

**Identification and characterisation of toxin-antitoxin systems  
(TA) in *Burkholderiapseudomallei***

Submitted by Aaron Trevor Butt to the University of Exeter  
as a thesis for the degree of  
Doctor of Philosophy in Biological Sciences  
In February 2013

This thesis is available for Library use on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: .....

## Abstract

The aim of this study was to identify and characterise type II toxin-antitoxin (TA) systems in *Burkholderiapseudomallei*, the causative agent of the human disease melioidosis.

8 putative TA systems were identified within the genome of *B. pseudomallei*K96243. 5 of these were located within genome islands. Of the candidate toxins, BPSL0175 (RelE1) or BPSS1060 (RelE2) caused growth to cease when expressed in *Escherichia coli*, whereas expression of BPSS0390 (HicA) or BPSS1584 (HipA) (in an *E. coli*  $\Delta$ hipBA background) caused a reduction in the number of culturable bacteria. HicA also caused growth arrest in *B. pseudomallei*K96243 $\Delta$ hicAB. These toxin induced phenotypes were enhanced by an <3kDa extracellular factor that accumulated in the spent medium during growth. Expression of the cognate antitoxins could restore growth and culturability of cells.

Expression of *hicA* in *E. coli* gave an increased number of persister cells in response to ciprofloxacin or ceftazidime. Site directed mutagenesis studies identified two key residues within the HicA toxin that were essential for both the reduced culturability and increased persistence phenotypes. Deletion of *hicAB* from *B. pseudomallei*K96243 did not affect persister cell or survival frequencies compared to the wild type following treatment with a variety of stress conditions.

Deletion of the  $\Delta$ hipBA locus from *B. pseudomallei* K96243 also had no effect on bacterial persistence or survival under the conditions tested.

<b>Contents</b>	<b>Page Number</b>
<b>Title page</b>	<b>1</b>
<b>Abstract</b>	<b>3</b>
<b>List of contents</b>	<b>4</b>
<b>List of figures</b>	<b>12</b>
<b>List of tables</b>	<b>15</b>
<b>Publications and posters</b>	<b>16</b>
<b>Declaration</b>	<b>17</b>
<b>Acknowledgements</b>	<b>18</b>
<b>Abbreviations</b>	
<b><u>19Chapter 1- Introduction</u></b>	
<b>1.0 <i>Burkholderiapseudomallei</i></b>	<b>23</b>
1.0.1 Genome	23
1.0.2 Virulence factors	27
1.0.2.1 Secretion systems	27
1.0.2.2 Adhesion	27
1.0.2.3 Flagella	28
1.0.2.4 Quorum sensing	28
1.0.2.5 Polysaccharides	28
1.0.2.6 Secreted factors	29
1.0.3 Antibiotic resistance and phenotypic tolerance	30
<b>1.1 Melioidosis</b>	<b>31</b>
1.1.1 Risk factors	31
1.1.2 Clinical features	32
1.1.3 Diagnosis	34
1.1.4 Treatment	34
1.1.5 Intracellular survival of <i>B. pseudomallei</i> .	35
1.1.6 Immune response	37
1.1.7 Vaccines	37
1.1.7.1 Live attenuated vaccines	38
1.1.7.2 Subunit vaccines	38

<b>1.2 Persister cells</b>	<b>39</b>
1.2.1 Eradication of persisters	42
<b>1.3 Toxin-antitoxin (TA) modules</b>	<b>45</b>
1.3.1 Background	45
1.3.2 Toxin-antitoxin structure	46
1.3.2.1 Type I	48
1.3.2.2 Type II	49
1.3.2.3 Type III	50
1.3.2.4 Type V	51
1.3.3 Targets of Type II TA toxins	52
1.3.3.1 DNA replication	52
1.3.3.2 Ribosome dependent mRNA interferases	52
1.3.3.3 Ribosome independent mRNA interferases	54
1.3.3.4 Ribosome inhibition	58
1.3.3.5 Cell division	58
1.3.3.6 Other targets	59
<b>1.4 Structural relationship of toxin and antitoxins</b>	<b>59</b>
1.4.1 Antitoxins	59
1.4.2 Toxins	61
<b>1.5 Species distribution of TA systems</b>	<b>63</b>
1.5.1 Distribution in <i>Burkholderia sp.</i>	65
<b>1.6 Aims of this study</b>	<b>66</b>
<b><u>Chapter 2- Materials and methods</u></b>	
<b>2.0 Bacterial strains</b>	<b>68</b>
<b>2.1 Culture media</b>	<b>68</b>
<b>2.2 Bacterial storage</b>	<b>68</b>
2.2.1 Freezer storage	68
2.2.2 Fridge storage	68
<b>2.3 Bioinformatic screening</b>	<b>70</b>
2.3.1 RASTA bacteria	70
2.3.2 Other data sources	70

<b>2.4 Molecular biology</b>	<b>70</b>
2.4.1 Polymerase chain reaction (PCR)	70
2.4.2 Gel electrophoresis	72
2.4.3 PCR purification	72
2.4.4 Determining DNA concentration	72
2.4.5 Digest of DNA using restriction enzymes	72
2.4.6 Extraction of digested DNA fragments and plasmids	73
2.4.7 Ligation of digested vector to insert DNA	73
2.4.8 Gateway vector cloning	74
2.4.9 Plasmid extraction	74
2.4.10 Chromosomal DNA extraction	74
2.4.11 Sequencing of PCR products and plasmid DNA	74
<b>2.5 Competent cells</b>	<b>75</b>
2.5.1 Electrocompetent cells	75
2.5.2 Calcium competent cells	75
<b>2.6 Electroporation</b>	<b>76</b>
<b>2.7 Transformation</b>	<b>76</b>
<b>2.8 Wanner mutagenesis</b>	<b>77</b>
<b>2.9 Conjugation</b>	<b>77</b>
<b>2.10 Toxicity assays with <i>E. coli</i> harbouring cloned toxin genes</b>	<b>78</b>
2.10.1 Expression at different cell densities	79
2.10.2 Preparing <i>E. coli</i> cultures at high or low cell densities	79
2.10.3 Preparing stationary phase spent media	79
2.10.4 Preparing exponential phase spent media	80
2.10.5 Acid treatment of spent media	80
2.10.6 Heat treatment of spent media	80
2.10.7 Fractionation of spent media	81
<b>2.11 Co-expression assays</b>	<b>81</b>
<b>2.12 Resuscitation assays</b>	<b>81</b>
<b>2.13 Live/dead staining</b>	<b>82</b>
<b>2.14 Minimum inhibitory concentration determination</b>	<b>83</b>
<b>2.15 Persister assays</b>	<b>83</b>

2.15.1 Stationary phase cultures	83
2.15.2 <i>E. coli</i> cultures expressing BPSS0390	84
2.15.3 <i>E. coli</i> cultures expressing BPSS0390 at different densities	85
<b>2.16 Deletion of <i>B. pseudomallei</i> TA loci</b>	<b>85</b>
<b>2.17 Expression of TA toxins in <i>Burkholderia</i></b>	<b>88</b>
2.17.1 Expression in <i>B. thailandensis</i>	88
2.17.2 Expression in <i>B. pseudomallei</i>	88
2.17.3 Expression in <i>B. pseudomallei</i> $\Delta$ BPSS0390-0391	89
2.17.4 Isolation of <i>B. pseudomallei</i> K96243 $\Delta$ BPSS0390- 0391 spent media	89
2.17.5 Expression in spent media	89
<b>2.18 Hydrogen peroxide stress assay</b>	<b>90</b>
<b>2.19 Heat stress experiments</b>	<b>91</b>
<b>2.20 pH stress experiments</b>	<b>91</b>
<b>2.21 Cadmium sulphate assay</b>	<b>91</b>
2.21.1 Cadmium in LB broth	91
2.21.2 Cadmium in LB agar	92
<b>2.22 Site directed mutagenesis</b>	<b>92</b>
<b>2.23 Protein gels</b>	<b>93</b>
<b>2.24 Western blots</b>	<b>93</b>
<b>2.25 Large scale protein expression</b>	<b>94</b>
2.25.1 Zym-5052 media	94
2.25.2 <sup>15</sup> N labelled N-5052 media	95
2.25.3 <sup>15</sup> N and <sup>13</sup> C N-5052 media	95
<b>2.26 Protein extraction</b>	<b>96</b>
2.26.1 Large scale expression	96
2.26.2 Small scale protein expression	96
<b>2.27 Protein extraction of histidine tagged proteins</b>	<b>97</b>
2.27.1 Affinity chromatography	97
2.27.2 De-salting columns	97
2.27.3 Enterokinase digestion	97
2.27.4 Concentrating protein	98

2.27.5 Size exclusion chromatography	98
2.27.6 Determining protein concentration	98
<b>2.28 Circular Dichroism</b>	<b>98</b>
<b>2.29 Crystallography</b>	<b>99</b>
<b>2.30 EMSA</b>	<b>99</b>
<b>2.31 RNase assay</b>	<b>100</b>
<b>2.32 Pull down assays</b>	<b>100</b>
<b>2.33 Stabilisation experiment</b>	<b>101</b>
<b>2.34 NMR</b>	<b>101</b>
<b><u>Chapter 3- Identification of type II TA systems in <i>B. pseudomallei</i></u></b>	
<b>3.0 Introduction</b>	<b>104</b>
3.0.1 Aims	105
<b>3.1 Bioinformatic screening</b>	<b>106</b>
3.1.1 RASTA-bacteria	106
3.1.1.1 Validation	106
3.1.1.2 Screening publicly available <i>B. pseudomallei</i> genomes	106
3.1.2 TA predictions and comparisons with other data sources	109
3.1.3 Distribution of candidate <i>B. pseudomallei</i> K96243 TA genes in assembled or partially assembled <i>B. pseudomallei</i> strains	112
3.1.4 Genomic location of candidate <i>B. pseudomallei</i> K96243 TA genes	114
3.1.5 Distribution of <i>B. pseudomallei</i> K96243 putative TA in <i>B. mallei</i> and <i>B. thailandensis</i>	114
3.1.6 BLAST searching <i>B. pseudomallei</i> K96243 TA candidates	116
<b>3.2 Microarray data</b>	<b>119</b>
3.2.1 Growth phase data	119
3.2.2 BALB/C mouse infection data	119
3.2.3 Hamster infection	120
3.2.4 NaCl treatment	120
3.2.5 Macrophage infection	120
3.2.6 Growth in iron	120
<b>3.3 Expression of putative <i>B. pseudomallei</i> toxins genes in <i>E. coli</i></b>	<b>121</b>
3.3.1 Expression of <i>hipA</i> <sub><i>E.coli</i></sub> in MG1655	121

3.3.2 Expression of <i>hipA</i> and <i>hipA</i> -his tag in <i>E. coli</i> MG1655 $\Delta$ <i>hipBA</i>	124
3.3.2.1 Creating $\Delta$ <i>hipBA</i> :: <i>Cm<sup>R</sup></i>	124
3.3.2.2 Expression of the pBAD-his- <i>hipA</i> and pBAD- <i>hipA</i> constructs in <i>E. coli</i> MG1655 $\Delta$ <i>hipBA</i> :: <i>Cm<sup>R</sup></i>	124
3.3.3 Expression of the 8 putative <i>B. pseudomallei</i> K96243 toxin genes in MG1655	127
3.3.4 Monitoring CFU following induction of BPSS1060, BPSL0175 and BPSS0390	130
3.3.5 Colony size following BPSS1060, BPSL0175 and BPSS0390 expression	131
3.3.6 Screening for homologs of the putative K96243 TA genes in <i>E. coli</i> MG1655	134
3.3.7 Expression of BPSS1584 in <i>E. coli</i> MG1655 $\Delta$ <i>hipBA</i>	134
<b>3.4 Expression of partner antitoxin genes</b>	<b>136</b>
3.4.1 Co-expression of toxin and antitoxin partner gene	136
3.4.2 Co-expression of different toxin and antitoxin families	140
3.4.2.1 Co-expression of BPSS0390 and BPSL0174	140
3.4.3 Resuscitation of growth by antitoxin	140
<b>3.5 Discussion</b>	<b>144</b>
<b><u>Chapter 4- Characterisation of BPSL0175 (RelE2) toxin activity</u></b>	
<b>4.0 Introduction</b>	<b>149</b>
4.0.1 Aims	150
<b>4.1 Expression of BPSL0175 at different cell densities</b>	<b>151</b>
<b>4.2 Expression of BPSL0175 in spent media</b>	<b>151</b>
4.2.1 Empty pBAD and LacZ controls	151
4.2.2 BPSL0175 expression	153
<b>4.3 Discussion</b>	<b>156</b>
<b><u>Chapter 5- Characterisation of the BPSS1583-1584 (HipBA) system</u></b>	
<b>5.0 Introduction</b>	<b>159</b>
5.0.1 Aims	161
<b>5.1 Phenotypic characterisation following BPSS1584 toxin expression</b>	<b>162</b>



5.1.1 Live/Dead screening following BPSS1584 expression in <i>ΔhipBA</i>	162
5.1.1.1 Fluorescence microscope imaging	162
5.1.1.2 Fluorescence plate reader	162
5.1.2 Expression of BPSS1584 at different cell densities	164
5.1.3 Expression of BPSS1584 in spent media	167
5.1.4 Expression of BPSS1584 in fractionated spent media	167
5.1.5 Expression of BPSS1584 in acid treated spent media	169
<b>5.2 Expression of BPSS1584 in <i>Burkholderiapseudomallei</i>K96243</b>	<b>172</b>
5.2.1 Generation of expression construct	172
<b>5.3 Characterisation of the <i>B. pseudomallei</i>K96243 <i>ΔhipBA</i> mutant</b>	<b>173</b>
5.3.1 Transcriptomics data	173
5.3.2 Heat stress assay	177
5.3.3 Persister assay with ciprofloxacin and ceftazidime	177
5.3.4 Acid stress	177
<b>5.4 Discussion</b>	<b>181</b>
<b><u>Chapter 6- Characterisation of the BPSS0390-0391 (HicAB) system</u></b>	
<b>6.0 Introduction</b>	<b>186</b>
6.0.1 Aims	187
<b>6.1 Phenotypic characterisation following BPSS0390 toxin expression in <i>E. coli</i> MG1655</b>	<b>188</b>
6.1.1 Live/Dead screening following BPSS0390 expression	188
6.1.2 Expression of BPSS0390 at different cell densities	188
6.1.3 Expression of BPSS0390 in spent media	191
6.1.3.1 Expression in exponential phase media	191
6.1.3.2 Comparing BPSS0390 expression at low density in different aged media	191
6.1.4 Expression of BPSS0390 in fractionated media	195
6.1.5 Heat treatment of spent media pre BPSS0390 expression	195
6.1.6 Acid treatment of spent media before BPSS0390 expression	197
6.1.7 Expression of BPSS0390 in <i>E. coli</i> with <i>B. thailandensis</i> spent media	197
6.1.8 Antibiotic treatment of <i>E. coli</i> MG1655 expressing BPSS0390	200

6.1.8.1 Ciprofloxacin	200
6.1.8.2 Ciprofloxacin at different cell densities	200
6.1.8.3 Ceftazidime	203
<b>6.2 Expression of BPSS0390 in <i>Burkholderia</i></b>	<b>205</b>
6.2.1 Creation and testing of the pScRhaB3-BPSS0390 construct	205
6.2.2 Expression in <i>B.thailandensis</i> E264	205
6.2.3 Expression in <i>B. pseudomallei</i> K96243	206
6.2.4 <i>B. pseudomallei</i> K96243 $\Delta$ BPSS0390-0391	209
6.2.4.1. Creation and confirmation of $\Delta$ BPSS0390-0391 mutant	209
6.2.4.2 Expression of BPSS0390 in $\Delta$ BPSS0390-0391	212
6.2.5 Expression of BPSS0390 in <i>B. pseudomallei</i> $\Delta$ BPSS0390-0391 grown in spent media	214
6.2.6 Expression of BPSS0390 in <i>B. pseudomallei</i> $\Delta$ BPSS0390-0391 with <i>E. coli</i> spent media	216
<b>6.3 Characterisation of <i>B. pseudomallei</i>K96243 <math>\Delta</math>BPSS0390-0391</b>	<b>218</b>
6.3.1 Persister assay with ciprofloxacin	218
6.3.2 Ciprofloxacin persister assay on different aged cultures	218
6.3.3 Transcriptomics data	221
6.3.4 Hydrogen peroxide stress	221
6.3.5 Cadmium sulphate stress	224
<b>6.4 Discussion</b>	<b>226</b>
<b><u>Chapter 7- Functional and structural characterisation of the BPSS0390 and BPSS0391 proteins</u></b>	
<b>7.0 Introduction</b>	<b>232</b>
7.0.1 Aims	233
<b>7.1 Identification of key BPSS0390 residues</b>	<b>234</b>
7.1.1 Conservation of residues in homologous proteins	234
7.1.2 Mapping key residues on the predicted BPSS0390 structure	234
7.1.3 Mutagenesis of potential key residues	237
7.1.3.1 His tagging the BPSS0390 toxin and screening toxicity phenotype	237
7.1.3.2 Site directed mutagenesis	237
7.1.4 Expression of BPSS0390 mutants in <i>E. coli</i> MG1655	239

7.1.4.1 Toxicity assay	239
7.1.4.2 Western blots to check expression of H24A and G22C	241
7.1.5 Co-expression of G14C, S23A or P41A with BPSS0391	241
7.1.6 Persister assays on <i>E. coli</i> MG1655 expressing BPSS0390 mutants	244
7.1.6.1 Ciprofloxacin	244
7.1.6.2 Ceftazidime	246
<b>7.2 Structure determination</b>	<b>248</b>
7.2.1 Purification of recombinant protein	248
7.2.1.1 His tagged BPSS0390	248
7.2.1.2 His tagged H24A mutant	248
7.2.2 Crystallisation trials	252
7.2.3 CD spectrophotometry	252
7.2.4 Stabilisation experiments	255
7.2.5 NMR	255
7.2.5.1 Sample preparation	255
7.2.5.2 NOE assignment	256
<b>7.3 The BPSS0391 antitoxin and BPSS0390-0391 complex</b>	<b>258</b>
7.3.1 Identification of key BPSS0391 residues	258
7.3.2 Expression and purification of the BPSS0390-0391 complex	258
7.3.3 Western blots following co-expression of BPSS0391 with G14C, P41A and S23G	260
7.3.4 Expression and purification of BPSS0391	263
7.3.5 Pull down assay	265
<b>7.4 RNA binding properties of the BPSS0390 toxin</b>	<b>267</b>
<b>7.5 Binding of BPSS0391<sup>his</sup> and BPSS0391 to DNA</b>	<b>267</b>
<b>7.6 Binding various concentrations of BPSS0390<sup>H24A</sup> to DNA</b>	<b>270</b>
<b>7.7 Discussion</b>	<b>272</b>
<b><u>Chapter 8- Final Discussion and conclusions</u></b>	<b>277</b>
<b>References</b>	<b>283</b>
<b>Appendix</b>	<b>300</b>

## List of figures

## Page number

### Chapter 1

<b>Figure 1.0</b> The phylogeny of the <i>Burkholderia</i> genus	24
<b>Figure 1.1</b> Heat map of Genomic Island distribution in <i>B. pseudomallei</i> strains	26
<b>Figure 1.2</b> World distribution of melioidosis	33
<b>Figure 1.3</b> <i>B. pseudomallei</i> infection and host response	36
<b>Figure 1.4</b> Persister cells survive antibiotic treatment	40
<b>Figure 1.5</b> Eradication of persister cells	44
<b>Figure 1.6</b> Structure and regulation of TA systems	47
<b>Figure 1.7</b> Possible method for <i>mazEF</i> dependent cell death	57
<b>Figure 1.8</b> Structure of 2 toxin families	62

### Chapter 2

<b>Figure 2.0</b> Generation of gene knockout constructs	87
--	----

### Chapter 3

<b>Figure 3.0</b> Genome location of the 8 putative TA loci and the genomic islands (GI) on which they are located.	115
<b>Figure 3.1</b> Generation of the pBAD expression constructs	122
<b>Figure 3.2</b> Expression of the <i>E. coli hipA</i> gene	123
<b>Figure 3.3</b> Schematic for the Wanner mutagenesis method	125
<b>Figure 3.4</b> Electrophoresis gel confirming <i>E. coli ΔhipBA</i>	126
<b>Figure 3.5</b> Expression of <i>E. coli hipA</i> in <i>E. coli ΔhipBA</i> and the effect on optical density	128
<b>Figure 3.6</b> The OD <sub>590nm</sub> growth profiles of the 8 putative TA toxins	129
<b>Figure 3.7</b> Effect of toxin expression on culturability	132
<b>Figure 3.8</b> Size of <i>E. coli</i> MG1655 following repression or expression of toxin	133
<b>Figure 3.9</b> Effect of BPSS1584 expression on <i>E. coli</i> MG1655 <i>ΔhipBA</i>	135
<b>Figure 3.10</b> Generation of the pME6032 expression constructs	138
<b>Figure 3.11</b> Co-expression of cognate toxin and antitoxin pairs	139
<b>Figure 3.12</b> Co-expression of <i>E. coli</i> MG1655 harbouring pBAD cloned BPSS0390 and pME6032 cloned BPSL0174.	142
<b>Figure 3.13</b> Resuscitation of toxin induced <i>E. coli</i> MG1655 growth by antitoxin	143

### Chapter 4

<b>Figure 4.0</b> Growth profile monitoring the CFU of <i>E. coli</i> MG1655 pBAD-BPSL0175 at different cell densities	149
<b>Figure 4.1</b> The effect of spent media or LB on <i>E. coli</i> growth at 2 different cell densities	154
<b>Figure 4.2</b> The effect of spent media or fresh LB on the CFU change of <i>E. coli</i> MG1655 pBAD-BPSL0175	155

### Chapter 5

<b>Figure 5.0</b> Fluorescent imaging following live/dead staining	<b>163</b>
<b>Figure 5.1</b> Ratio of fluorescence units, after mixing various amounts of test and dead <i>E. coli</i> cells.	<b>165</b>
<b>Figure 5.2</b> Growth profile monitoring the CFU of <i>E. coli</i> MG1655 $\Delta$ <i>hipBA</i> pBAD-BPSS1584 at different cell densities	<b>166</b>
<b>Figure 5.3</b> The effect of spent media or LB on <i>E. coli</i> growth at 2 different cell densities	<b>168</b>
<b>Figure 5.4</b> The effect of fractionated spent media or LB on <i>E. coli</i> growth at 2 different cell densities	<b>170</b>
<b>Figure 5.5</b> The effect of HCl treated spent media on <i>E. coli</i> growth at 2 different cell densities	<b>171</b>
<b>Figure 5.6</b> Methodology for creation of the pBHR-paraBPSS1584BAD plasmid	<b>174</b>
<b>Figure 5.7</b> Growth profiles measuring the optical density at 590nm of pBHR-paraBPSS1584BAD containing bacterial strains	
<b>Figure 5.8</b> Fold change in CFU numbers of <i>B. pseudomallei</i> K96243 and <i>B. pseudomallei</i> K96243 $\Delta$ <i>hipBA</i> heat treated for 2 hours at 65°C	<b>178</b>
<b>Figure 5.9</b> Persister frequency of <i>B. pseudomallei</i> K96243 and <i>B. pseudomallei</i> K96243 $\Delta$ <i>hipBA</i> with 100 x MIC of antibiotic for 24 hours	<b>179</b>
<b>Figure 5.10</b> CFU fold change of <i>B. pseudomallei</i> K96243 and <i>B. pseudomallei</i> $\Delta$ <i>hipBA</i> subjected to pH stress	<b>180</b>

## Chapter 6

<b>Figure 6.0</b> Fluorescent imaging following live/dead staining	<b>189</b>
<b>Figure 6.1</b> Growth profile of BPSS0390 expressing <i>E. coli</i> MG1655 pBAD-BPSS0390 at 3 different cell densities	<b>190</b>
<b>Figure 6.2</b> The effect of exponential media or LB on <i>E. coli</i> growth at 2 different cell densities	<b>192</b>
<b>Figure 6.3</b> The effect of LB, exponential media and stationary spent media on the growth of low density <i>E. coli</i> cultures.	<b>193</b>
<b>Figure 6.4</b> The effect of fractionated spent media or on <i>E. coli</i> growth at 2 different cell densities	<b>195</b>
<b>Figure 6.5</b> The effect of heat treated spent media on <i>E. coli</i> growth at 2 different cell densities	<b>196</b>
<b>Figure 6.6</b> The effect of HCl treated spent media on <i>E. coli</i> growth at 2 different cell densities	<b>198</b>
<b>Figure 6.7</b> The effect of <i>B. thailandensis</i> spent media or LB on <i>E. coli</i> growth at 2 different cell densities	<b>199</b>
<b>Figure 6.8</b> Persister frequency following ciprofloxacin treatment of BPSS0390 repressed or expressed <i>E. coli</i> MG1655 pBAD-BPSS0390 pME6032-BPSS0391 cultures	<b>201</b>
<b>Figure 6.9</b> Persister frequency following ciprofloxacin treatment of BPSS0390 expressed <i>E. coli</i> MG1655 pBAD-BPSS0390 pME6032-BPSS0391 cultures at different cell densities	<b>202</b>
<b>Figure 6.10</b> Persister frequency following ceftazidime treatment of BPSS0390 repressed or expressed <i>E. coli</i> MG1655 pBAD-BPSS0390 pME6032-BPSS0391 cultures	<b>204</b>
<b>Figure 6.11</b> Growth profile of <i>B. thailandensis</i> E264/pSCrhaB3-BPSS0390	<b>207</b>
<b>Figure 6.12</b> Growth profile of <i>B. pseudomallei</i> K96243/pSCrhaB3-BPSS0390	<b>208</b>

<b>Figure 6.13</b> Methodology of BPSS0390-0391 deletion	<b>210</b>
<b>Figure 6.14</b> Gel image of potential <i>ΔBPSS0390-0391</i> mutants following sucrose selection	<b>201</b>
<b>Figure 6.15</b> Growth profiles of <i>B. pseudomallei</i> K96243 <i>ΔBPSS0390-0391</i> /pSCrhaB3-BPSS0390	<b>213</b>
<b>Figure 6.16</b> The effect of spent media or LB on growth of <i>B. pseudomallei</i> K96243 <i>ΔBPSS0390-0391</i> /pSCrhaB3 or <i>B. pseudomallei</i> K96243 <i>ΔBPSS0390-0391</i> /pSCrhaB3-BPSS0390 at 2 different cell densities	<b>215</b>
<b>Figure 6.17</b> The effect of <i>E. coli</i> spent media or LB on growth of <i>B. pseudomallei</i> K96243 <i>ΔBPSS0390-0391</i> /pSCrhaB3 or <i>B. pseudomallei</i> K96243 <i>ΔBPSS0390-0391</i> /pSCrhaB3-BPSS0390 at 2 different cell densities	<b>217</b>
<b>Figure 6.18</b> Persister frequency of <i>B. pseudomallei</i> K96243 or <i>B. pseudomallei</i> K96243 <i>ΔBPSS0390-0391</i> treated with 100 x MIC (200μg/ml) of ciprofloxacin	<b>219</b>
<b>Figure 6.19</b> Persister frequency of early or late stationary phase <i>B. pseudomallei</i> K96243 or <i>B. pseudomallei</i> K96243 <i>ΔBPSS0390-0391</i> treated with 100 x MIC (200μg/ml) of ciprofloxacin	<b>220</b>
<b>Figure 6.20</b> Survivor frequency of <i>B. pseudomallei</i> K96243 or <i>B. pseudomallei</i> K96243 <i>ΔBPSS0390-0391</i> treated with 15mM hydrogen peroxide	<b>223</b>
<b>Figure 6.21</b> CFU fold change of <i>B. pseudomallei</i> K96243 and <i>B. pseudomallei</i> K96243 <i>ΔBPSS0390-0391</i> treated with cadmium sulphate	<b>225</b>

## Chapter 7

<b>Figure 7.0</b> Growth of <i>E. coli</i> MG1655 /pBAD/his-BPSS0390	<b>236</b>
<b>Figure 7.1</b> Growth of <i>E. coli</i> MG1655 /pBAD/his-BPSS0390	<b>238</b>
<b>Figure 7.2</b> CFU fold change of <i>E. coli</i> MG1655 following expression of BPSS0390 mutants	<b>240</b>
<b>Figure 7.3</b> Combined western blots following repression or expression of BPSS0390 mutants	<b>242</b>
<b>Figure 7.4</b> CFU fold change in <i>E. coli</i> cell numbers following co-expression of BPSS0391 with the BPSS0390 site directed mutants	<b>243</b>
<b>Figure 7.5</b> Persister frequency of <i>E. coli</i> MG1655 strains expressing pBAD/his cloned BPSS0390 mutants using ciprofloxacin	<b>245</b>
<b>Figure 7.6</b> Persister frequency of <i>E. coli</i> MG1655 strains expressing pBAD/his cloned BPSS0390 mutants using ceftazidime	<b>247</b>
<b>Figure 7.7</b> Western blot of <i>E. coli</i> MG1655 /pBAD <i>E. coli</i> MG1655 /pBAD/his-LacZ and <i>E. coli</i> MG1655 /pBAD/his-BPSS0390	<b>250</b>
<b>Figure 7.8</b> SDS-PAGE gels showing the purification steps of the H24A protein	<b>251</b>
<b>Figure 7.9</b> CD spectroscopy of the his tagged BPSS0390 protein	<b>254</b>
<b>Figure 7.10</b> HSQC Spectra	<b>257</b>
<b>Figure 7.11</b> Co-expression and purification of his tagged BPSS0390 and BPSS0391	<b>261</b>
<b>Figure 7.12</b> Western blots following co-expression of BPSS0391 with the toxic alleles of BPSS0390	<b>262</b>
<b>Figure 7.13</b> SDS-PAGE gels showing the purification steps of the BPSS0391 antitoxin	<b>264</b>
<b>Figure 7.14</b> SDS-PAGE gel showing the result of a pull down assay	<b>266</b>
<b>Figure 7.15</b> Agarose gel showing the migration pattern of MS2 RNA incubated with various proteins	<b>268</b>
<b>Figure 7.16</b> EMSA assay incubating DNA with purified his tagged BPSS0391	<b>269</b>

and BPSS0391 protein	
<b>Figure 7.17</b> EMSA assay incubating DNA with BPSS0390H24A protein at various concentrations	<b>271</b>

## List of tables

### Chapter 1

<b>Table 1.0</b> Type II toxin-antitoxin families	<b>53</b>
---	-----------

### Chapter 2

<b>Table 2.0</b> Bacterial strains used in this study	<b>69</b>
---	-----------

### Chapter 3

<b>Table 3.0</b> Testing the validity of RASTA bacteria	<b>107</b>
<b>Table 3.1</b> Information about the 5 reference <i>B. pseudomallei</i> strains	<b>108</b>
<b>Table 3.2</b> List of predicted TA genes in the 5 reference <i>B. pseudomallei</i> strains	<b>110</b>
<b>Table 3.3</b> Distribution of predicted <i>B. pseudomallei</i> K96243 TA genes in other strains	<b>113</b>
<b>Table 3.4</b> Distribution of predicted <i>B. pseudomallei</i> K96243 TA genes in <i>B. mallei</i> and <i>B. thailandensis</i> .	<b>117</b>
<b>Table 3.5</b> Homology of the predicted <i>B. pseudomallei</i> K96243 TA systems with other proteins.	<b>118</b>

### Chapter 5

<b>Table 5.0.</b> Stress conditions in which BPSS1583 and BPSS1584 were up or down regulated in <i>B. pseudomallei</i> K96243	<b>176</b>
---	------------

### Chapter 6

<b>Table 6.0</b> Stress conditions in which BPSS0390 and BPSS0391 were up or downregulated in <i>B. pseudomallei</i> K96243	<b>222</b>
---	------------

### Chapter 7

<b>Table 7.0</b> The most highly conserved residues in 75 proteins homologous to BPSS0390	<b>235</b>
<b>Table 7.1</b> Crystallisation of H24A protein	<b>253</b>
<b>Table 7.2</b> The most conserved residues in 89 homologous BPSS0391 protein sequences	<b>259</b>