

LIST OF FIGURES AND TABLES

CHAPTER 1

Figure 1. 1: Targets of antibiotics.....	2
Figure 1. 2: Multidrug-resistance efflux pumps.....	5
Figure 1. 3: Schematic diagram of synthesis and degradation of (p)ppGpp.....	9
Figure 1. 4: A “hop” model for mechanism of RelA mediated (p)ppGpp synthesis.....	13
Figure 1. 5: A switch model for ACP mediated ppGpp synthesis from SpoT.	15
Figure 1. 6: ppGpp and its’ effect on global gene expression.....	16
Figure 1. 7: Structure of the RNAP core enzyme with the main channel.....	18

CHAPTER 3

Table 3. 1: List of microbial strains used in the study.....	37
Table 3. 2: List of plasmids and constructs used in the study.....	38
Table 3. 3: Antibiotics used in the study.....	40
Table 3. 4: List of Primers used in PCR studies.....	41
Table 3. 5: List of miscellaneous reagents.....	45
Table 3. 6: PCR Programme.....	48

CHAPTER 4

Figure 4. 1: Restriction map of pTE1 plasmid.....	47
Figure 4. 2: Confirmation of <i>relA</i> and/or <i>rumA</i> clones by different restriction enzymes.....	49
Figure 4. 3: Genomic Nucleotide Coordinates of the <i>relA rumA</i> DNA in pTE1 and pTE2.....	50
Figure 4. 4: Recapitulation of multiple tolerance phenotype of <i>relA</i> and <i>rumA</i> DNA.....	51
Figure 4. 5: Schematic diagram of <i>relA</i> SDM mutagenesis method.	52
Figure 4. 6: A multiple antibiotic tolerance requires functional <i>relA</i>	53
Table 4. 1: <i>Escherichia coli</i> strains/Plasmids used in this study.....	45
Table 4. 2: List of Primers used in this work.....	46
Table 4. 3: MIC of various constructs for different antibiotics.....	54

CHAPTER 5

Figure 5. 1: Comparing the effect of exchange of <i>spoT</i> allele between <i>relA</i> ⁺	64
--	----

Table 5. 1: Bacterial strains and plasmids used in this chapter.....	60
Table 5. 2: Doubling time and growth rate of different mutants.....	65
Table 5. 3: Comparison of <i>relA1</i> and <i>relA2009</i> on 3-AT/SMG supplemented media.....	66

CHAPTER 6

Figure 6. 1: Arrangement on <i>E coli</i> chromosome of <i>relA</i> , <i>rumA</i> and <i>barA</i> genes.....	71
Figure 6. 2: Confirmation of clones by <i>SalI</i> restriction enzyme digestion.....	76
Figure 6. 3: Confirmation of <i>CAT</i> insertion in the plasmid by <i>EcoRI</i> restriction digestion.	78
Figure 6. 4: Comparison between wild type <i>rumA</i> and $\Delta rumA1$ DNA sequence	84
Figure 6. 5: Construction of chromosomal $\Delta rumA1$ mutant.	85
Figure 6. 6: Analysis of genomic DNA by PCR using RumAF1-RumARPst1 primers.	86
Figure 6. 7: Serine hydroxamate sensitivity of <i>relA2009</i> mutant.....	88
Figure 6. 8: Confirmation of <i>CAT</i> cassette insertion in <i>rumA</i> and <i>barA</i>	91
Figure 6. 9: Determination of orientation of <i>CAT</i> insertion in <i>rumA</i> gene at <i>SalI</i> and <i>MluI</i>	92
Figure 6. 10: Slow growth phenotype of <i>rumASalI::CAT</i> (KP8) mutant.....	93
Figure 6. 11: Growth rate measurement of <i>rumASalI::CAT</i> mutant and other derivatives. -	95
Figure 6. 12: Swapping of <i>spoT</i> alleles between MC4100 and MG1655 strains.	98
Figure 6. 13: Assessment of <i>relA</i> phenotype.	101
Figure 6. 14: Growth of suppressors of <i>rumASalI::CAT</i> mutant.....	102
Figure 6. 15: Complementation of <i>rumASalI::CAT</i> mutant by pKC1 plasmid.....	103
Figure 6. 16: Quantification of RelA protein of KP8 (<i>rumASalI::CAT</i>) mutant.....	106
Figure 6. 17: Measurement of RelA protein in pKC1 transformants of KP8.	107
Figure 6. 18: <i>relA</i> mRNA transcript quantitated by RT-PCR.	108
Figure 6. 19: Cloned <i>spoT</i> ⁺ gene suppresses slow growth defect of KP8.	109
Figure 6. 20: Analysis of pTE18 by restriction enzymes.	110
Figure 6. 21: Ability of the derivatives of pTE18 (<i>spoT</i> ⁺ in pBR322) plasmid	111
Figure 6. 22: Confirmation of <i>spoT</i> gene in pTE18 by PCR.....	111
Figure 6. 23: $\Delta dksA$ <i>rumASalI::CAT</i> double mutants do not form colonies.	113

Figure 6. 24: Quantification of RelA protein in <i>dksA rumASall::CAT</i> mutants.-----	114
Figure 6. 25: Effect of $\Delta rpoZ::Kan$ mutation.-----	115
Figure 6. 26: $\Delta rpoS::KAN$ mutation effect on growth of KP8 (<i>rumASall::CAT</i>).-----	117
Figure 6. 27: Effect of <i>rif^R</i> mutation in <i>rpoB</i> on growth of KP8 (<i>rumASall::CAT</i>). -----	118
Table 6. 1: Some examples of methylated nucleotides in rRNAs	70
Table 6. 2: Strains and plasmids used in this chapter.....	73
Table 6. 3: List of Primers used in this work.....	75
Table 6. 4: Growth competition between wildtype and $\Delta rumA1$ mutant	87
Table 6. 5: Doubling time and growth rate of MC4100 and its derivatives.....	96
Table 6. 6: Doubling time and growth rate of different mutants.....	99