Appendices

APPENDIX A

Media

1. Media composition and Preparation

1.1 De Man, Rogasa, and Sharpe agar (MRS agar)

Peptone	10.0	g
Beef extract	10.0	g
Yeast extract	5.0	g
Glucose	20.0	g
Tween 80	1.0	g
K_2HPO_4	2.0	g
$CH_3COONa.3H_2O$	5.0	g
Triammonium citrate	2.0	g
$MgSO_4.12H_2O$	0.2	g
$MnSO_4$	0.2	g
Agar	15.0	g
Distilled water	1,000	ml

Medium was adjusted pH to 6.2 and sterilized at 121^{o}C for 15 min.

1.2 Basal medium

Peptone	5.0	g
Yeast extract	1.0	g
K_2HPO_4	0.3	g
KH_2PO_4	0.1	g
$MgSO_4$	0.2	g
$(NH_4)_2SO_4$	2.5	g

Glucose	10.0	g
Distilled water	1,000	ml

Medium was sterilized at 121°C for 15 min

1.3 Eosin Methylene Blue agar (EMB agar)

Peptone	10.0	g
Lactose	5.0	g
Sucrose	5.0	g
K_2HPO_4	2.0	g
Eosin	0.4	g
Methylene blue	0.065	g
Agar	15.0	g
Distilled water	1,000	ml

The 37.5 g powder of commercial EMB agar was dissolved in 1,000 ml distilled water and sterilized at 121°C for 15 min.

1.4 Salmoella-Shigella agar (SS-agar)

Beef extract	5.0	g
Peptone	5.0	g
Lactose	10.0	g
Bacto-bile salts 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 1,000 ml distilled water and heated to boiling with frequent agitation to dissolve the medium completely.

1.5 Nutrient broth (NB)

Peptone	10.0	g
Beef extract	10.0	g
NaCl	5.0	g
Distilled water	1,000	ml

Medium was sterilized at 121^{o}C for 15 min.

1.6 Nutrient agar (NA)

Peptone	10.0	g
Beef extract	10.0	g
NaCl	5.0	g
Agar	15.0	g
Distilled water	1,000	ml

Medium was sterilized at 121^{o}C for 15 min.

APPENDIX B

Determination of total sugar, reducing sugar and carbazole assay

1. Total sugar determination by phenol-sulfuric method

(Dubois *et al.,* 1956)

Reagents

(1) Conc. Sulfuric acid (95.5%)

(2) 5% Phenol solution

Methods

(1) Standard curve of sugar was prepared using the serial concentration of glucuronic acid solution (0-300 μg/ml) in distilled water. The 500 μl of each concentration was transferred to test tube and added with 500 μl of 5% Phenol solution. The mixtures were shaken and followed by the addition of 2.5 ml conc. Sulfuric acid. All mixtures were homogenized by vortex and subsequently stand for 10 minute. The absorbance (490 nm) of the reaction mixture was measured. Finally, the relation between A_{490} and glucuronic concentration was plotted.

(2) Determination of total sugar in samples, sugar concentration in sample solution was determined as the method described above. The reaction mixture composed with 500 μl of sample solution, 500 μl of 5% Phenol solution and 2.5 ml conc. Sulfuric acid solution.

Table 8 Absorbance at 490 nm by glucuronic acid solution at several concentrations.

Glucuronic acid concentration (μg/ml)	A_{490}
0	0
30	0.097
60	0.204
90	0.362
120	0.483
150	0.576
180	0.706
210	0.790
240	0.932
270	1.075
300	1.142

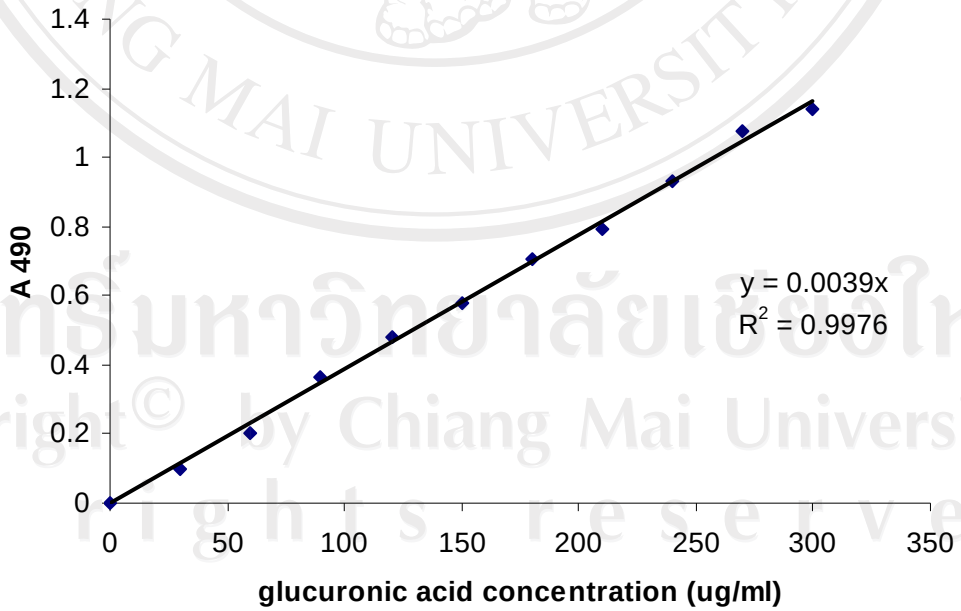


Figure 25 Standard curve of total sugar by phenol-sulfuric method using glucuronic acid as standard sugar.

2. Reducing sugar determination by dinitrosalicylic acid method (DNS method)

Reagent

DNS solution: Dissolve 2.5 g of 3,5 dinitrosalicylic acid (DNS) in 50 ml of 2 N NaOH. Add sodium potassium tartrate (75g) and stir until completely dissolve. Finally, adjust the volume to 250 ml.

Methods

(1) Standard curve preparation of reducing sugar was prepared using serial concentration of glucuronic acid solution (0-700μg/ml) in distilled water. The 500 μl of each concentration was filled into test tube and added with 500 μl of DNS solution and subsequently boiled for 15 minute. After that, cooling and addition with 4.0 ml of distilled water was performed. After homogenizing of reaction mixture, the absorbance at 540 nm was measured. The relation between glucose concentration and A_{540} was plotted.

(2) To determine amount of reducing sugar in sample solution, the 500 μl of sample solution was determined with the method as described above similar to standard curve preparation. After A_{540} measurement, reducing sugars concentration was calculated by comparing to standard curve.

Table 9 Absorbance at 540 nm by glucuronic acid solution at several concentrations

Glucuronic acid concentration (μg/ml)	A_{540}
0	0
50	0.025
100	0.069
200	0.146
300	0.230
400	0.307
500	0.386
600	0.473
700	0.550
800	0.629
900	0.714

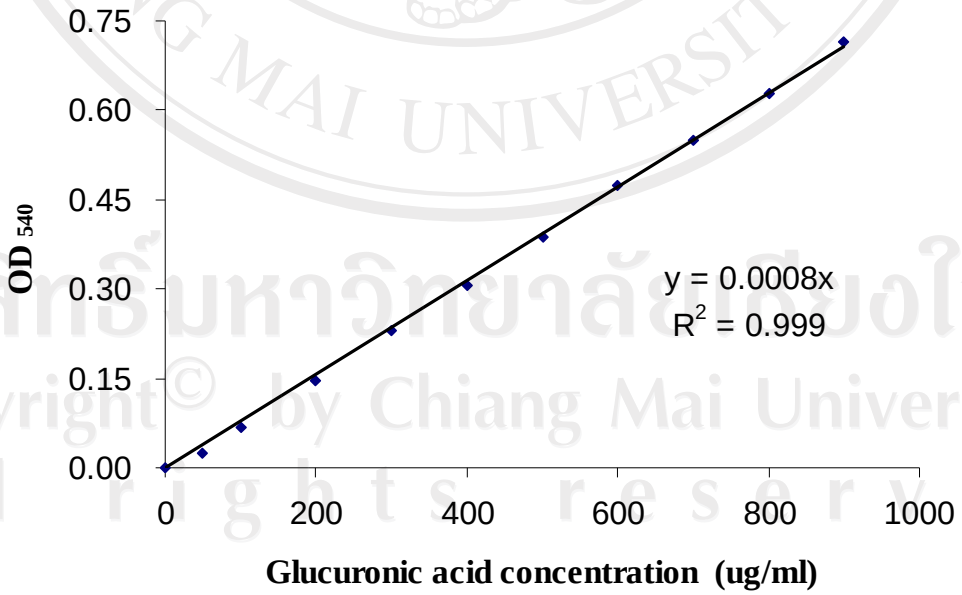


Figure 26 Standard curve of reducing sugar by DNS method using glucuronic acid as standard sugar.

3. Carbazole assay (Dische, 1947)

Reagents

(A) Dissolve 0.95 g of sodium tetraborate decahydrate in 2.0 ml of hot water and add 98 ml of ice-cold concentrated sulfuric acid carefully with stirring. This reagent is stable indefinitely if refrigerated.

(B) Dissolve 125 mg of carbazole in 100 ml of absolute ethanol to give a stable reagent.

Method

(1) Cool the sample, standard (preparation of reducing sugar was prepared using serial concentration of glucuronic acid solution (0-70μg/ml) in distilled water) and controls (250μl) in an ice bath. After that add ice-cold reagent A (1.5 ml) with mixing and cooling in the ice bath and heat the mixtures at 100^{O}C for 10 min. Cool rapidly in the ice-bath.

(2) Add 50 μl of reagent B and mix well. Then, reheat at 100^{O}C for 15 min. After cool rapidly to room temperature and determine the absorbance at 525 nm. Finally, the relation between A_{525} and glucose concentration was plotted.

(3) Determination of uronic acid in samples, sugar concentration in sample solution was determined as the method described above.

Table 10 Absorbance at 525 nm by glucuronic acid solution at several concentrations

Glucuronic acid concentration (μg/ml)	A_{525}
0	0
5	0.081
10	0.178
20	0.321
30	0.494
40	0.628
50	0.791
60	0.952
70	1.129

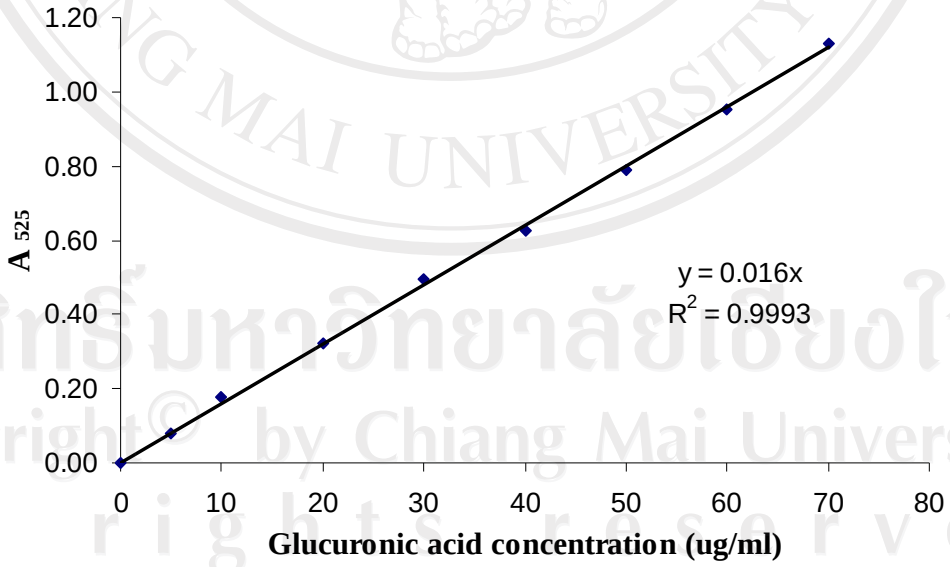
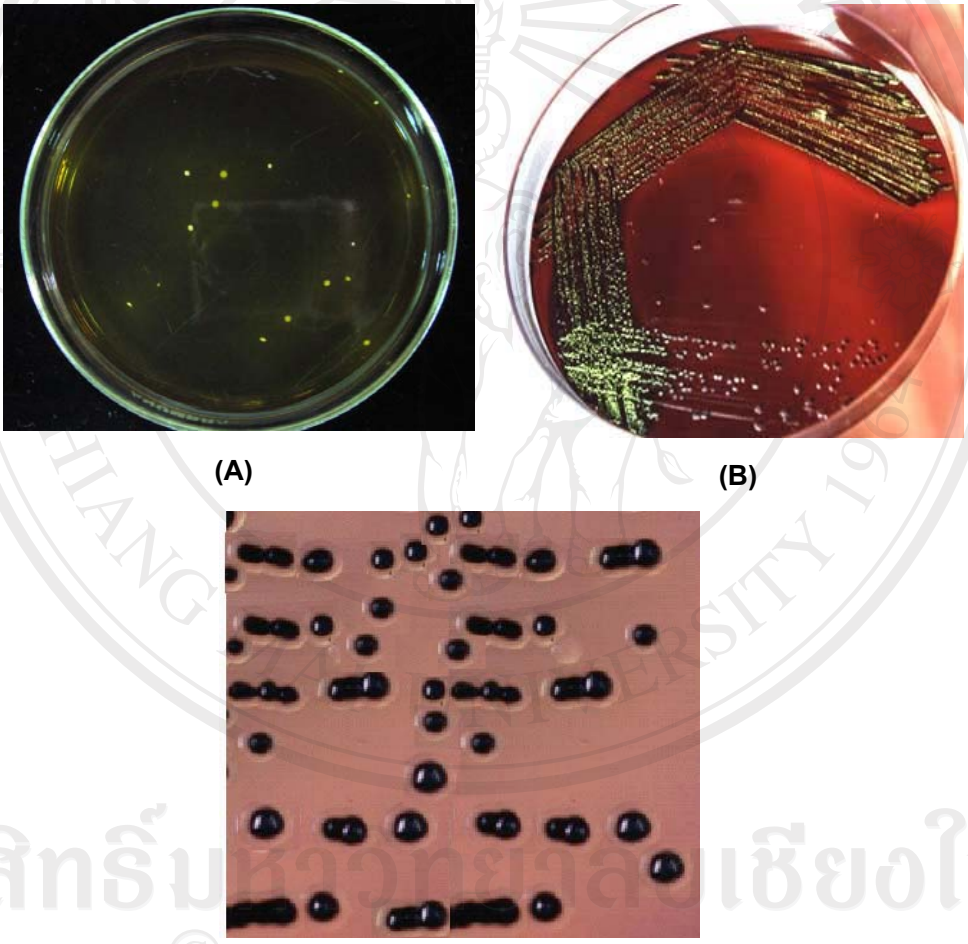


Figure 27 Standard curve of uronic acid by carbazole method using glucuronic acid as standard sugar

APPENDIX C

Colony of some microorganisms on selective media



(A) (B)

(C)

Figure 28 The morphology of some strain on selective media. (A) Colony of LAB (oval shape colony with yellow zone around colony) on MRS agar. (B) Colony of *E. coli* and their metallic sheen colonies on EMB agar. (C) Colony of *S. havana* (black colony) on SS agar.

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