

TABLE OF CONTENTS

LIST OF ABBREVIATIONS	i
LIST OF FIGURES.....	iii
LIST OF TABLES	iv
ABSTRACT	v
1. INTRODUCTION & REVIEW OF LITERATURE	7
1.1 Fine tuning the gene expression in bacteria: An introduction	7
1.1.1 Modulation of transcription	7
1.1.2 Modulation of translation.....	8
1.2 Small regulatory RNAs	9
1.2.1 Classification of small regulatory RNAs	10
1.3 Mechanism of sRNA mediated gene regulation	14
1.3.1 Positive and negative regulation of translation.....	14
1.3.2 Positive and negative regulation of mRNA stability	16
1.4 Identification and characterization of small RNAs	17
1.4.1 Biocomputational approach	17
1.4.2 RNomics approach	18
1.4.3 Differential RNA-sequencing	18
1.4.4 RIP-seq	19
1.4.5 CLIP-seq	19
1.4.6 Grad-seq	19
1.5 Identification of sRNA targets for discovery of sRNA function	19
1.5.1 Computational Approaches.....	20
1.5.2 Experimental approaches	20
1.6 <i>Staphylococcus aureus</i> , an opportunistic pathogen	22
1.7 Virulence factors of <i>S. aureus</i>	22
1.7.1 Cell wall associated virulence factors	22
1.7.2 Secreted virulence factors	25
1.8 Regulation of virulence factors in <i>S. aureus</i>	27
1.8.1 Two Component System	27
1.8.2 SarA	30
1.8.3 Alternative sigma factors, SigB	31

1.9	sRNA discovery in <i>Staphylococcus aureus</i>	31
1.10	sRNA regulon in <i>S. aureus</i>	32
1.10.1	sRNAs involved in metabolic regulation	32
1.10.2	sRNA linking quorum sensing and virulence regulation	35
1.10.3	sRNAs expressed from the pathogenicity island	35
2.	PRESENT STUDY	37
3.	MATERIALS AND METHODS	39
3.1	Bacterial strains, plasmids, and culture conditions.....	39
3.2	Bioinformatics tools	40
3.2.1	IntaRNA	40
3.2.2	Mfold	40
3.2.3	Primer3	40
3.2.4	BPROM.....	40
3.3	Molecular biology methods.....	40
3.3.1	Genomic DNA extraction	40
3.3.2	Plasmid DNA extraction	40
3.3.3	Restriction enzyme digestion.....	41
3.3.4	Polymerase chain reaction	41
3.3.5	Agarose gel electrophoresis and DNA elution	41
3.3.6	Ligation	42
3.3.7	Transformation in <i>E. coli</i>	43
3.3.8	Transformation of <i>S. aureus</i>	43
3.4	Construction of <i>sprX</i> knockdown strain.....	44
3.5	Proteomic studies	44
3.5.1	Preparation of proteins for two-dimensional gel electrophoresis	44
3.5.2	Isoelectric focusing (IEF), 2D-PAGE and protein identification	44
3.5.3	SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE).....	45
3.5.4	Colloidal Coomassie staining with CBB G-250.....	46
3.5.5	Silver Staining.....	46
3.6	Preparation of extracellular and cell wall associated autolysins.....	46
3.6.1	Zymography	47
3.7	RNA isolation and quantitative real time PCR	47
3.8	Triton X-100-induced autolysis assay	47

3.9	Observation of cell morphology and analysis of cell clustering.....	48
3.10	Biofilm assay.....	48
3.11	<i>In vitro</i> transcription and RNA labeling.....	48
3.12	Electrophoretic Mobility Shift Assay (EMSA).....	49
4.	RESULTS AND DISCUSSION	50
4.1	SprX, highly conserved small RNA expresses in <i>S. aureus</i>	50
4.2	Construction of a modified strain of SprX	50
4.3	Expression analysis of small RNA SprX in <i>S. aureus</i> Newman	52
4.4	SprX differentially regulates the protein profile in <i>S. aureus</i> Newman.....	53
4.5	SprX regulates the expression of IsaA	55
4.6	SprX binds at 3' coding region of <i>isaA</i> mRNA	56
4.7	SprX modulates the expression of autolysins in <i>S. aureus</i> Newman	56
4.8	<i>sprX</i> knockdown, an autolysin deficient strain, exhibits altered phenotypes ...	59
4.9	SprX regulates the genes involved in cell wall metabolism in <i>S. aureus</i> Newman.....	62
4.10	SprX interacts with WalR, a positive regulator of autolysin	64
4.11	Role of SprX in the complex network of regulation of autolysins	65
5.	SUMMARY	70
6.	REFERENCES	72
APPENDIX I	92
APPENDIX II	93
APPENDIX III	95
PUBLICATION	96
POSTER PRESENTATION	96