

Sr. No.	Title	Page No.
<b>1</b>	<b>Chapter 1: Introduction</b>	<b>2</b>
1.1	Cyanobacteria	2
1.1.1	<i>Anabaena</i> PCC 7120	3
1.2	Characteristics of enzymes involved in DNA cleavage and recombination.	13
1.2.1	Site specific recombinases	13
1.2.2	Type IB Topoisomerase	17
1.2.3	Restriction endonucleases	22
1.3	Link between Endonucleases and Site Specific Recombinases	24
1.4	Objectives	26
<b>2</b>	<b>Chapter 2: Materials and Methods</b>	<b>27</b>
2.1	Bacterial Strains / Plasmids	28
2.2	Culture Condition	28
2.3	Molecular Biology Tools and Techniques	35
2.4	DNA Sequencing	36
2.5	Online Sequence and Structural Analysis	36
2.5.1	BLAST	36
2.5.2	Restriction Analysis	36
2.5.3	Oligonucleotide Sequence Analysis	36
2.5.4	Theoretical modeling of XisA protein	36
2.6	Site Directed Mutagenesis	37
2.7	Physiological Experiments	37
2.7.1	PCR Based Recombinase Assay	37
2.7.2	Endonuclease Assay	37
2.8	Statistical Analysis	37
<b>3</b>	<b>Chapter: 3 Expression and Purification of Functional XisA Protein</b>	<b>38</b>
3.1	Introduction	38
3.1.1	Toxicity hypothesis of XisA protein	38
3.1.2	Rationale of the present study	38
3.2	Materials and Methods	38
3.2.1	<i>E. coli</i> Strains, Plasmids and Oligonucleotide Primers Used in the Study	38
3.2.2	Construction of recombinant <i>pxisA</i> (pET28a + <i>xisA</i> ) expression vector	40
3.2.3	Toxicity assessment of <i>xisA</i> gene overexpression in <i>E. coli</i>	41
3.2.4	<i>In vivo</i> detection of XisA protein endonuclease activity by restriction digestion	41
3.2.5	Expression and purification of XisA protein	42
3.2.6	Confirmation of purified XisA protein by MALDI-TOF analysis	42
3.2.7	Theoretical modelling of XisA protein	43
3.3	Results	43
3.3.1	Cloning and heterologous expression of <i>xisA</i> gene	43

3.3.2	Purification and confirmation of XisA protein	43
3.3.3	Effect of <i>xisA</i> gene overexpression in <i>E. coli</i>	45
3.3.4	<i>In vivo</i> detection of XisA functionality	47
3.3.5	Predicting secondary and 3D structure of XisA protein	48
3.4	Discussion	49
<b>4</b>	<b>Chapter 4: Development of PCR based assay to monitor the recombinase activity of XisA protein <i>in vivo</i></b>	<b>52</b>
4.1	Introduction	53
4.1.1	Selection of substrate plasmid	54
4.1.2	Rationale of the present study	54
4.2	Materials and Methods	55
4.2.1	<i>E. coli</i> Strains, Plasmids and Oligonucleotide Primers Used in the Study	56
4.2.2	Strategy to monitor XisA protein recombinase activity by PCR assay	56
4.3	Results	59
4.3.1	Monitoring recombinase activity of XisA protein by PCR assay	59
4.4	Discussion	59
<b>5</b>	<b>Chapter 5: Determining minimal regions of XisA protein displaying recombinase and endonuclease activities</b>	<b>62</b>
5.1	Introduction	63
5.1.1	Rationale of the present study	65
5.2	Materials and Methods	68
5.2.1	<i>E. coli</i> Strains, Plasmids and Oligonucleotide Primers Used in the Study	68
5.2.2	Strategy to design sequential N-terminal truncation products of <i>xisA</i> gene.	70
5.2.3	Construction of vectors expressing sequential N-terminal truncation products of <i>xisA</i> gene	71
5.2.4	Heterologous over expression and purification of sequential N-terminal truncation products of <i>xisA</i> gene	72
5.2.5	Detection of Recombinase activity of N-terminal truncation products of XisA protein	73
5.2.6	Monitoring the recombinase activity of N-terminal truncation products of XisA protein	73
5.2.7	Monitoring endonuclease activity of various sequential N-terminal truncation products of XisA protein by antibiotic sensitivity assay	74
5.3	Results	75
5.3.1	Cloning and heterologous expression of sequential N-terminal truncation products of <i>xisA</i> gene	75
5.3.2	Purification of sequential N-terminal truncation products of XisA protein	76
5.3.3	Determining terminal region of XisA protein exhibiting recombinase and endonuclease activities	76
5.4	Discussion	79
<b>6</b>	<b>Chapter 6: Role of predicted basic region of leucine zipper (bZIP) of XisA protein in recombinase and endonuclease activities</b>	<b>82</b>

6.1	Introduction	83
6.1.1	Rationale of the present study	83
6.2	Materials and methods	85
6.2.1	<i>E. coli</i> Strains, Plasmids and Oligonucleotide Primers Used in the Study	85
6.2.2	Strategy to design bLZ deletion products of <i>xisA</i> gene	87
6.2.3	Construction of vectors expressing sequential N-terminal truncation products of <i>xisA</i> gene	87
6.2.4	Construction of expression vector of <i>jun luciferase zipper(jLZ)</i> complementation chimera of <i>xisA</i> gene bLZ deletion products	89
6.2.5	Monitoring recombinase and endonuclease potential of bLZ deleted XisA protein products and their jLZ complements	91
6.3	Results	91
6.3.1	Cloning and bLZ deletion products of <i>xisA</i> gene in pET28a	91
6.3.2	Cloning of jLZ complemented bLZ deleted products of <i>xisA</i> gene in pET28a	91
6.3.3	Effect of bLZ deletion and jLZ complementation on recombinase and endonuclease activities of XisA.	93
6.4	Discussion	93
<b>7</b>	<b>Chapter 7: Role of active site residues in recombinase and endonuclease activities of XisA protein</b>	<b>96</b>
7.1	Introduction	97
7.1.1	Rationale of the study	98
7.2	Materials and methods	99
7.2.1	<i>E. coli</i> Strains, Plasmids and Oligonucleotide Primers Used in the Study	99
7.2.2	Strategy to design XisA1 protein active site mutants	101
7.2.3	Construction of vectors synthesizing XisA1 active site mutants	101
7.2.4	Expression of <i>pxisA1</i> mutants and purification of encoded active site mutants of XisA1 protein.	102
7.2.5	Monitoring recombinase and endonuclease activities of active site mutants of XisA protein.	102
7.3	Results	104
7.3.1	Site directed mutagenesis of XisA protein active site residues	104
7.3.2	DNA sequencing to confirm <i>xisA1</i> mutants	104
7.3.3	Purification of XisA1 active site mutants	104
7.3.4	Effect of active site mutations on recombinase and endonuclease activities of XisA1 protein	106
7.4	Discussion	107
	<b>Summary</b>	<b>110</b>
	<b>References</b>	<b>115</b>
	List of Publications	
	Awards	
	Orals/Poster presentations	
	Synopsis	