

APPENDIX

APPENDIX I

Background data for *Salmonella* Typhimurium Strains

A. Isolated from animals

Strain	Source	Place	Date of Isolation	Age
STM 8322/03	Chicken	Perak	16/9/03	56 days
STM 6324/03	Chicken	Perak	23/7/03	40 weeks
STM 3068/98	Chicken	-	-	-
STM 402/05	Chicken	Sabah	25/1/05	22 weeks
STM 1204/05	Chicken	Selangor	2/3/05	-
STM 1621/05	Chicken	Johor	16/3/05	-
STM 2560/05	Chicken	Johor	20/5/05	-
STM 2625/05	Chicken	Selangor	14/4/05	-
STM 3503/05	Chicken	Perak	26/5/05	-
STM 3865/05	Chicken	Perak	1/6/05	18 weeks
STM 3866/05	Chicken	Selangor	1/6/05	9-10 weeks
STM 4524/05	Chicken	Selangor	15/6/05	-
STM 5229/05	Chicken	Sabah	13/7/05	-
STM 254/98	Chicken	-	-	-
STM 7456/04	Cattle	Sabah	11/10/04	2 years
STM 2555/05	Cattle	Selangor	20/5/05	-
STM 2559/05	Cattle	Selangor	20/5/05	-
STM 2593/04	Cattle	Lucky Frozen	14/4/05	-
STM 3079/05	Cattle	Kedah	9/5/05	-
STM 5231/05	Cattle	Sabah	13/7/05	12 days
STM 2553/05	Cattle	Selangor	20/5/05	-
STM 3000/05	Fish	Penang	3/5/05	-
STM 3077/05	Swine	Perak	1/6/05	2 months
STM 1377/05	Frog	Butterworth	10/3/05	-

B. Isolated from animals (No Background)

Strain	Source	Place	Date of Isolation	Age
STM 1234/04	Animal	-	-	-
STM 5532/04	Animal	-	-	-
STM 5553/04	Animal	-	-	-
STM 981/04	Animal	-	-	-
STM 6344/95	Animal	-	-	-
STM 3215/03	Animal	-	-	-
STM 2599/05	Animal	-	-	-

C. Isolated from Humans (Clinical)

Strain	Age	Sex	Date of Isolation	Source
STM 0504/69	-	-	26/11/69	-
STM 0287/69	-	-	26/11/69	-
STM 95893/70	2 years	Male	4/2/70	-
STM 30822/70	2 years	Female	4/2/70	-
STM 87098/70	5 years	Female	13/3/70	-
STM 79495/70	8 years	Male	4/2/70	-
STM 110187/70	5 years	Male	1/4/70	-
STM 113254/70	5 months	Male	24/3/70	-
STM 287232/77	-	-	21/1/77	-
STM 196/05	28 years	Female	2005	Blood
STM 447/05	3 months	Female	2005	Stool
STM 32/05	7 months	Female	2005	Stool
STM 01/06	-	Male	16/1/06	Stool
STM 02/06	-	Female	30/4/06	Stool

APPENDIX II

A. Extraction Reagents

Reagents	Manufacturer Supplier
i-genomic CTB, DNA Extraction Mini Kit	
- Buffer CG	
- Buffer CB	
- Buffer CW	iNtRON Biotechnology, South Korea
- Buffer CE	
- RNase A Solution	
- Proteinase K Solution	
Absolute Alcohol	BDH Lab Supplies, England

B. Digestion Reagents

Reagents	Manufacturer Supplier
10X Buffer C	Promega, Madison, Wisconsin, USA
Acetylated BSA (10 μ g/ μ l)	Promega, Madison, Wisconsin, USA
<i>Hae</i> III Restriction Enzyme (10 μ / μ l)	Promega, Madison, Wisconsin, USA

C. PCR Reagents

Reagents	Manufacturer Supplier
10X PCR Buffer	iNtRON Biotechnology, South Korea
MgCl ₂ (25 mM)	iNtRON Biotechnology, South Korea
dNTPs (2.5 mM)	iNtRON Biotechnology, South Korea
<i>Taq</i> DNA Polymerase	iNtRON Biotechnology, South Korea
6X Blue/ Orange Loading Dye	Promega, Madison, Wisconsin, USA
Primers for confirmation PCR	Operan, Germany
Primers for Restricted AP-PCR	BioNeer, Korea

D. Rest of Reagents

Reagents	Manufacturer Supplier
Agarose Powder	Promega, Madison, Wisconsin, USA
Etidium Bromide (EtBr)	Sigma Chemical Company, USA
Glycerol	Invitrogen, USA
Lambda DNA/HindIII Marker	Promega, Madison, Wisconsin, USA
100bp Marker	Promega, Madison, Wisconsin, USA
Ethylenediamine-Tetraacetic Acid (EDTA)	Sigma Chemical Company, USA
Orthoboric Acid	BDH Lab Supplies, England
Trizma Base	Sigma Chemical Company, USA
Tris	BioBasic, Canada

Agarose gel (1%) for PCR

Agarose gel	0.32 g
0.5X TBE Buffer	32 ml

The agarose mixture was heated slowly until the agarose particles completely dissolved. Then it was kept in water bath equilibrated 55 °C-60 °C before pour for casting the gel.

APPENDIX III

A. Growth Media

Growth Media	Manufacturer Supplier
Luria Bertani (LB) Agar	
- Tryptone	Oxoid Ltd., England
- Yeast Extract	Oxoid Ltd., England
- Sodium Chloride (NaCl)	BDH Lab Supplies, England
- Bacteriological Agar	Oxoid Ltd., England
Luria Bertani (LB) Broth	
- Tryptone	Oxoid Ltd., England
- Yeast Extract	Oxoid Ltd., England
- Sodium Chloride (NaCl)	BDH Lab Supplies, England
Xylose-Lysine-Desoxycholate Agar (XLD Agar)	Oxoid Ltd., England
Hektoen Enteric Agar	Oxoid Ltd., England

Luria Bertani (LB) Agar

- Tryptone	3.0 g
- Yeast Extract	1.5 g
- NaCl	1.5 g
- Bacteriological Agar	4.5 g
- Disstilled Water	300 ml

Luria Bertani (LB) Broth

- Tryptone	3.0 g
- Yeast Extract	1.5 g
- NaCl	1.5 g
- Disstilled Water	300 ml

The media above were dissolved in 300 ml of ddH₂O. The medium was boiled to dissolve completely and sterilized by autoclaving at 121 °C for 15 minutes. Then it was cooled to 50 °C-55 °C, and poured into sterile Petri dishes plates.

Xylose-Lysine-Desoxycholate Agar (XLD Agar)

- XLD Agar Powder 15.9 g

- Distilled Water 300 ml

Hektoen Enteric Agar

- Hektoen Enteric Agar Powder 22.5 g

- Distilled Water 300 ml

For XLD Agar and Hektoen Enteric Agar, the mixture was heated gently along with frequent agitation until the medium start to boil and simmer for 30 seconds to dissolve the agar. The medium was cooled to 50 °C, mixed well and poured into plates. The medium should not be autoclaved and overheated.

APPENDIX IV

50% Glycerol

- Ultra Pure Glycerol 50 ml
- ddH₂O 50 ml

The mixture was sterilized by autoclaving at 121 °C for 15 minutes and stored at room temperature.

Etidium Bromide (EtBr)

- Etidium Bromide 30 µl
- ddH₂O 300 ml

This solution was stored in a dark bottle at room temperature, and diluted to µg/ml with distilled water before used.

10X Tris-Borate EDTA (TBE), pH 8.3

- Trizma Base 121.2 g
- Orthoboric/Boric Acid (BDH) 61.8 g
- EDTA 0.74 g
- Deionised Water 1000 ml

The above ingredients were dissolved in 500 ml of deionised water by stirring on the heated stirrer plate. pH was adjusted to 8.3 and top up to 1000 ml and autoclaved at 121 °C; 15 psi for 15 minutes.

0.5X Tris-Borate EDTA (TBE)

- 10X TBE 50 ml

- Deionised Water 950 ml

100 ml of 10X TBE was aliquoted into clean sterile glassware and top up to 1000 ml by using the deionised water.

1 M Tris, pH 8.0 (Molecular Weight = 121.14 g)

- Tris 36.342 g

- Deionised Water 250 ml

36.342 g of Tris powder were dissolved in 250 ml of deionised water by stirring on the heated stirrer plate. pH was adjusted to 8.0 and top up to 300 ml and autoclaved at 121 °C; 15 psi for 15 minutes.

0.5 EDTA, pH 8.0 (Molecular Weight = 372.24 g)

- EDTA 55.83 g

- Deionised Water 250 ml

55.83 g of EDTA powder were dissolved in 250 ml of deionised water by stirring on the heated stirrer plate. pH was adjusted to 8.0 and top up to 300 ml and autoclaved at 121 °C; 15 psi for 15 minutes.

Tris-EDTA (TE) Buffer (10 mM Tris; 1 mM EDTA; pH 8.0)

- 1 M Tris, pH 8.0 10 ml

- 0.5 EDTA, pH 8.0 2 ml

Top up with deionised water to 1000 ml, and autoclaved at 121 °C; 15 psi for 15 minutes.