

**SUBCLONING, CHARACTERIZATION AND SEQUENCING  
OF THE STREPTOMYCIN RESISTANCE GENE OF A  
MULTIPLE ANTIBIOTIC RESISTANCE TRANSPOSON  
FROM *Salmonella typhi***

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*Dedicated to,*

*The loving memory of my Daddy (late), .....*

*the wellspring of wisdom and perseverance and*

*to my Mummy,.....*

*for her devotion and her constant demonstrations  
of love.*

## ABSTRACT

*Salmonella typhi* S8 is resistant to ampicillin (Ac), chloramphenicol (Cm), co-trimoxazole (Ctm), streptomycin (Sm), and tetracycline (Tc). The Ac, Cm, Ctm, and Sm resistance traits have been shown to be mediated by a multiple antibiotic resistance transposon located on a large conjugative plasmid. Transposition of the transposon encoding Ac<sup>R</sup> (ampicillin resistance), Cm<sup>R</sup>, Ctm<sup>R</sup>, and Sm<sup>R</sup>, designated TnX8, was done by Wong (1999) to a recipient replicon, plasmid pUB307. The Ac<sup>R</sup>, Cm<sup>R</sup>, Ctm<sup>R</sup>, and Sm<sup>R</sup> genes were subcloned into plasmid pKan by digesting the recombinant plasmid, designated pCL8 (pUB307::TnX8), with *SalI*. This subcloning strategy generated different recombinant pKans that harbour different antibiotic resistance genes. Plasmid pCLS55 is a recombinant pKan that harbours a *SalI* insert bearing Sm<sup>R</sup> genes from the multiple antibiotic resistance transposon of *S. typhi* S8.

The main objective of this study was to subclone and characterize, by DNA sequencing, the Sm<sup>R</sup> genes derived from the transposon of *S. typhi* S8, in pCLS55. Successive subclonings into pUC19 and M13mp18 vectors, and construction of deletions localized the Sm<sup>R</sup> genes on a 2.5 kb *EcoRI* DNA fragment. Southern hybridization, performed before DNA sequencing, confirmed that the 2.5 kb *EcoRI* Sm<sup>R</sup> inserts carried by M13pSMR13 and M13pSMS16 originated from the plasmid in *S. typhi* S8.

A total of 2,128 nucleotides was determined based on complete DNA sequencing of the 2.5 kb *EcoRI* Sm<sup>R</sup> insert in recombinant M13mp18. The nucleotide sequences were analyzed and the gene structure (containing *str* genes, *strA* and *strB*) within the 2.5 kb *EcoRI* Sm<sup>R</sup> insert from pCLS55 was determined. These sequences were identical to the *strA* and *strB* genes encoding Sm resistance in plasmid RSF1010 (Scholz *et al.*, 1989) and in Tn5393 of *Erwinia amylovora* (Chiou and Jones, 1993).

The nucleotide sequences obtained in this study indicated that there are three open reading frames (ORF) in the 2.5 kb *EcoRI* Sm<sup>R</sup> fragment. The first ORF of 267 amino acids was designated ORF A'. The deduced amino acid sequence corresponded to a molecular mass of 29,595 Daltons and has high similarity with the amino acid sequence of Sm phosphotransferase protein A, which is also encoded by RSF1010

and Tn5393. This ORF translation starts at UUG (TTG in the DNA sequence) and this suggests that this gene did not originally come from members of the family *Enterobacteriaceae*.

The second ORF, designated ORF B', encodes a polypeptide of 278 amino acids; which corresponded to a molecular mass of 30,824 Daltons and has high similarity with the amino acid sequence of Sm phosphotransferase protein B.

The Sm<sup>R</sup> genes were also observed to contain part of the transposase gene belonging to IS26 and its variants. This was observed in the third ORF, ORF C', in the nucleotide sequences obtained. Partial DNA sequencing of the 5.5 kb *SalI* insert in pCLS55 showed that the two ends of this insert each contained part of the transposase gene of IS26 and its variants.

The Sm<sup>R</sup> genes in pCLS55 appeared to be borne on a composite element, flanked by IS26 at its ends. The presence of the composite IS26 element within TnX8 supports the hypothesis that multiple antibiotic resistance transposons evolved by insertion of antibiotic resistance determinants, which are themselves transposable.

## ABSTRAK

*Salmonella typhi* S8 adalah rintang terhadap ampisilin (Ac), kloramfenicol (Cm), ko-trimoxazole (Ctm), streptomisin (Sm), dan tetrasiklin (Tc). Ciri-ciri kerintangan terhadap Ac, Cm, Ctm, dan Sm adalah diperantarakan oleh suatu transposon pelbagai kerintangan antibiotik yang berlokasi pada suatu plasmid konjugatif yang besar. Transposisi transposon *TnX8*, transposon yang mengkodkan  $Ac^R$  (kerintangan terhadap ampisilin),  $Cm^R$ ,  $Ctm^R$ , dan  $Sm^R$ , kepada replikon penerima iaitu plasmid pUB307 telah dilakukan oleh Wong (1999).

Hasil transposisi tersebut iaitu plasmid rekombinan pUB307, yang didesignasi sebagai pCL8 (pUB307::TnX8), dicerna dengan enzim pencerna *SaI* untuk mensubklonkan gen-gen  $Ac^R$ ,  $Cm^R$ ,  $Ctm^R$ , dan  $Sm^R$  ke dalam plasmid pKan. Strategi penubklonan ini menjana beberapa pKan rekombinan yang mengandungi gen-gen kerintangan antibiotik yang berlainan. Plasmid pCLS55 adalah sejenis pKan rekombinan yang mengandungi gen-gen  $Sm^R$  dalam satu fragmen *SaI* bersaiz 5.5 kb daripada transposon pelbagai kerintangan *S. typhi* S8.

Objektif utama kajian ini adalah untuk mensubklon dan mencirikan, melalui penjujukan DNA, gen-gen  $Sm^R$  dalam pCLS55 yang diterbitkan dari transposon *S. typhi* S8. Penubklonan berturutan kedalam vektor pUC19 dan sejurusnya kedalam vektor M13mp18, serta penjanaan delesi berjaya melokasikan gen-gen  $Sm^R$  pada satu fragmen *EcoRI* bersaiz 2.5 kb. Sebelum penjujukan DNA, hibridisasi Southern dilakukan dan ia menunjukkan bahawa asalan fragmen  $Sm^R$  *EcoRI* bersaiz 2.5 kb ini adalah sememangnya dari plasmid *S. typhi* S8.

Nukeotida-nukleotida berjumlah 2,128 ditentukan berdasarkan penjujukan DNA seluruhan fragmen *EcoRI* bersaiz 2.5 kb yang terkandung dalam M13mp18 rekombinan. Jujukan nukleotida yang diperolehi dianalisis dan struktur gen (yang mengandungi gen-gen *str*, iaitu *strA* dan *strB*) dalam fragmen *EcoRI* bersaiz 2.5 kb daripada pCLS55 berjaya ditentukaan. Jujukan-jujukan ini didapati seiras dengan gen-gen *strA* dan *strB* yang mengkodkan kerintangan terhadap Sm dalam plasmid RSF1010 (Scholz *et al.*, 1989) dan dalam Tn5393 yang termaktub dalam *Erwinia amylovora* (Chiou and Jones, 1993).

Jujukan nukleotida yang diperolehi dalam kajian ini menunjukkan bahawa ada tiga bingkai bacaan terbuka (ORF, "open reading frame") dalam fragmen Sm<sup>R</sup> *EcoRI* bersaiz 2.5 kb. Bingkai bacaan pertama yang berdesignasi ORF A' mengandungi 267 asid amino dan sejajar dengan berat molekul 29,595 Dalton. Ia mempunyai kesamaan tinggi dengan jujukan asid amino protin A fosfotransferase Sm yang dikodkan oleh RSF1010 dan Tn5393. Translasi ORF ini bermula dengan UUG (TTG dalam jujukan DNA) dan mencadangkan bahawa gen *strA* ini tidak berasal daripada ahli famili *Enterobacteriaceae*.

Bingkai bacaan kedua berdesignasi ORF B' mengkodkan polipeptida sebesar 278 asid amino dan sejajar dengan berat molekul 30,824 Dalton. Analisa jujukan asid amino ini sejajar dengan protin B fosfotransferase Sm yang dikodkan oleh gen *strB* RSF1010 dan Tn5393. Gen-gen Sm<sup>R</sup> ini didapati juga mengandungi sebahagian gen enzim transposisi IS26 dan variasinya. Ini diperhatikan dalam bingkai bacaan ketiga, yakni ORF C', dalam jujukan nukleotida yang diperolehi dalam kajian ini. Penjujukan DNA separa juga dilakukan terhadap fragmen *SaII* bersaiz 5.5 kb dari pCLS55. Analisa jujukan nukleotid separa yang diperolehi ini juga menunjukkan kehadiran gen enzim transposisi IS26 dan variasinya.

Gen-gen Sm<sup>R</sup> dalam pCLS55 didapati termaktub dalam elemen komposit kerana ia diapiti oleh IS26 pada kedua-dua hujungnya. Kehadiran elemen komposit IS26 dalam Tn $\chi$ 8 mengukuhkan lagi hipotesis bahawa transposon pelbagai kerintangan antibiotik berevolusi melalui insersi penentu-penentu antibiotik, yang mempunyai keupayaan untuk bertransposisi secara sendiri.

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## ABBREVIATIONS

Most of the abbreviations used are standard. However, attention is drawn to the following:

$\mu\text{g}$	microgram
$\mu\text{l}$	microlitre
$\mu\text{m}$	micrometer
A	adenine (in DNA nucleotide sequence)
Ac	ampicillin
Ac <sup>R</sup>	ampicillin resistant or resistance
bp	base pair
BPB	bromophenol blue
C	cytosine (in DNA nucleotide sequence)
Cm	chloramphenicol
Cm <sup>R</sup>	chloramphenicol resistant or resistance
CsCl	cesium chloride
Ctm	cotrimoxazole
Ctm <sup>R</sup>	cotrimoxazole resistant or resistance
dATP	deoxyadenosine 5'-triphosphate
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetate
EtBr	ethidium bromide
g	gram
G	guanine (in DNA nucleotide sequence)
hr	hour
int	integron or integrase
IR	inverted repeats
IS	insertion sequence
kb	kilobase pair or kilobase
Km	kanamycin



Km <sup>R</sup>	kanamycin resistant or resistance
LB	Luria-Bertani
M	molar
mA	milliampere
mg	milligram
MH	Mueller Hinton
min	minute
ml	millilitre
mM	millimolar
MW	molecular weight
N	Normal
ng	nanogram
nm	nanometer
°C	degree Celsius
OD	optical density
ORFS	open reading frame
<i>ori</i>	origin
P	promoters
p.s.i	pounds per square inch
PEG	polyethylene glycol
rec	recombination
RF	replicative form (of M13 DNA)
RHSs	recombination hot spots
rpm	revolutions per minute
SDS	sodium dodecyl sulphate
sec	second
Sm	streptomycin
Sm <sup>R</sup>	streptomycin resistant or resistance
Smz	sulphamethoxazole
Smz <sup>R</sup>	sulphamethoxazole resistant or resistance
SSC	sodium chloride-sodium citrate
Su	sulfonamide
Su <sup>R</sup>	sulfonamide resistant or resistance

T	thymine (in DNA nucleotide sequence)
TBE	Tris-borate-EDTA
Tc	tetracycline
Tc <sup>R</sup>	tetracycline resistant or resistance
TE	Tris-EDTA
TEMED	N,N,N',N' - tetramethylenediamine
TGE	transposable genetic element
Tn	transposon
Tp	trimethoprim
Tp <sup>R</sup>	trimethoprim resistant or resistance
Tris	Tris (hydroxymethyl) methylamine
UV	ultraviolet
V	volt
W	watt

# LIST OF CONTENTS

	<b><u>Page</u></b>
<b>ABSTRACT</b>	i
<b>ABSTRAK</b>	iii
<b>ACKNOWLEDGEMENTS</b>	v
<b>ABBREVIATIONS</b>	vi
<b>LIST OF CONTENTS</b>	ix
<b>LIST OF FIGURES</b>	xvi
<b>LIST OF TABLES</b>	xxiii
<b>CHAPTER ONE: INTRODUCTION</b>	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
<b>CHAPTER TWO: MATERIALS AND METHODS</b>		<b>39</b>
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 <sup>4</sup> <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 <sup>R</sup> 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 <sup>R</sup> genes	59
2.19.3	Preparation of competent <i>E. coli</i> DH5 $\alpha$ 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 <sup>R</sup> genes	60
2.19.5	Transfection of <i>E. coli</i> DH5 $\alpha$ 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 <sup>R</sup> 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 <sup>R</sup> gene(s) to a smaller fragment	67
2.22.3	Flowchart of experiments to subclone the Sm <sup>R</sup> gene(s) into M13mp18 vector, sequence the Sm <sup>R</sup> gene(s), and analyses of the nucleotide sequences	68
<b>CHAPTER THREE: RESULTS</b>		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 <sup>R</sup> gene(s)	77
3.6	Plasmid profiles of Ac <sup>R</sup> Sm <sup>R</sup> 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 <sup>R</sup> DNA fragment from pSR3	85
3.11	Plasmid profiles of the Ac <sup>R</sup> Sm <sup>R</sup> 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 <sup>R</sup> 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 <sup>R</sup> 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
<b>CHAPTER FOUR: DISCUSSION AND CONCLUSION</b>		124
4.1	Discussion	124
4.1.1	Localization of the Sm <sup>R</sup> genes	124
4.1.2	Southern hybridization	125
4.1.3	Nucleotide sequences of the 2.5 kb Sm <sup>R</sup> genes	125
4.1.4	IS26-like segment	127
4.2	Conclusion	128
<b>REFERENCES</b>		132



## LIST OF FIGURES

		<u>Page</u>
Figure 1	: Structure of streptomycins	8
Figure 2	: Chemical structures of the aminoglycosides	9
Figure 3	: Action of aminoglycoside-inactivating enzymes	11
Figure 4	: A schematic diagram displaying the possible route of acquisition of antibiotic resistance genes by bacteria under the selective pressure of antibiotics used	15
Figure 5	: Evolutionary relationships of Tn21 and Tn21-related transposons	20
Figure 6	: Two modes of transposition operative in bacteria	22
Figure 7	: Physical maps showing the regions common to Tn21, R46, and R388	24
Figure 8	: Structure of relevant Km <sup>R</sup> transposons	26
Figure 9	: Sequence alignment of the termini of IS26R, IS1, and IS102/IS903	28
Figure 10	: General structure of <i>sull</i> -associated integrons	30
Figure 11	: General structure of integrons	32
Figure 12	: Cloning vectors M13mp18 and M13mp19	34
Figure 13	: The orientation of inserted DNA fragments harbouring antibiotic resistance gene(s) in M13mp18 36	
Figure 14	: Summary of the principles of the ECL Direct Nucleic Acid Labelling and Detection System	57
Figure 15	: Principles of the C-test	62
Figure 16	: Ethidium bromide-stained 0.5% (w/v) agarose gel of pKan and pCLS55	70
Figure 17	: Ethidium bromide-stained 0.7% (w/v) agarose gel of <i>Sal</i> I-digested pKan and <i>Sal</i> I-digested pCLS55	71
Figure 18	: Ethidium bromide-stained 0.7% (w/v) agarose gel of pCLS55 digested with various restriction	

	endonucleases	72
Figure 19	: Ethidium bromide-stained 0.8% (w/v) agarose gel of <i>Pst</i> I-digested pKan and <i>Pst</i> I-digested pCLS55	74
Figure 20	: The restriction map of pKan (after Micklos and Freyer, 1990)	75
Figure 21	: The physical map of pCLS55	76
Figure 22	: Ethidium bromide-stained 0.8% (w/v) agarose gel of <i>Pst</i> I-digested pCLS55 and <i>Pst</i> I-digested pUC19	78
Figure 23	: Ethidium bromide-stained 0.5% (w/v) agarose gel of ccc pUC19, pCLS55, and pUC19 recombinants extracted by the alkaline lysis method of Birnboim (1983) from <i>E. coli</i> DH5 $\alpha$ transformants	81
Figure 24	: Ethidium bromide-stained 0.7% (w/v) agarose gel of <i>Pst</i> I-digested recombinant plasmids isolated from 10 Ac <sup>R</sup> Sm <sup>R</sup> transformants	82
Figure 25	: Strategy used to subclone and locate the DNA fragment harbouring the Sm <sup>R</sup> gene(s)	83
Figure 26	: Ethidium bromide-stained 0.5% (w/v) agarose gel of <i>Sa</i> I-digested recombinant plasmids isolated from 10 Ac <sup>R</sup> Sm <sup>R</sup> transformants	84
Figure 27	: Construction of pSR3 and pSR4	86
Figure 28	: Ethidium bromide-stained 0.5% (w/v) agarose gel of ccc pUC19, pSR3, and recombinant plasmids extracted from <i>E. coli</i> DH5 $\alpha$ transformants harbouring circularized 5.2 kb <i>Sa</i> I fragment from pSR3 (pSR3a-e)	89
Figure 29	: Ethidium bromide-stained 0.5% (w/v) agarose gel of <i>Sa</i> I-digested recombinant pUC19 derived from circularized 5.2 kb <i>Sa</i> I fragment from pSR3 (pSR3a-e)	90
Figure 30	: Ethidium bromide-stained 0.7% (w/v) agarose gel of <i>Eco</i> RI-digested recombinant pUC19 (pSR3a-e) derived from circularized 5.2 kb <i>Sa</i> I fragment from pSR3	91

Figure 31	:	Ethidium bromide-stained 0.6% (w/v) agarose gel of gel-eluted <i>EcoRI</i> -digested M13mp18 and the 2.5 kb <i>EcoRI</i> fragment harbouring Sm <sup>R</sup> gene(s) from pSR3a	93
Figure 32	:	Ethidium bromide-stained 0.6% (w/v) agarose gel of recombinant M13mp18 RF DNA exhibiting Sm <sup>R</sup> and Sm <sup>S</sup> phenotypes	97
Figure 33	:	Ethidium bromide-stained 0.6% (w/v) agarose gel of <i>EcoRI</i> -digested recombinant M13mp18 RF DNA	98
Figure 34	:	Ethidium bromide-stained 0.6% (w/v) agarose gel of recombinant M13 after the C-test	99
Figure 35	:	Construction of M13pSMR13 and M13pSMS16	101
Figure 36	:	Hybridization with the 2.5 kb <i>EcoRI</i> -digested fragment probe	102
Figure 37	:	Ethidium bromide-stained 0.7% (w/v) agarose gel showing the single-stranded DNA of M13mp18 (lane 1), M13pSMR13 (lane 2), and M13pSMS16 (lane 3)	103
Figure 38	:	Complete nucleotide sequence obtained by using M13pSMR13 as the template and three primers: the M13 universal primer and two oligonucleotide primers, R13 5'-CGTCCGCCATCTGTGCAATGCGTC-3' (nucleotide positions 690 to 713 in M13pSMR13) and R132 5'-GCGAAGGCGCGCTCTGCTTCATCT-3' (nucleotide positions 1456 to 1479 in M13pSMR13)	105
Figure 39	:	Complete nucleotide sequence obtained by using M13pSMS16 as the template and three primers: the M13 universal primer and two oligonucleotide primers, R16 5'-CGGCTCGGAACAGCAGATCGCTAT-3' (nucleotide positions 708 to 731 in M13pSMS16) and R162 5'-GAAGGCGCGCTCTGCTTCATCT-3' (nucleotide positions 1246 to 1269 in M13pSMS16)	107
Figure 40:		Comparison of the M13pSMR13 (top sequence) and M13pSMS16 (below sequence) nucleotides	109
Figure 41:		Complete nucleotide sequence of the 2.5 kb <i>EcoRI</i>	

	fragment (represented by the characters below the dots shown in the 5'- to 3'-orientation)	113
Figure 42	: (A) and (B) show the 2.5 kb <i>EcoRI</i> fragment harboured by M13pSMR13 and M13pSMS16, respectively	117
Figure 43	: Ethidium bromide-stained 0.5% (w/v) agarose gel of ccc pUC19 and recombinant pUC19 (with 5.5 kb <i>SaII</i> -digested fragment inserted into <i>SaII</i> -digested pUC19) from <i>E. coli</i> transformants extracted by the alkaline lysis method of Birnboim (1983)	119
Figure 44	: Ethidium bromide-stained 0.6% (w/v) agarose gel of <i>SaII</i> -digested recombinant plasmids isolated from Ac <sup>R</sup> Sm <sup>R</sup> transformants	121
Figure 45	: Partial nucleotide sequence from pSR55 by using the M13 universal primer	122
Figure 46	: Proposed genetic organization of the 5.5 kb <i>SaII</i> fragment from pCLS55	129

## LIST OF TABLES

	<b><u>Page</u></b>
Table 1 : List of antibiotics	41
Table 2 : Enzymes used in this study	41
Table 3 : Antibiotic stock solutions	44
Table 4 : Protocols for ligation of the <i>Pst</i> I digested pCLS55 and pUC19	59
Table 5 : Protocols for ligation of the 2.5 kb <i>Eco</i> RI DNA fragment harbouring the Sm <sup>R</sup> genes with <i>Eco</i> RI- digested M13mp18	64
Table 6 : Fragments produced by single or double restriction endonuclease digestions of pCLS55	73
Table 7 : Results of the shotgun cloning of the four DNA fragments (5.75, 2.23, 0.92, and 0.8 kb) obtained from <i>Pst</i> I-digested pCLS55 into pUC19	79
Table 8 : Results of the subcloning of the Sm <sup>R</sup> DNA fragment from pSR3 and pSR4	87
Table 9 : Results of ligation of the 2.5 kb <i>Eco</i> RI fragment from pSR3a with <i>Eco</i> RI-digested RF DNA of M13mp18	94
Table 10 : Results showing the number of colonies growing on LB agar plates containing Sm after an overnight incubation at 37°C of cells from 30 randomly selected plaques/ colonies from tube A or B	95
Table 11 : Results of the subcloning of the 5.5 kb <i>Sal</i> I fragment from pCLS55 into pUC19	118