

CONTENTS

List of Abbreviations	i
List of Figures	iii
List of Tables	vi
Abstract	vii
Chapter 1 Introduction	
1.1. Regulation of gene expression in prokaryotes	1
1.2. Small noncoding RNAs as post transcriptional regulators	1
1.3. Mechanism of regulation of small noncoding RNAs	2
1.3.1. Requirement of bases for pairing sRNA with mRNA	2
1.3.2. Translational repression	3
1.3.3. Translational activation	3
1.3.4. Transcription termination	4
Review of Literature	
1.4. Identification of small noncoding RNAs in bacteria	5
1.4.1. Bioinformatic approaches	6
1.4.2. Experimental RNomics	8
1.5. Computational prediction of mRNA targets for sRNA	10
1.6. Classification of small noncoding RNA	12
1.6.1. cis-encoded RNAs	12
1.6.1.1. Plasmid encoded <i>cis</i> - acting RNA	12
1.6.1.2. Chromosomally encoded <i>cis/trans</i> -RNA	13
1.6.1.2.1. Riboswitches	13
1.6.1.2.2. Thermosensors	14
1.6.2. trans-encoded RNAs	15
1.6.2.1. Hfq mediated sRNA regulation	16
1.6.2.2. Protein binding sRNAs	16
1.6.3. CRISPR RNAs	17
1.7. Dual role of sRNAs	19
1.8. Small RNAs in pathogenic bacteria	20
1.9. <i>Staphylococcus aureus</i>- A human pathogen	24
1.9.1. Methicillin Susceptible <i>Staphylococcus aureus</i> strain Newman- a clinical isolate	24
1.10. Virulence factors of <i>S. aureus</i>	25
1.10.1. Surface Adhesins/molecules	25
1.10.2. Secreted toxins and enzymes	29
1.11. Regulation of virulence factors	31
1.11.1. The Agr regulon	32
1.11.2. Sar	33
1.11.3. Rot	33
1.11.4. Sae	34

Studies on noncoding RNAs in the regulation of pathogenicity in Staphylococcus aureus

1.11.5. Sigma σ^B	34
1.11.6. Small noncoding RNA	35
1.12. Small noncoding RNAs in <i>Staphylococcus aureus</i>	35
1.12.1. Pathogenicity island (PaIs) encoded sRNAs	35
1.12.2. Current perspectives of <i>S. aureus</i> noncoding RNAs	35
1.12.3. Staphylococcal Regulatory RNA database (SRD)	39
1.13. Use of antisense reagents as possible anti-infective therapy	39
Chapter 2 The present investigation	
2.1. Definition of the problem	42
2.2. Objectives	43
2.3. The present study	43
Chapter 3 Materials and Methods	
3.1. Bacterial strains, plasmids and primers	45
3.1.1. Bacterial strains	45
3.1.2. Plasmids	46
3.1.3. Primers	47
3.2. Media, chemicals, enzymes, biochemicals and kits	47
3.3. Media and Culture conditions	48
3.4. Bioinformatic tools used in this study	48
3.4.1. Promoter prediction	48
3.4.2. Rho independent transcription terminators	48
3.4.3. Staphylococcal Regulatory RNA database (SRD)	49
3.4.4. Target prediction tools	49
3.4.5. Sequence retrieval	49
3.4.6. Primer design	49
3.4.7. <i>in silico</i> restriction analysis	49
3.4.8. Multiple sequence alignment	50
3.4.9. RNA secondary structure prediction	50
3.5. Molecular biology techniques	50
3.5.1. Isolation of genomic DNA from <i>Staphylococcus aureus</i>	50
3.5.2. Isolation of plasmid from <i>E. coli</i> and <i>S. aureus</i>	51
3.5.3. Polymerase Chain Reaction (PCR) conditions	51
3.5.4. Restriction enzyme digestion	52
3.5.5. Ligation	52
3.5.6. DNA transformation in <i>E. coli</i> and <i>S. aureus</i>	52
3.5.7. DNA sequencing	53
3.5.8. DNA/RNA Hybridization studies	53
3.5.8.1. DNA/RNA Probe labeling	53
3.5.8.2. RNA isolation	53
3.5.8.3. Transfer of RNA to membrane	54
3.5.8.4. Transfer of DNA to membrane	54
3.5.8.5. Hybridization and detection	55
3.5.9. RT- PCR	55

Studies on noncoding RNAs in the regulation of pathogenicity in Staphylococcus aureus

3.5.9.1. Semi quantitative Reverse Transcriptase PCR	55
3.5.9.2. Real time PCR	56
3.6. Estimation of protein by Folin Lowry method	56
3.7. Physiological assays	56
3.7.1. Quantitative biofilm production by microtiter plate assay	57
3.7.2. Confocal microscopy of biofilm formation and clumping of cells	57
3.7.3. Delta hemolysis Assay	57
3.7.4. Alpha hemolysis Assay	58
3.7.5. Extraction of carotenoid pigment	58
3.7.6. Antibiotic susceptibility Test	59
3.8. in vitro transcription of RNA	59
3.9. Electrophoretic gel mobility shift assay	60
3.10. Animal infection studies	60
Chapter 4 Results and Discussion	
4.1. Biocomputational predictions of sRNAs and its targets	61
4.1.1. Identification of small RNAs in <i>S. aureus</i> Newman	62
4.1.2. <i>in silico</i> prediction of mRNA targets	62
4.2. SprX- a small noncoding RNA	
4.2.1. <i>in silico</i> characterization of SprX1	67
4.2.1.1. Sequence analysis of Newman SprX with other <i>S. aureus</i> strains	67
4.2.1.2. <i>in silico</i> predicted targets of SprX1 selected for this study	70
4.2.2. Functional characterization of SprX1	71
4.2.2.1. Overexpression of SprX1 in <i>S. aureus</i> Newman	71
4.2.2.2. Disruption of <i>sprX1</i> using kanamycin marker	73
4.2.2.3. Expression analysis of altered level of SprX1 constructs	75
4.2.2.3.1. Analysis of SprX1 overexpression by Northern blot	75
4.2.2.3.2. Analysis of <i>sprX1</i> disruption mutant by PCR, Northern and Southern blot	76
4.2.2.4. Effects of altered expression of SprX1 on various pathogenicity factors	78
4.2.2.4.1. Effect of SprX1 on growth physiology	78
4.2.2.4.2. Influence of SprX1 on staphylococcal delta hemolysin	79
4.2.2.4.3. SprX1 plays a significant role in biofilm formation	81
4.2.2.4.3.1. Imaging of increased adherence of biofilm and clumping of cells	83
4.2.2.4.4. Other predicted targets of SprX1	84
4.2.3. Electrophoretic gel mobility shift assay	84
4.2.3.1. Generation of SprX1 template by PCR	84
4.2.3.2. Cloning of interaction region of delta hemolysin, clumping factorB and non specific PhrD in pBSKS ⁺	85
4.2.3.3. <i>in vitro</i> sRNA – mRNA interaction assay	87
4.2.4. Animal infection studies using Swiss albino BALB/c mice as model organism	88

Studies on noncoding RNAs in the regulation of pathogenicity in Staphylococcus aureus

4.2.5. Overall influence of SprX1 on pathogenicity of <i>S. aureus</i> Newman	90
4.3. SprB- a small noncoding RNA	
4.3.1. <i>in silico</i> characterization of SprB and its targets	93
4.3.2. Functional characterization of SprB	95
4.3.2.1. Overexpression of SprB in <i>S. aureus</i> Newman	95
4.3.2.2. Expression analysis of SprB	97
4.3.2.3. Effects of SprB on various growth physiology	97
4.3.2.3.1. Influence of SprB on clumping factorA/B and biofilm formation	98
4.3.2.3.2. Influence of SprB on staphylocoagulase	100
4.3.2.3.3. Influence of SprB on staphyloxanthin pigment production	100
4.3.2.3.4. Influence of SprB on antibiotic susceptibility	101
4.3.3. Virulence studies of SprB in mice model of infection	103
4.3.4. Overall influence of SprB in <i>S. aureus</i> Newman	105
Significance of SprX1/SprB in <i>S. aureus</i> Newman	107
Summary	108
Conclusion	112
References	113
Appendix I	132
Appendix II	135
Publication and poster presentations	138