APPENDIX I

Apparatus and Chemicals

1.1 Apparatus

(1) Ultrasonicator, model 2200, manufactured by Branson, U.S.A.

(2) Autoclave SS-240, Tomy Seiko Co.Ltd., Tokyo, Japan

(3) Dispenser Dispet TM Nichiyo Co., Ltd., Japan

(4) Incubator B5050 Heraeus, West Germany

(5) Stereomicroscope VMZ-4SA-2W, Olympus Optical Co. Ltd., Japan

(6) Microscope Olympus model CHS, Olympus Optical Co. Ltd., Japan

(7) AIR metrics MiniVol Portable sampler, USEPA, USA

(8) Automatic balance, Sartorius Ag, Goettingen, Germany

(9) Electrophoresis unit, BIORAD

(10) Fluorescence microscope, ZEISS

(11) 47 mm Teflon impregnated gas fiber filters, type A20, Pallfex Product Corp., Putnam, CT, USA

1.2 Chemicals

(1) Agarose, normal melting point (NMP), Sigma Chemical Co., U.S.A.

(2) Agarose, low melting point (LMP), Sigma Chemical Co., U.S.A

(3) Dichloromethane, organic residue analysis grade, J.T. Baker Inc., U.S.A

(4) Anhydrous sodium su;fate, GR grade, Merk, Germany

(5) Ampicillin (U.S.A) L.B.I.

(6) Bacto agar Difco laboratories, U.S.A.

(7) d-Biotin, Sigma Chemical Co., U.S.A.

(8) Dimethylsulfoxide (spectroscopic grade), E. Merck, Germany

(9) Dipotassium hydrogenphosphate AR, Fluke A.G., Buchs, Switzerland

(10) Disodium hydrogenphosphate AR, E. Merk, Germany

(11) D-Glucose-6-phosphate, Sigma Chemical Co., U.S.A.

(12) Ethelenediamine tetraacetic acid (EDTA) disodium salt, Sigma Chemical Co., U.S.A.

(13) Ethidium bromide,Sigma Chemical Co., U.S.A.

(14) Magnesium chloride AR, May and Baker Ltd., England

(15) Magnesium sulfate ($MgSO_4.7H_2O$) AR, Fluke A.G., Buchs, Switzerland

(16) β-Naphoflavane, Aldrich chemical company Inc., U.S.A.

(17) β-Nicotinamide adenine dinucleotide (b-NADH), Orental yeast, company, Japan

(18) β -Nicotinamide adenine dinucleotide phosphate, reduced form (β-NADPH), Orental yeast company, Japan

(19) Oxoid nutrient broth No. 2, Oxide Ltd., England

(20) Phenobarbital sodium (U.S.P) Wako pure chemical industries, Ltd., Japan

(21) Sodium ammonium hydrogen phosphate ($NaNH_4PO_4.4H_2O$), E. Merk, Germany

(22) Sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$) AR, E. Merk, Germany

(23) Sodium lauroyl sarcosinate, Sigma Chemical Co., U.S.A.

Appendix II

Preparation of some reagents

2.1 Preparation of minimal glucose agar plate

The components of 2,000ml minimal glucose agar medium were

Bacto-Difcoagar	30 g
D-glucose	40 g
Distilled water	1,800 ml
10 x Vogel-Bonner medium E	200 ml

The ingredients should be autoclaved separately. When the solution has cooled slightly, mixed together well and pored 30 ml into each plate. Half hour later, all prepared plates were put in the incubator at 37^0 C for four days before they used in experiment.

The components of 1,000 ml Vogel-Bonner medium E (ten-fold solution) are:

$MgSO_4.7H_2O$	2 g
Citric acid.H_2O	20 g
K_2HPO_4	100 g
$NaNH_4HPO_4$	35 g
Distilled water	1,000 ml

2.2 Preparation of top agar containing histidine and biotin

A: The components of 10 ml top agar

Bacto-Difco agar	0.6 g
NaCl	0.5 g
Distilled water	100 ml

The solution was sterilized by autoclave at 1 lb, 120^0 C , 20 min

B: The components of 100 ml 0.5 mM histidine/biotin

D-biotin	124 mg
L-Histidine $HClH_2O$	105 mg
Distilled water	100 ml

2.3 Lysing solution

Ingredients per 1000 ml:

2.5 M NaCl	146.1 g
100 mM EDTA	37.2 g
10 mM Tris	1.2 g

(set pH to 10 with ~ 8 g solid NaOH)

1 % Na lauroyl sarcosinate	10 g

q.s. to 890 ml with dH_2O, store at room temperature.

Final lysing solution (100 ml):

Add fresh 1 ml Triton X-100 and 10 ml DMSO to 89 ml lysing solution, and then refrigerate (4^{0}C) for 60 min before use.

2.4 Electrophoresis buffer

300 mM NaOH/1 mM EDTA

Prepare from stock solutions:

a) 10 N NaOH (200 g/500 ml dH_2O)

b) 200 mM EDTA (14.89 g/200 ml dH_2O pH 10)

store at room temperature

For 1x buffer(made fresh before each run;Total volume depends on gel box capacity) mix 45 ml NaOH plus 7.5 ml EDTA,

q.s. to 1500 ml,mix well

2.5 Neutralization buffer

0.4 M Tris 48.5 g

q.s. to 1000 ml with dH_2O

set pH to 7.5 with HCl and store at room temperature

2.6 Staining solution

Ethidium bromide (10x stock: 200 microgram/ml)

10 mg in 50 ml dH_2O

store at room temperature

for 1x stock (20 microgram/ml) mix 1 ml with 9 ml dH_2O and filter

2.7 Sodium phosphate buffer

1 M NaH_2PO_4

1 M Na_2HPO_4

mix until pH 7.0

VITA

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