

LIST OF CONTENTS

	<u>Page</u>
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	v
ABBREVIATIONS	vi
LIST OF CONTENTS	ix
LIST OF FIGURES	xvi
LIST OF TABLES	xxiii
CHAPTER ONE: INTRODUCTION	1
1.1 <i>Salmonella typhi</i>	1
1.2 Typhoid fever	1
1.3 Diagnosis and treatment of typhoid fever	2
1.4 Antibiotics	4
1.4.1 Characteristics of antibiotics	4
1.4.2 Aminoglycosides	5
1.4.3 Streptomycin (Sm)	7
1.4.4 Mechanisms of Sm resistance	10
1.4.4.1 Sm-modifying enzymes	10
1.4.4.2 Alterations in ribosomal Sm binding site	12
1.4.4.3 Mutations interfering with Sm uptake	12
1.5 Epidemiology of antibiotic resistance	13
1.6 Origin of antibiotic resistance	14
1.7 Transfer of antibiotic resistance genes	16
1.8 R plasmids	16
1.9 Transposable genetic elements (TGE)	18
1.10 Transposon Tn21	21
1.11 IS elements	25
1.12 Integrations	29

1.13	M13mp18 and M13mp19 bacteriophages	33
1.14	Previous studies on pST8	35
1.15	Objectives of this study	37
CHAPTER TWO: MATERIALS AND METHODS		39
2.1	Bacterial strains	39
2.2	Plasmids and phage	39
2.3	Materials	39
2.4	Media, antibiotic solutions, and stock solutions	42
2.4.1	Luria-Bertani (LB) medium (Sambrook <i>et al.</i> , 1989)	42
2.4.2	SOB medium	42
2.4.3	SOC medium	42
2.4.4	Mueller Hinton (MH) agar	42
2.4.5	B agar (Sambrook <i>et al.</i> , 1989)	42
2.4.6	2X TY	43
2.4.7	Antibiotic stock solutions	43
2.4.8	Solutions for agarose gel electrophoresis	43
2.4.8.1	Tris-borate EDTA (TBE) buffer, pH 8.3 (Sambrook <i>et al.</i> , 1989)	43
2.4.8.2	50X Tris-acetate EDTA (TAE) buffer	44
2.4.8.3	6X Bromophenol blue (BPB) loading dye	44
2.4.9	Common solutions for DNA extraction, cloning, and transformation	45
2.5	Solutions for hybridization experiments	45
2.5.1	Solutions for Southern blotting	45
2.5.1.1	Depurination solution	45
2.5.1.2	Denaturation solution	45
2.5.1.3	Neutralization solution	45
2.5.1.4	Solutions for hybridization and blot washing	45
2.5.1.5	Hybridization buffer	45
2.5.1.6	Primary wash buffer (without urea)	46
2.5.1.7	Secondary wash buffer (2X SSC)	46

2.5.2	Solutions for labelling DNA probes	46
2.5.3	Signal generation and detection	46
2.6	Solutions for M13mp18 subcloning	46
2.6.1	Xgal	46
2.6.2	IPTG (0.1 M)	47
2.6.3	20% (w/v) PEG, 2.5 M NaCl	47
2.6.4	SDS-formamide dye mix (Young, 1984)	47
2.7	Sterilization techniques	47
2.7.1	Heat sterilization	47
2.7.2	Steam sterilization	48
2.7.3	Membrane sterilization	48
2.8	Plating techniques	48
2.9	Maintenance and purification of bacterial strains	48
2.10	Small scale rapid extraction of plasmid DNA (miniprep)	48
2.11	Large-scale extraction and purification of covalently closed circular (ccc) DNA	49
2.12	Estimation of DNA concentration	51
2.13	Restriction endonuclease digestions of DNA	51
2.14	Agarose gel electrophoresis	52
2.15	Recovery of DNA fragments from agarose gels	52
2.15.1	Electroelution (Sambrook <i>et al.</i> , 1989)	52
2.15.2	Recovery of DNA fragments from agarose gels by the GENECLEAN II kit	53
2.16	Ligation of DNA fragments with compatible ends	53
2.17	Subcloning of the streptomycin resistance gene(s) (Sm^R) from <i>Pst</i> I digested pCLS55 into pUC19	54
2.17.1	Comparison of pKan and pCLS55 after restriction endonuclease digestions	54
2.17.2	Preparation of ⁴ <i>Pst</i> I digested pCLS55 and pUC19	54
2.17.3	Shotgun subcloning of the fragment harbouring the Sm^R gene (s) into plasmid vector pUC19	54

2.17.4	Preparation of competent <i>E. coli</i> cells	54
2.17.5	Transformation	55
2.17.6	Analysis of transformants	55
2.18	Southern blotting and hybridization	56
2.18.1	Southern transfer of DNA (Southern, 1975) from agarose gel to nylon membrane	56
2.18.2	Direct labelling of DNA probes	56
2.18.3	Southern hybridization	58
2.18.4	Post-hybridization membrane washing	58
2.18.5	Signal generation and detection	58
2.19	Subcloning of the 2.5 kb <i>EcoRI</i> DNA fragment harbouring the Sm ^R genes into M13mp18	59
2.19.1	Preparation of the <i>EcoRI</i> digested M13mp18	59
2.19.2	Gel-elution of the 2.5 kb <i>EcoRI</i> DNA fragment harbouring the Sm ^R genes	59
2.19.3	Preparation of competent <i>E. coli</i> DH5 α F' cells	59
2.19.4	Ligation of the <i>EcoRI</i> digested M13mp18 with the 2.5 kb <i>EcoRI</i> DNA fragment harbouring the Sm ^R genes	60
2.19.5	Transfection of <i>E. coli</i> DH5 α F' with the RF DNA of M13mp18	60
2.19.6	Complementary or C-test to confirm the opposite orientations of inserts in recombinant M13mp18	61
2.19.7	Purification of single-stranded template DNA of recombinant M13mp18	61
2.20	Subcloning of the 5.5 kb <i>SalI</i> DNA fragment from pCLS55 into <i>SalI</i> digested pUC19	63
2.20.1	Preparation of the <i>SalI</i> digested pCLS55 and pUC19	63
2.20.2	Ligation of the 5.5 kb <i>SalI</i> digested DNA fragment with <i>SalI</i> digested puC19	63
2.20.3	Transformation	63
2.20.4	Analysis of transformants	63

2.21	DNA sequencing	63
2.21.1	Non-isotopic automated DNA sequencing	63
2.21.2	Polyacrylamide gel electrophoresis	64
2.21.3	Analysis of nucleotide sequences	65
2.22	Flowcharts	66
2.22.1	Flowchart of experiments to subclone the Sm ^R gene(s) into pUC19	66
2.22.2	Flowchart of experiments to construct a restriction map of the 5.5 kb insert and to locate Sm ^R gene(s) to a smaller fragment	67
2.22.3	Flowchart of experiments to subclone the Sm ^R gene(s) into M13mp18 vector, sequence the Sm ^R gene(s), and analyses of the nucleotide sequences	68
CHAPTER THREE: RESULTS		69
3.1	Comparison between pKan and pCLS55	69
3.2	Restriction patterns of pKan and pCLS55	69
3.3	Complete digestion of pCLS55 and pUC19 with <i>Pst</i> I	77
3.4	Elution of the excised fragments from pCLS55 and linearized pUC19	77
3.5	Shot-gun subcloning of the Sm ^R gene(s)	77
3.6	Plasmid profiles of Ac ^R Sm ^R transformants	80
3.7	Restriction patterns of recombinant pUC19 after <i>Pst</i> I and <i>Sal</i> I digestions	80
3.8	Complete digestion of pSR3 and pSR4 with <i>Sal</i> I	85
3.9	Elution of the excised fragments from <i>Sal</i> I-digested pSR3 and pSR4	85
3.10	Subcloning of the Sm ^R DNA fragment from pSR3	85
3.11	Plasmid profiles of the Ac ^R Sm ^R transformants	88
3.12	Restriction analyses of recombinant pUC19 obtained after circularization of the 5.2 kb <i>Sal</i> I fragment from pSR3	88

3.13	Complete digestion of pSR3a and RF DNA of M13mp18 with <i>EcoRI</i>	88
3.14	Subcloning of the 2.5 kb <i>EcoRI</i> fragment harbouring the Sm ^R genes into M13mp18	92
3.15	Screening of the recombinant M13mp18	92
3.16	Confirmation of the opposite orientation of inserts in recombinant M13mp18 RF DNA	96
3.17	Southern hybridization with recombinant plasmids and phages	100
3.18	Isolation of single-stranded DNA from M13pSMR13 and M13pSMS16	100
3.19	Nucleotide sequences from M13pSMR13 and M13pSMS16	104
3.20	Analysis of the nucleotide sequence from M13pSMR13 and M13pSMS16	104
3.21	Subcloning of the 5.5 kb fragment from <i>SalI</i> -digested pCLS55 harbouring Sm ^R gene into <i>SalI</i> -digested pUC19	116
3.22	<i>SalI</i> -digestion of the recombinant pUC19	120
3.23	Isolation and sequencing of double-stranded pSR55	120
3.24	Partial nucleotide sequences from pSR55	120
3.25	Analysis of the nucleotide sequences from pSR55	123
CHAPTER FOUR: DISCUSSION AND CONCLUSION		124
4.1	Discussion	124
4.1.1	Localization of the Sm ^R genes	124
4.1.2	Southern hybridization	125
4.1.3	Nucleotide sequences of the 2.5 kb Sm ^R genes	125
4.1.4	IS26-like segment	127
4.2	Conclusion	128
REFERENCES		132