS (Sodium dodesyl sulfate) solution

SDS	0.1	g
EDTA	0.37	g
Tris	0.12	g
Sodium chloride	0.58	g
Distilled water	100	ml

Adjust pH 7.5 by 10 N sodium hydroxide, and autoclave.

2.4 Electrophoresis buffer

Tris	9.68	g
EDTA	0.74	g
Sodium acetate	2.72	g
Distilled water	2	L

Adjust pH 7.8 by acetic acid, and autoclave

2.5 Loading buffer

Saccharose	4	g
Bromo phenol blue	2.5	mg
Distilled water	10	ml

2.6 0.1 M Sodium phosphate buffer

0.2 M Disodium hydrogen phosphate

0.2 M Sodium dihydrogen phosphate

Adjust to pH 7.5 and 8.0 and sterilise by autoclaving.

2.7 Zymolyase solution

2 M Sorbitol	10	ml
0.1 M Phosphate buffer (pH 7.5)	1.5	ml
10 % Mercaptoethanol	0.2	ml
Zymolyase-100 T	4	mg
Distilled water	8.3	ml

Mix each solution just before use. The solution is sterilised by filtration.

Vita

Ms. Wimolsiri Porntaveewat was born on January 20, 1959 in Bangkok. She received Bachelor ' s Degree of Science in the field of Food Science and Technology from Faculty of Agriculture, Chiangmai University in 1981.