

Table of Figures

Title of Figures	Page No.
Fig. 1.1 Evolution of earth's atmospheric oxygen content through time (lyons et al., 2014).	2
Fig. 1.2 Different morphological forms in cyanobacteria.	3
Fig. 1.3 Morphological changes during heterocyst formation.	4
Fig. 1.4 Structure and mechanism of nitrogenase showing reduction of N ₂ to two molecules of NH ₃ .	5
Fig. 1.5 Arrangement of DNA elements in vegetative cells of <i>Anabaena</i> PCC 7120.	7
Fig. 1.6 Excision of DNA elements in <i>Anabaena</i> PCC 7120 heterocyst.	10
Fig. 1.7 Identity and similarity between XisA and XisC excisases of <i>Anabaena</i> PCC 7120.	11
Fig. 1.8 Proposed model for the expression of <i>Anabaena</i> PCC 7120 <i>xisA</i> gene in <i>E. coli</i> .	13
Fig. 1.9 Crystal structure of site specific recombinases.	15
Fig. 1.10 Catalytic mechanism of tyrosine recombinases.	16
Fig. 1.11 Domain architect of tyrosine recombinase.	16
Fig. 1.12 Structure and mechanism of catalysis of serine recombinases.	18
Fig. 1.13 Crystal structure of topoisomerase IB.	19
Fig. 1.14 Catalytic similarity between YRs and Topoisomerase IB.	19
Fig. 1.15 Catalytic domain of Type II restriction endonuclease (<i>EcoRII</i>).	24
Fig. 1.16 Crystal structures of Type IIe restriction endonucleases.	24
Fig. 2.1 Plasmid maps of pAM461 and pMX25.	30
Fig. 2.2 Restriction maps of plasmids used in the study.	32
Fig. 3.1 Schematic representation of <i>xisA</i> gene cloning strategy.	40
Fig. 3.2 PCR amplification of <i>xisA</i> gene.	44
Fig. 3.3 Restriction digestion analysis of <i>xisA</i> gene bearing recombinant vectors.	44

Fig. 3.4 Overexpression and purification of XisA protein.	45
Fig. 3.5 MALDI tof mass spectrometry analysis of anabaena pcc 7120 XisA protein.	46
Fig. 3.6 <i>E. coli</i> growth curves at different IPTG concentration.	47
Fig. 3.7 Agarose gel showing <i>Eco</i> RI and <i>Hind</i> III digestion patterns for confirmation of pAM461 and validation of <i>xisA</i> functionality.	48
Fig. 3.8 Secondary structure analysis of XisA protein.	49
Fig. 3.9 Sequence analysis and theoretical modelling of XisA protein.	50
Fig. 4.1 : Earlier rearrangement assay to monitor <i>nifD</i> element excision.	53
Fig. 4.2 Schematic representation of pAM461 rearrangement	54
Fig. 4.3 Strategy to design primers for PCR based <i>in vivo</i> monitoring of excision by XisA	56
Fig. 4.4 Detection of pAM461 rearrangement using substrate and product specific PCR primers.	57
Fig. 4.5 <i>in vivo</i> colony per detection of pAM461 rearrangement in <i>E. coli</i> expression host.	58
Fig. 4.6 Experimental design and to monitor XisA recombinase activity <i>in vivo</i> by PCR based assay.	59
Fig. 4.7 PCR based monitoring of excision.	60
Fig. 5.1 Abortive deletion events arising during <i>nifD</i> rearrangement in <i>E. coli</i> (pMX25) resulting in blue white sectoring colonies indication endonuclease activity if XisA.	64
Fig. 5.2 Strategy for constructing chimeric XisAC, harbouring N-terminal of XisA and C-terminal of Xisc.	66
Fig. 5.3 Monitoring recombinase and endonuclease activities of N-terminal, C-terminal and chimer of XisA and XisC.	67
Fig. 5.4 Strategy for selecting targets target for XisA gene sequential truncation.	70
Fig. 5.5 Schematic representation of <i>xisA</i> gene sequential N-terminal truncation products.	71
Fig. 5.6 Schematic representation of cloning <i>xisa</i> gene sequential N-terminal truncation products in pET28(a).	72
Fig. 5.7 A typical agarose gel profile of PCR based recombinase assay of <i>xisA</i>	74
Fig. 5.8 Methodology to monitor endonuclease activity of XisA and derived variants.	74
Fig. 5.9 PCR amplification of wild type <i>xisA</i> gene and its sequential N-terminal truncation variants.	75
Fig. 5.10 BglIII digestion pattern pJET1.2 containing <i>xisA</i> gene sequential N-terminal truncation variants.	75

Fig. 5.11 Restriction digestion pattern of pET28(a) containing <i>xisA</i> gene sequential N-terminal truncation variants.	76
Fig. 5.12 Expression and purification of N-terminal truncation products of XisA protein.	77
Fig. 5.13 Agarose gel profile displaying pcr based functionality detection of <i>E. coli</i> strains synthesising N-terminal truncation products of XisA protein.	78
Fig. 5.14 Monitoring <i>in vivo</i> recombinase and endonuclease activity XisA protein N-terminal truncation product.	80
Fig. 6.1 X-ray structure of the bZIP dimer GCN4 bound to dna.	83
Fig. 6.2 Prediction of basic region of leucine zipper (<i>bZIP</i>) in XisA	84
Fig. 6.3 XisA nucleotide sequence displaying predicted <i>bLZ</i> region.	88
Fig. 6.4 Schematic representation of <i>xisA</i> gene <i>bLZ</i> deletion products.	88
Fig. 6.5 Schematic representation of <i>jLZ</i> complemented to <i>xisA</i> gene <i>bLZ</i> deletion products.	89
Fig. 6.6 Schematic representation of cloning <i>jLZ</i> complemented <i>bLZ</i> deleted products of <i>xisA</i> gene in pET28(a).	90
Fig. 6.7 Amplification and cloning of <i>bLZ</i> deleted and <i>jLZ</i> complimented <i>xisA</i> gene products.	92
Fig. 6.8 <i>in vivo</i> functional characterization of <i>bLZ</i> deleted and <i>jLZ</i> complementation products of XisA protein.	94
Fig. 7.1 Active site mutagenesis studies of <i>Anabaena</i> PCC 7120 XisC.	97
Fig. 7.2 Schematic representation of <i>EcorII</i> Y308F causing loss in the cleavage activity of the enzyme.	98
Fig. 7.3 Amino acid sequence of XisA1 protein with position of active site residues shaded in red	101
Fig. 7.4 Schematic representation of XisA1 gene targets used for active site mutagenesis of XisA1 protein.	101
Fig. 7.5 Schematic representation of the strategy used for site directed mutagenesis of <i>pxisA1</i>	103
Fig. 7.6 Mutagenic PCR of <i>xisA1</i> .	104
Fig. 7.7 Chromatograms of sequenced <i>xisA1</i> gene mutants.	105
Fig.7.8 SDS PAGE analysis of <i>xisA1</i> protein active site mutants.	106
Fig. 7.9 Agarose gel profile displaying PCR based functionality detection of <i>E. coli</i> strains synthesising XisA1 mutants.	106
Fig. 7.10 Monitoring <i>in vivo</i> recombinase and endonuclease activity of XisA1 protein active site mutants.	108
Fig. S1 Schematic representation of multimer forming potential of XisA sequential N-terminal truncation products	112

Fig. S2 Schematic representaion of functionality loss of BLZ deleted XisA	113
Fig. S3 A brief timeline of <i>Anabaena</i> PCC 7120 research	114