APPENDIX A

The characteristic of commercial chitosan

Table 12 Technical chitosan product and impurities

Crab shell chitosan	
Mw 150,000	75-85% DD Viscosity ~100 mPa.s (1% in 1% acetic acid, 20 °C)
Mw 400,000	85-90% DD Viscosity 200-400 mPa.s (1% in 1% acetic acid, 20 °C)

APPENDIX B

Microbiological media and diluents

1. Nutrient agar per litre

Beef Extract	3.0	g
Peptone	5.0	g
NaCl	5.0	g
Agar	15.0	g
pH	7.4	

2. Nutrient broth

Beef Extract	3.0	g
Peptone	5.0	g
NaCl	5.0	g
Distrilled water	1000	ml

3. Eosin Methylene Blue (EMB) agar per litre

Peptone	10	g
K_2HPO_4	2.0	g
Eosin Y	0.4	g
Lactose	5.0	g
Sucrose	5.0	g
Methylene Blue	0.065	g
Agar	13.5	g

pH	7.2

4. DeMan Rogasa Sharpe (MRS) agar per liter

Peptone	10.0	g
Meat Extract	8.0	g
Yeast Extract	4.0	g
Lactose	10.0	g
Sucrose	10.0	g
Sodium acetate	5.0	g
diammonium citrate	2.0	g
K_2HPO_4	2.0	g
$MgSO_4$	0.2	g
$MnSO_2$	0.05	g
Tween 80 (Polysorbate)	1.0	ml
Bromcresol purple (2%)	2.0	ml
Agar	14.0	g
pH	6.2	

5. *Salmonella-Shigella* agar (SS-agar)

Beef extract	5.0	g
Peptone	5.0	g
Lactose	10.0	g
Bacto-bile salt No3	8.0	g
Sodium citrate	8.5	g
Sodium thiosulfate	8.5	g
Ferric citrate	1.0	g
Brilliant green	0.33	g
Neutral red	25	mg
Agar	15.0	g
pH	7.0	

The 63 g powder of commercial SS-agar was dissolved in 1000 ml distrilled water and heated.

6. Typtone glucose yeast agar (plate count agar) per litre

Pancreatic digest of casein	5.0	g
Yeast extract	2.5	g
Glucose	1.0	g
Agar	15.0	g
pH	7.0	

7. *B. cereus* medium (BCM) per 110 ml

D-Mannitol	1.0	g
$(NH_4)_2PO_4$	0.1	g
KCl	0.02	g
$MgSO_4.7H_2O$	0.02	g
Yeast extract	0.02	g
Bromcresol Purple	4.0	g
Egg yolk emulsion, 20%	10.0	ml
Agar	2.0	g
pH	7.0	

- Egg yolk Emulsion 20% per 100 ml

Chicken egg yolks	11	
Whole chicken egg	1	
NaCl (0.9% solution)	80.0	ml

Eggs were soaked with 1:100 dilution of saturated mercuric chloride solution for 1 min. Eggs were cracked and separated yolks from whites. Mixed egg yolks with 1 chicken egg. Measured 20 ml of egg yolk emulsion and

added to 80 ml of 0.9% NaCl solution. Mixed thoroughly and sterilized by filter sterilize. Warm to 45-50°C.

APPENDIX C

Growth rate of seven food spoilage bacteria

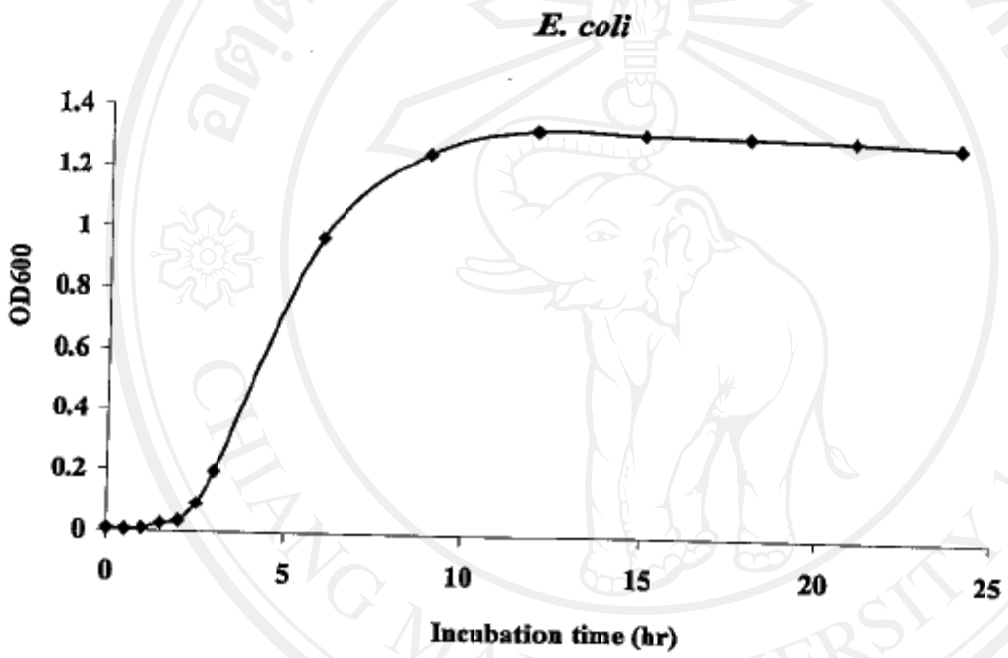

Figure 18 The growth rate of *E. coli*

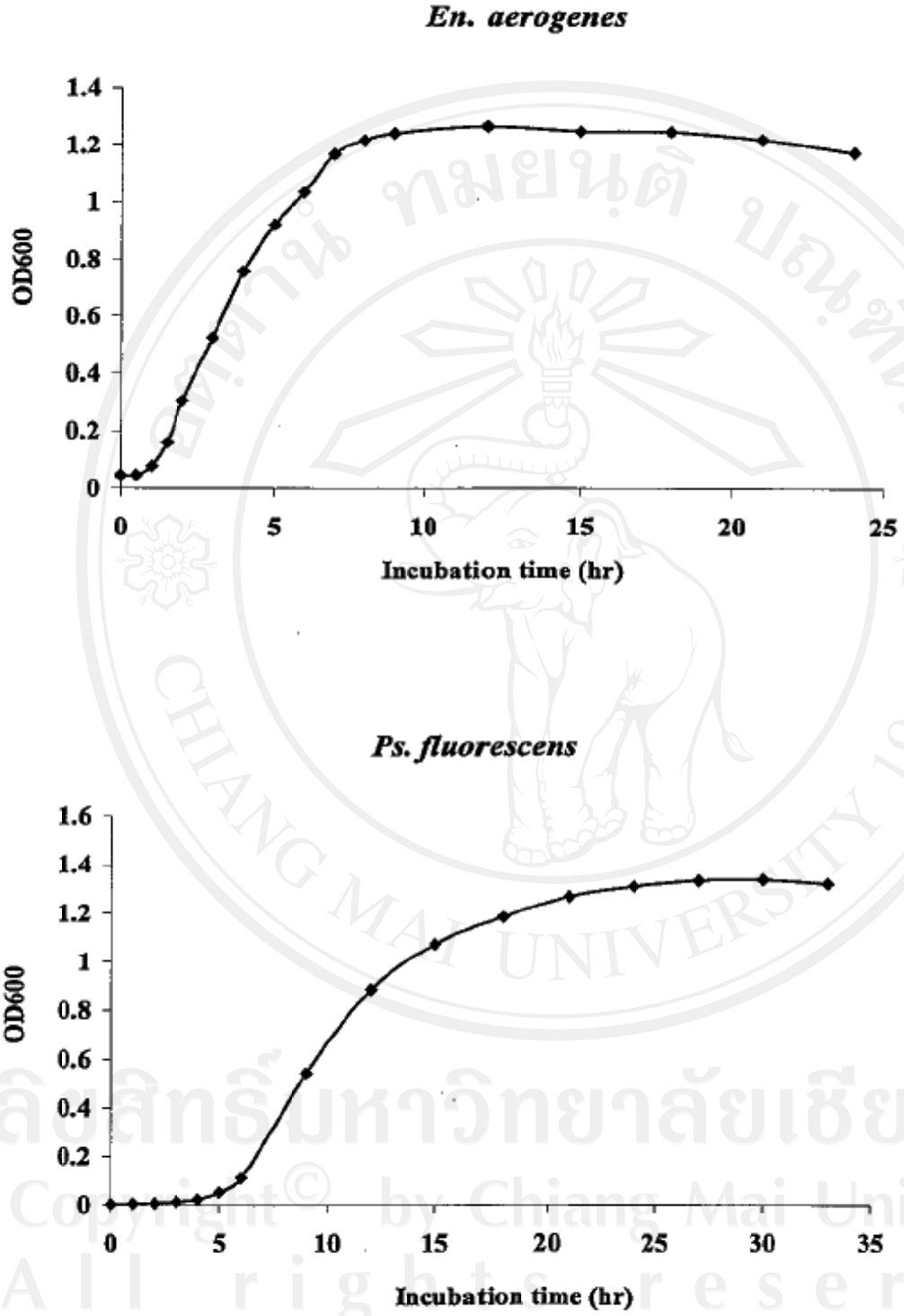

Figure 19 The growth rate of *En. aerogenes* and *Ps. fluorescens*

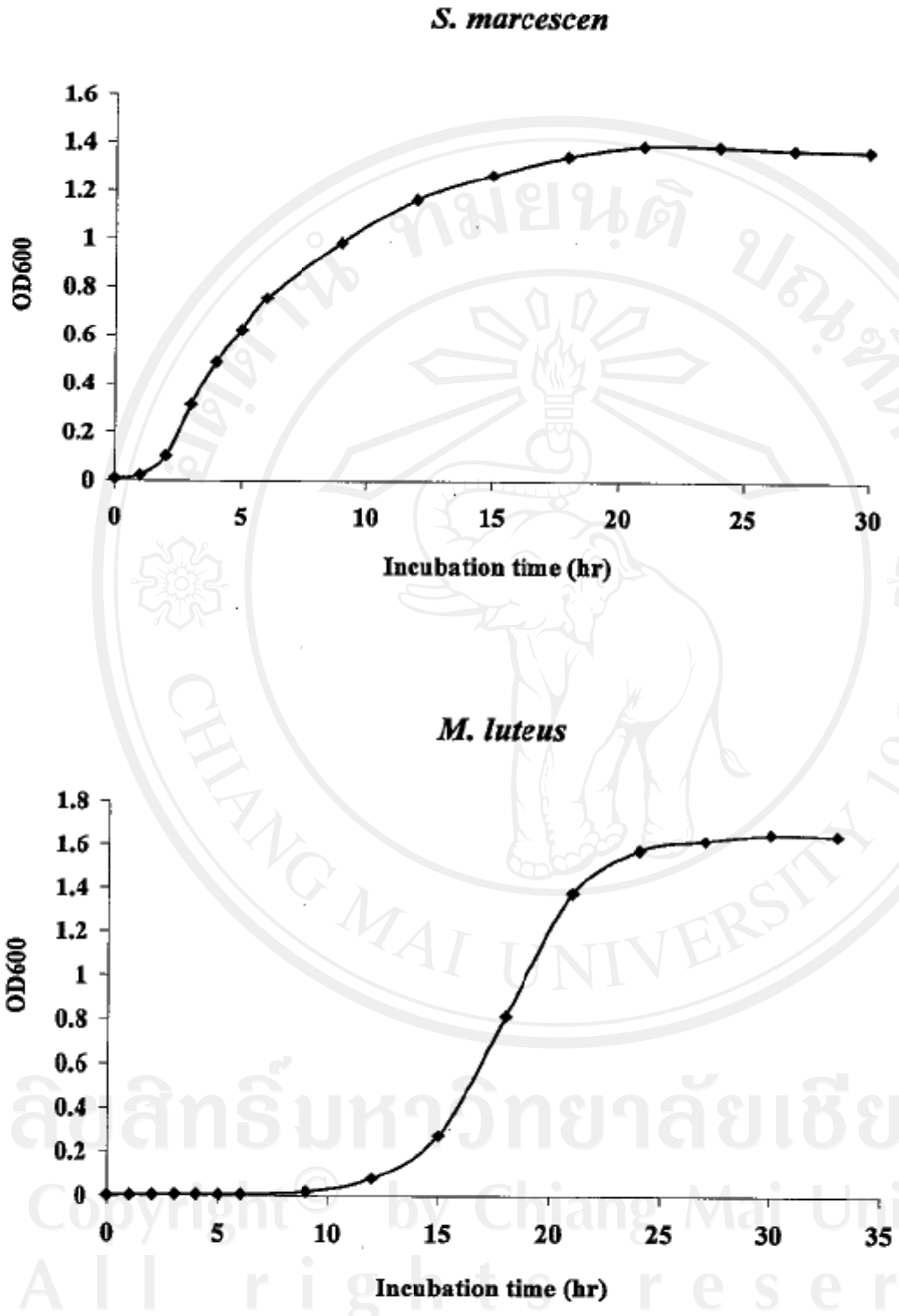

Figure 20 The growth rate of *S. marcescen* and *M. luteus*

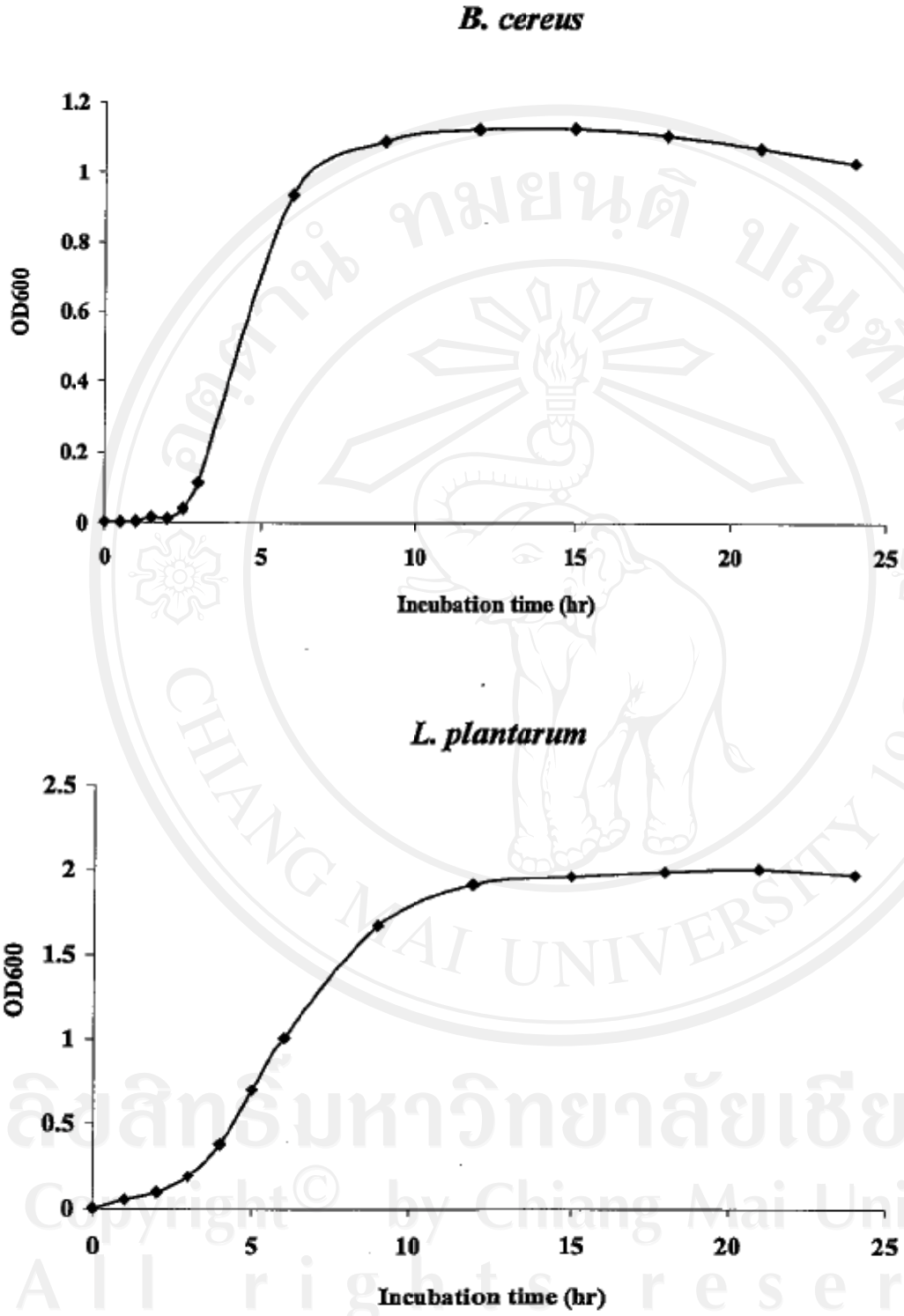

Figure 21 The growth rate of *B. cereus* and *L. plantarum*

APPENDIX D

Standard graph for enumeration of bacteria

Methods

1) One full loop of stock culture on nutrient agar slants of each bacteria was inoculated into 10 ml of nutrient broth, incubated with shaking 180 rpm at 37°C for 24 hours for inocula preparing.
2) 0.5 ml of these culture were inoculated into 50 ml nutrient broth and incubated with shaking 180 rpm at 37°C for 24 hours.
3) Samples were taken and measured for the growth and viable cell counts of bacteria at 1 hr intervals by monitoring the turbidity and plate count method for obtained a standard plot of turbidity versus cell counts.
4) The turbidity of these samples were monitored in terms of optical density (OD) in a spectrophotometer at 600 and 660 nm. The blank used in this monitoring was sterile nutrient broth. Simultaneously the actual cell counts in each culture was determined by plate count method. Each samples were done to dilution plating and plate which gave visible colonies between 30-300 colonies were counted for calculate the colony forming units (CFU) in the original sample.

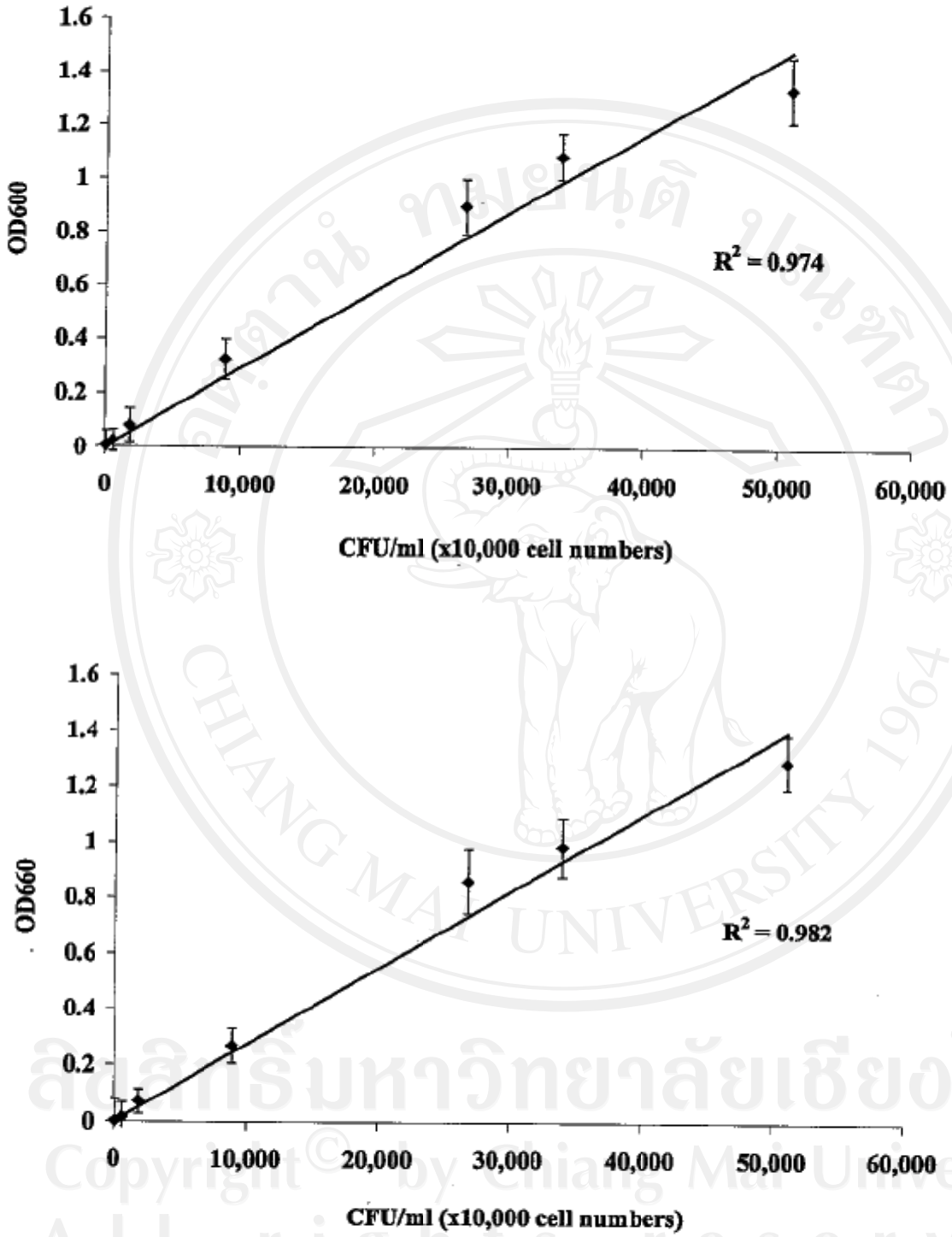

Figure 22 Standard graph of *B.cereus* at OD_{600} and OD_{660}

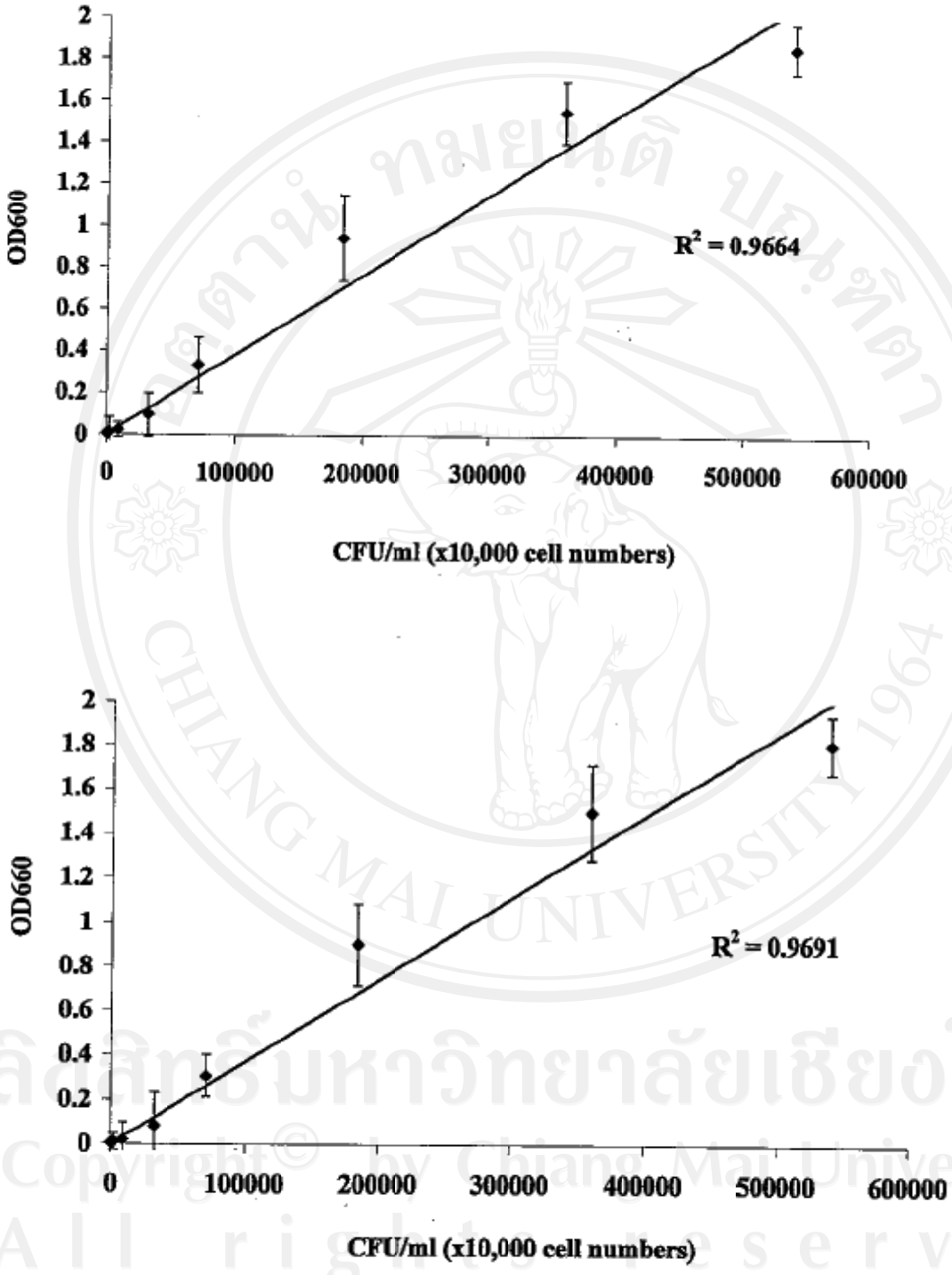

Figure 23 Standard graph of *M. luteus* at OD_{600} and OD_{660}

APPENDIX E

Growth inhibition percentages formular

$$\% \text{ Growth inhibition} = 100 - \left[\frac{\text{Cell number in chitosan}}{\text{Cell number in control}} \right] 100$$

APPENDIX F

Colonies of food spoilage bacteria on isolation plate.

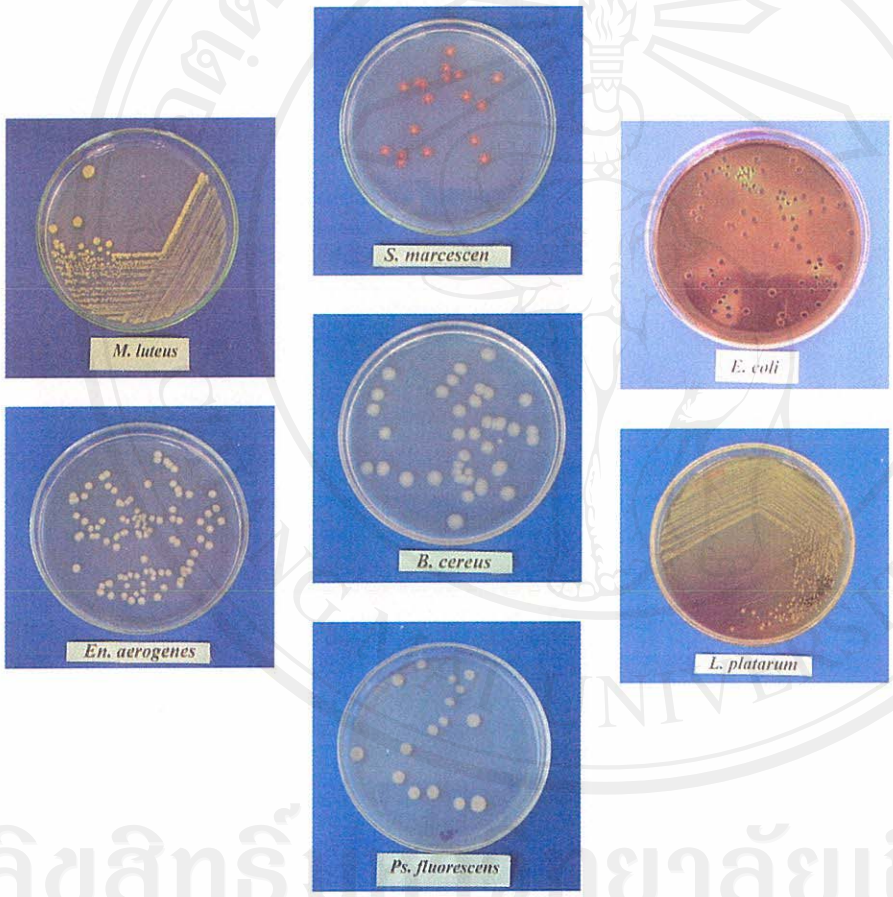

Figure 24 Colonies of seven food spoilage bacteria tested after 24 hr incubation.

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