

RyjB, a novel riboregulator in *Escherichia coli*: Regulation of its expression and mechanism of its action

NAMRA SIDDIQUI



DEPARTMENT OF CHEMISTRY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

JULY 2023

RyjB, a novel riboregulator in *Escherichia coli*: Regulation of its expression and mechanism of its action

by

NAMRA SIDDIQUI

Department of Chemistry

Submitted

in partial fulfillment of the requirements of the degree of Doctor of Philosophy

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

JULY 2023

***This work is dedicated to my
beloved family***

CERTIFICATE

This is to certify that the thesis entitled “**RyjB, a novel riboregulator in *Escherichia coli*: Regulation of its expression and mechanism of its action**” being submitted by **Ms. NAMRA SIDDIQUI** to the Indian Institute of Technology Delhi for the award of the degree of ***Doctor of Philosophy*** in chemistry is a record of bonafide research work carried out by her. Ms. Namra Siddiqui has worked under my guidance and supervision and has fulfilled the requirements for the submission of the thesis, which, to my knowledge, has reached the requisite standard. The results contained in this dissertation have not been submitted in part or full to any other university or institute for the award of any degree or diploma.

Date:

Place:

Dr. Tanmay Dutta

Associate Professor

Department of Chemistry

Indian Institute of Technology,
Delhi

Acknowledgments

"The secret to success is not to try to avoid or get rid of or shrink from your problems; the secret is to grow yourself so that you are bigger than any problem."

- T. Haru Eker

A doctoral degree encompasses much more than a mere academic credential. It demands an immense amount of time, patience, and perseverance, which is truly commendable. The journey can leave you vulnerable, but it also offers the potential to rebuild you as a resilient and humbled researcher. I would like to express my heartfelt gratitude to all those who supported me throughout this arduous yet rewarding journey.

First and foremost, I am deeply indebted to my mentor, **Prof. Tanmay Dutta** for his encouragement, keen supervision and ever enthusiastic approach to each and every finding that I made. This piece of work would have been a distant dream for me without his unsparing donation of advice, ideas, and precious time and it is the foundation of this research. I thank him for his continuous belief in me and my abilities through all the highs and lows of this journey. I owe him lots of gratitude for enlightening a scientific attitude in me that has made a permanent impression on my career and life. He is a man with great intelligence, and it won't be exaggeration to call him a genius in field of biochemistry. He has always been very supportive in these five years of my Ph.D. journey.

I would like to thank **Prof. S. Pandey**, Head of the Department of Chemistry, as well as **Prof. N. Kurur**, **Prof. A. Elias**, and **Prof. A.K. Ganguli**, former Heads of the Chemistry Department, for providing me with all the necessary facilities during my tenure.

I would also like to extend my sincere thanks to **Prof. Sunil Kumar Khare**, **Prof. Shashank Deep**, and **Prof. Ashok Kumar Patel**, who served as my SRC members and generously offered their time, kind support, and insightful comments. In particular, I am grateful to Khare Sir for granting me access to their lab instruments whenever I needed them.

No words suffice to thank my incredible senior lab member **Dr. Amit Kumar Gupta** and **Dr.**

Saumya Singh whose insightful suggestions helped me in greatly improving my thesis work. Especially I want to thank **Amit Sir** for the insightful discussions at critical stages of my work that helped me to resolve many problems with his conceptual clarity and deep knowledge of the subject. I wouldn't have been able to complete my thesis without him.

Additionally, I would like to express my gratitude to the **Department of Chemistry at IIT Delhi** for their cooperation and support throughout my research journey. I am also thankful to the **Ministry of Human Resource and Development, Delhi** for providing a healthy stipend, which enabled me to sustain myself financially during these five years of research.

I am grateful to **IIT Delhi** for providing financial support for attending national and international conferences, and I acknowledge this support. It has been an enriching experience, and I am grateful for the opportunities that have been extended to me during this journey.

This work would not have been possible without the immense support and guidance of all those people who were immensely helpful throughout this thesis. I extend my gratitude to all my friends - **Palak, Reena, Vishakha, Naved, Nitin Sir, Kamini, Suhail, Risha and Raman**. I can never thank these people enough for their selfless love for me.

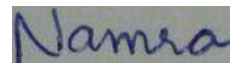
I owe enormous words of appreciation to my lab mates - **Pratyusha, Bipasa, Stanzin, Anam, Aashma, Nitin** for always cheering me and willingly helped me out with their abilities. I will remember the company of all the past and present students of IIT and companions including, who made my stay memorable.

I am deeply grateful to my family, especially my mother **Mrs. Fareeda**, for being a constant support in my times of hardship. My brother **Mohd. Tariq**, my sister **Surraiya**, and my father **Mr. Khursheed Ahmad** have also played a vital role in my accomplishments. Without their unwavering support, I wouldn't have been able to achieve what I have today. They always supported in pursuing my passion and never stopped me from testing my ideas and taking risks in life. I owe a great deal of thanks to my parents whose blessings were indispensable in the completion of my Ph.D. journey.

They say that a child's smile has the power to make you forget any problem. I am grateful to

Ismail, Filzah, Sara, and Shreyanvi, who are very special children that always bring a smile to my face even in times of despair. I owe them my thanks for the happiness they bring to my life.

It is by Gods willingness that I hereby place on record my deep sense of gratitude and reverence to all those who have helped and encouraged me to complete this work.



Namra Siddiqui

Abstract

Noncoding RNA molecules (ncRNAs) are transcripts, which directly act on their targets rather than coding for amino acids. These RNA molecules are present in all life forms and regulate diverse cellular functions. ncRNAs engage in a variety of mechanisms to regulate such as methylation of rRNA, inhibition of translation, and transcription and sequestration of regulatory proteins. Bacterial noncoding RNAs are generally signified as small RNAs (sRNAs). With the advancements in bacterial small RNA (sRNA) research, these sRNAs are found to be involved in many regulatory roles crucial for gene expression related to virulence in pathogenic bacteria, thus, controlling pathogenicity. Bacterial sRNAs are generally induced in response to changing environmental conditions and participate in numerous regulatory networks to fine tune the expressions of genes, which promote the cell survival in response to environmental cues. We have taken an insightful approach in the current study to investigate the regulation of expression of a novel sRNA RyjB. RyjB although was detected in all growth phases of *Escherichia coli* cells, but it was more prevalent in the exponential than the stationary phase of growth. RyjB was transcriptionally regulated neither by stationary phase sigma factor $E\sigma^S$ nor by $E\sigma^E$ under normal and stress conditions. RNA chaperones Hfq and ProQ are not necessary for the post-transcriptional abundance of RyjB, at least in the exponential phase of growth.

Interestingly, RyjB abundance appeared to be increased as the cells progressed through the exponential phase and decreased in the stationary phase, although RyjB was found to be acid stress-inducible small RNA. Nearly 2.5-fold upregulation was observed after 45 min of acid stress. The probability of RpoS involvement in *ryjB* expression under acid stress was eliminated as RyjB status was found to be identical in both wild-type and $\Delta rpoS$ cells. The mechanism how RyjB was up-regulated to promote the cell viability under acid stress drew immense attention. PhoP, an integrant of the two-component regulatory system PhoPQ, has been reported to control many genes involved

in major cellular functions such as magnesium homeostasis, cell envelope composition, bacterial virulence, and acid resistance. Comparison of RyjB expression in wild-type and $\Delta phoP$ cells revealed that elimination of *phoP* allele from the chromosome resulted in a constant low-level expression of RyjB. Thus, *ryjB* appeared to be a PhoP-dependent acid stress-inducible sRNA.

RyjB is synthesized from a complementary strand to the one from which *sgcA* is synthesized and shares a 4-nt complementary region with *sgcA* transcript. This suggests that RyjB might act as an antisense RNA for *sgcA* expression. This finding supports the notion that RyjB:SgcA base pairing is a post-transcriptional event, thus, the potential role of RyjB is to increase the stability of *sgcA* transcript against intracellular ribonuclease-mediated degradation.

The final part of the thesis delineates that SgcA is a protein that is involved in a phospho-transfer reaction and has a potential role in cellular response to oxidative and osmotic stress. A remarkable growth difference observed between WT and $\Delta sgcA$ cells suggests that SgcA may have an important role in cellular growth and/or survival. SgcA plays a role in sugar transport within the cell, and its absence leads to decreased glucose uptake in the cell, which was confirmed by 2-NBDG assay. The observation that overexpression of RyjB in the cells leads to increased glucose uptake suggests that RyjB may be involved in regulating the expression of SgcA. Specifically, it has been postulated that RyjB may stabilize the mRNA of *sgcA*, enhancing the gene's expression and, subsequently, glucose uptake. The putative phosphotransferase protein SgcA lacks a crystal structure, making it challenging to comprehend its structural properties. To understand the secondary structure of SgcA, we performed CD and ICP-MS analysis, which allowed us to estimate the elements present in the protein. Overall, these findings suggest that SgcA is a protein that is involved in cellular growth, survival, and stress response, likely through its enzymatic activity in a phospho-transfer reaction. Further studies may be needed to fully elucidate SgcA's function and mechanisms of action.

सारांश

नॉनकोडिंग आरएनए अणु (एनसीआरएनए) प्रतिलेख हैं, जो अमीनो एसिड के लिए कोडिंग के बजाय सीधे अपने लक्ष्य पर कार्य करते हैं। ये आरएनए अणु सभी जीवन रूपों में मौजूद हैं और विविध सेलुलर कार्यों को नियंत्रित करते हैं। एनसीआरएनए विनियमित करने के लिए विभिन्न तंत्रों में संलग्न होते हैं जैसे कि आरआरएनए का मिथाइलेशन, अनुवाद का निषेध, और नियामक प्रोटीन का प्रतिलेखन और ज़ब्ती। बैक्टीरियल नॉनकोडिंग आरएनए को आम तौर पर छोटे आरएनए (एसआरएनए) के रूप में दर्शाया जाता है। बैक्टीरियल छोटे आरएनए (एसआरएनए) अनुसंधान में प्रगति के साथ, ये एसआरएनए रोगजनक बैक्टीरिया-रिया में विषाणु से संबंधित जीन अभिव्यक्ति के लिए महत्वपूर्ण कई नियामक भूमिकाओं में शामिल पाए जाते हैं, इस प्रकार, रोगजनकता को नियंत्रित करते हैं। बैक्टीरियल एसआरएनए आम तौर पर बदलती पर्यावरणीय परिस्थितियों के जवाब में प्रेरित होते हैं और जीन की अभिव्यक्तियों को ठीक करने के लिए कई नियामक नेटवर्क में भाग लेते हैं, जो पर्यावरणीय संकेतों के जवाब में कोशिका अस्तित्व को बढ़ावा देते हैं। हमने एक नए sRNA RyjB की अभिव्यक्ति के नियमन की जांच के लिए वर्तमान अध्ययन में एक व्यावहारिक दृष्टिकोण अपनाया है। RyjB हालांकि एस्चेरिचिया कोली कोशिकाओं के सभी विकास चरणों में पाया गया था, लेकिन यह विकास के स्थिर चरण की तुलना में घातीय में अधिक प्रचलित था। RyjB को सामान्य और तनाव की स्थिति में न तो स्थिर चरण सिग्मा कारक E σ S और न ही E σ E द्वारा ट्रांसक्रिप्शनल रूप से विनियमित किया गया था। आरएनए चैपरोन एचएफक्यू और प्रोक्यू आरवाईजेबी की पोस्ट-ट्रांसक्रिप्शनल बहुतायत के लिए आवश्यक नहीं हैं, कम से कम विकास के घातीय चरण में।

दिलचस्प बात यह है कि जैसे-जैसे कोशिकाएं घातीय चरण के माध्यम से आगे बढ़ें, RyjB बहुतायत में वृद्धि हुई और स्थिर चरण में कमी आई, हालांकि RyjB को एसिड तनाव-प्रेरित छोटा आरएनए पाया गया। 45 मिनट के एसिड तनाव के बाद लगभग 2.5 गुना अपनियमन देखा गया। एसिड तनाव के तहत ryjB अभिव्यक्ति में RpoS की भागीदारी की संभावना समाप्त हो गई क्योंकि RyjB स्थिति जंगली-प्रकार और

$\Delta rpoS$ कोशिकाओं दोनों में समान पाई गई। एसिड तनाव के तहत सेल व्यवहार्यता को बढ़ावा देने के लिए RyjB को कैसे अपग्रेड किया गया, इस तंत्र ने अत्यधिक ध्यान आकर्षित किया। PhoP, दो-घटक नियामक प्रणाली PhoPQ का एक अभिन्न अंग है, जो मैग्नीशियम होमियोस्टैसिस, सेल आवरण संरचना, जीवाणु विषाणु और एसिड प्रतिरोध जैसे प्रमुख सेलुलर कार्यों में शामिल कई जीनों को नियंत्रित करने की सूचना दी गई है। जंगली-प्रकार और $\Delta phoP$ कोशिकाओं में RyjB अभिव्यक्ति की तुलना से पता चला कि गुणसूत्र से phoP एलील के उन्मूलन के परिणामस्वरूप RyjB की निरंतर निम्न-स्तरीय अभिव्यक्ति हुई। इस प्रकार, ryjB एक PhoP-निर्भर एसिड तनाव-प्रेरक sRNA प्रतीत होता है।

RyjB को एक पूरक स्ट्रैंड से संश्लेषित किया जाता है जिससे sgcA संश्लेषित होता है और sgcA प्रतिलेख के साथ 4-एनटी पूरक क्षेत्र साझा करता है। इससे पता चलता है कि RyjB sgcA अभिव्यक्ति के लिए एक एंटीसेंस RNA के रूप में कार्य कर सकता है। यह खोज इस धारणा का समर्थन करती है कि RyjB: SgcA बेस पेयरिंग एक पोस्ट-ट्रांसक्रिप्शनल घटना है, इस प्रकार, RyjB की संभावित भूमिका इंटरसेल्युलर राइबोन्यूक्लिज़-मध्यस्थता गिरावट के खिलाफ sgcA ट्रांसक्रिप्ट की स्थिरता को बढ़ाना है।

थीसिस का अंतिम भाग बताता है कि एसजीसीए एक प्रोटीन है जो फॉस्फो-ट्रांसफर प्रतिक्रिया में शामिल होता है और ऑक्सीडेटिव और ऑस्मोटिक तनाव के लिए सेलुलर प्रतिक्रिया में इसकी संभावित भूमिका होती है। WT और $\Delta sgcA$ कोशिकाओं के बीच देखा गया एक उल्लेखनीय वृद्धि अंतर बताता है कि सेलुलर विकास और/या अस्तित्व में SgcA की महत्वपूर्ण भूमिका हो सकती है। SgcA कोशिका के भीतर शर्करा परिवहन में एक भूमिका निभाता है, और इसकी अनुपस्थिति से कोशिका में ग्लूकोज की मात्रा कम हो जाती है, जिसकी पुष्टि 2-NBDG परख द्वारा की गई थी। यह अवलोकन कि कोशिकाओं में RyjB की अधिक अभिव्यक्ति से ग्लूकोज की मात्रा बढ़ जाती है, यह पता चलता है कि RyjB SgcA की अभिव्यक्ति को विनियमित करने में शामिल हो सकता है। विशेष रूप से, यह माना गया है कि RyjB sgcA के mRNA को स्थिर कर सकता है, जीन की अभिव्यक्ति को बढ़ा सकता है और, परिणामस्वरूप, ग्लूकोज ग्रहण कर सकता

है। कल्पित फॉस्फोट्रांसफेरेज प्रोटीन एसजीसीए में क्रिस्टल संरचना का अभाव है, जिससे इसके संरचनात्मक गुणों को समझना चुनौतीपूर्ण हो जाता है। एसजीसीए की द्वितीयक संरचना को समझने के लिए, हमने सीडी और आईसीपी-एमएस विश्लेषण किया, जिससे हमें प्रोटीन में मौजूद तत्वों का अनुमान लगाने में मदद मिली। कुल मिलाकर, इन निष्कर्षों से पता चलता है कि एसजीसीए एक प्रोटीन है जो सेलुलर विकास, अस्तित्व और तनाव प्रतिक्रिया में शामिल है, संभवतः फॉस्फो-ट्रांसफर प्रतिक्रिया में इसकी एंजाइमेटिक गतिविधि के माध्यम से। SgcA के कार्य और क्रिया के तंत्र को पूरी तरह से स्पष्ट करने के लिए आगे के अध्ययन की आवश्यकता हो सकती है।

Contents

Certificate	i
Acknowledgment	ii
Abstract	v
List of Figures	xvii
List of Tables	xix
List of Abbreviations	xx

CHAPTER I.	INTRODUCTION	1-37
-------------------	---------------------	-------------

1.1.	Introduction	1
1.2.	Review of literature	3
1.2.1.	Gene regulation	3
1.2.1.1.	Regulation of transcription	3
1.2.1.2.	Regulation of translation	4
1.3.	Central dogma and RNA	5
1.4.	RNA as a regulatory molecule	6
1.4.1.	Evolution of RNA as regulators	7
1.5.	Non-coding RNA in Eukaryotes	8
1.6.	Non-coding RNA in Prokaryotes	9
1.6.1.	Riboswitches	10
1.6.2.	Small RNAs	11

1.6.3.	RNA mining: Approaches to identify novel small RNAs in bacteria	12
1.6.3.1.	Direct labeling and sequencing	12
1.6.3.2.	Genetic screens	12
1.6.3.3.	Biocomputational screens	13
1.6.3.4.	Co-purification with Proteins or Target RNA	13
1.6.3.5.	Microarray Detection	13
1.6.3.6.	RNomics and Next gen sequencing (RNA-seq)	14
1.7.	sRNAs in <i>E. coli</i>	14
1.7.1	CsrB	15
1.7.2.	McaS	16
1.7.3.	SokX	16
1.7.4.	RyjB	17
1.8.	Small RNA in various pathogenic bacteria	21
1.8.1.	sRNAs in <i>Salmonella typhimurium</i>	21
1.8.2.	sRNAs in <i>Mycobacterium tuberculosis</i>	22
1.8.3.	sRNAs in <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Listeria monocytogenes</i> , and <i>Vibrio cholerae</i> .	23
1.9.	sRNA mechanisms	25

1.9.1.	sRNAs that modulate protein activity	25
1.9.2.	sRNAs that modulate transcription	26
1.9.3.	sRNAs modulating translation	27
1.9.3.1.	Base pairing small RNAs	27
1.9.3.1.1.	<i>cis</i> -encoded base pairing sRNAs	27
1.9.3.1.2.	<i>trans</i> -encoded base pairing sRNAs	29
1.10.	Proteins required for sRNA function	32
1.10.1.	Hfq Protein	32
1.10.2.	Ribonucleases	33
1.11.	Stress response of bacterial small RNA	34
1.12.	Elucidation of sRNA targets	36
1.13.	Objectives	38
1.14.	Scope of the thesis	39

CHAPTER II.	MATERIAL AND METHODS	42-56
--------------------	-----------------------------	--------------

2.1.	Materials	42
2.2.	Bacterial strain construction and growth conditions	45
2.2.1.	Growth survival assay	46
2.2.2.	Phage P1 transductions	46

2.2.3.	Phage titers	46
2.2.4.	Transduction procedure	47
2.3.	Isolation of Plasmid and genomic DNA	47
2.3.1.	PCR	47
2.3.2.	Agarose gel electrophoresis	48
2.4.	Total RNA Isolation	48
2.4.1.	Reverse Transcription PCR	49
2.4.2.	Real time PCR (RT-qPCR)	49
2.4.3.	Northern Blotting	50
2.5.	In-vitro transcription	50
2.6.	Western Blotting	51
2.7.	Cloning and overexpression of RyjB, PhoP and RpoE	51
2.8.	Cloning of SgcA	52
2.9.	Overexpression & Purification of SgcA	53
2.10.	Circular Dichroism	53
2.11.	Probe and Primer	54
2.11.1.	Gene mapping	54
2.11.2.	Calculation of doubling times	54
2.12.	Statistical analysis	54

CHAPTER III.	RyjB in <i>E. coli</i>; Regulation of its expression, effect of ribo-nucleases and stresses	57-78
---------------------	--	--------------

3.1.	Introduction	57
------	---------------------	-----------

3.2.	Results	58
3.2.1.	Growth phase-dependent expression of RyjB in <i>E. coli</i>	58
3.2.1.1.	Regulation of RyjB expression	58
3.2.1.2.	Role of Hfq in RyjB expression	61
3.2.1.3.	Role of ProQ in RyjB expression	64
3.2.2.	Ribonucleases in <i>E. coli</i>	66
3.2.2.1.	Regulatory role of RNase BN on RyjB expression	67
3.2.2.2.	Regulatory role of RNase D on RyjB expression	68
3.2.3.	Effect of stresses on RyjB expression	70
3.2.3.1.	Effect of Magnesium stress on RyjB expression	70
3.2.3.2.	Effect of pH stress on RyjB expression	72
3.2.3.3.	Effect of osmotic stress on RyjB expression	74
3.2.3.4.	Effect of oxidative stress on RyjB expression	75
3.3.	Discussion	76

CHAPTER IV.	Role of RyjB in pH homeostasis of <i>E. coli</i>	79-96
--------------------	---	--------------

4.1.	Introduction	79
4.2.	Results	81
4.2.1.	RyjB is an acid stress inducible sRNA	81
4.2.2.	Effect of RyjB overexpression on cell viability	83
4.2.3.	RyjB overexpression activates glutamate decarboxylase/anti-porter-dependent acid resistance system	84
4.2.4.	PhoP upregulates RyjB under acid stress	86

4.2.5.	Ectopic expression of Phop in $\Delta phoP$ cells restores RyjB induction	88
4.2.6.	Role of RpoE in the transcription of RyjB	90
4.3.	Discussion	92
CHAPTER V.	Antisense mechanism of RyjB on the expression of <i>sgcA</i>	97-112
5.1.	Introduction	97
5.2.	Results	99
5.2.1.	Overexpression of RyjB induces RyjB expression	99
5.2.2.	Overexpression of truncated RyjB (4-nt off) abstrain <i>sgcA</i> population	102
5.2.3.	Stability of <i>ryjB</i> and <i>sgcA</i> in WT <i>E. coli</i> cells	104
5.2.4.	RNase E is responsible for degradation of <i>sgcA</i> mRNA	106
5.2.5.	Ectopic expression of RyjB in WT restores <i>sgcA</i> induction	109
5.3.	Discussion	110
CHAPTER VI.	Role of RyjB in sugar transport into the cell And characterization of <i>SgcA</i>	113-128
6.1.	Introduction	113
6.2.	Results	115
6.2.1.	<i>sgcA</i> mutation decreases the growth rate of <i>E. coli</i> cells	115
6.2.2.	Role of <i>sgcA</i> in sugar transport system	117
6.2.3.	Effect of osmotic and oxidative stress on <i>sgcA</i> expression	119

6.2.4.	Characterization of SgcA	121
6.2.4.1.	Cloning, overexpression, and purification of SgcA	121
6.2.4.2.	Secondary structure of SgcA protein	124
6.2.4.3.	Estimation of elements present in SgcA protein	125
6.3.	Discussion	126
	Conclusions & Future Perspective	129
	REFERENCES	134
	LIST OF PUBLICATIONS & CV	157

List of Figures

Figure No.	Description	Page No.
Fig. 1.1.	BLAST analysis	3
Fig. 1.2.	Different kind of riboregulators in bacteria	10
Fig. 1.3.	sRNAs modulating protein activity	26
Fig. 1.4.	sRNAs modulating transcription	27
Fig. 1.5.	<i>cis</i> -encoded antisense sRNAs	29
Fig. 1.6.	<i>trans</i> -encoded sRNAs	31
Fig. 1.7.	Hfq crystal structure	32
Fig. 1.8.	Hfq mediated sRNA-mRNA base pairing	33
Fig. 3.1.	Accumulation of RyjB at different growth phases of <i>E. coli</i> cells	60
Fig. 3.2.	Role of Hfq in RyjB expression	63
Fig. 3.3	Role of ProQ in RyjB expression	65
Fig. 3.4A.	Role of Ribonuclease BN in RyjB expression	68
Fig. 3.4B.	Role of Ribonuclease D in RyjB expression	70
Fig. 3.5.	Cellular levels of RyjB in WT and $\Delta phoP$ cells under high magnesium conditions	71
Fig. 3.6.	Amount of RyjB in WT cells under pH stress	73
Fig. 3.7.	Amount of RyjB in WT cells under osmotic stress	75
Fig. 3.8.	Amount of RyjB in WT cells under oxidative stress	76

Fig. 4.1.	Amount of RyjB in WT and $\Delta rpoS$ cells under pH stress	82
Fig. 4.2.	Growth phase representation of MG1655 cells	84
Fig. 4.3.	Effect of RyjB overexpression on GADR system	86
Fig. 4.4.	Effect of PhoP on RyjB expression under acid stress	88
Fig. 4.5.	Effect of ectopic expression of PhoP on <i>ryjB</i> expression	89
Fig. 4.6.	Effect of ectopic expression of RpoE on <i>ryjB</i> expression	91
Fig. 5.1.	Effect of RyjB overexpression on <i>sgcA</i> accumulation in the cell	101
Fig. 5.2.	Effect of truncated RyjB overexpression on <i>sgcA</i> accumulation in the cell	103
Fig. 5.3.	Stability of RyjB and <i>sgcA</i> transcripts in WT <i>E. coli</i> cells.	106
Fig. 5.4.	Accumulation of RyjB and <i>sgcA</i> transcript in WT and RNase E temperature strain (<i>rne^{ts}</i>)	109
Fig. 5.5.	Effect of ectopic expression of RyjB on <i>sgcA</i> expression in WT cells	110
Fig. 6.1.	Effect of Frameshift mutation on the growth rate of <i>E. coli</i> MG1655 cells	116
Fig. 6.2.	Confocal microscopy	119
Fig. 6.3.	Amount of <i>sgcA</i> mRNA in WT cells under different stresses	120
Fig. 6.4.	Cloning of <i>sgcA</i> gene	121
Fig. 6.5.	Protein purification and Western blot depicting purified SgcA	123
Fig. 6.6.	Secondary structure analysis of SgcA protein	124
Fig. 6.7.	ICP-MS analysis of SgcA protein	126

List of Tables

Table No.	Description	Page No.
Table 1.1	List of characterized sRNAs	19
Table 1.2	List of <i>E. coli</i> sRNAs with their known targets and function	36
Table 2.1	Strains and plasmids used in this study	43
Table 2.2	Antibiotics concentration	44
Table 2.3	Reagents	44
Table 2.4	PCR conditions	47
Table 2.5	RT-PCR conditions	49
Table 2.6	Oligonucleotide sequence	54
Table 2.7	Generation time of <i>E. coli</i> MG1655 cells under normal and acid stress conditions with or without the overexpression of RyjB	84
Table 2.8	Generation time of <i>E. coli</i> MG1655 cells with or without <i>sgcA</i> mutation under normal conditions.	116

List of Abbreviations

BLAST	Basic Local Alignment Search Tool
Nt	Nucleotide
WT	Wild type
Amp	Ampicillin
Cat	Chloramphenicol acetyl transferase
Kan	Kanamycin
PFU	Plaque-forming units
RNA	Ribonucleic acid
sRNA	Small RNA
RNase	Ribonuclease
DEPC	Diethyl Pyro carbonate
rRNA	Ribosomal RNA
mRNA	Messenger RNA
tRNA	Transfer RNA
DNA	Deoxyribonucleic acid
cDNA	Complementary DNA
DNase	Deoxyribonuclease
RT-qPCR	Quantitative Real-time PCR
RT-PCR	Reverse Transcriptase PCR
PAGE	Poly acrylamide gel electrophoresis
DTT	Dithiothreitol
TEMED	Tetramethyl ethylenediamine
IPTG	Isopropyl β - d-1-thiogalactopyranoside
PMSF	Phenyl Methyl Sulfonyl Fluoride
PVDF	Polyvinylidene fluoride
DTT	Dithiothreitol
CFU	Colony Forming Units
pH	Negative logarithm of hydrogen ion
MgCl ₂	Magnesium chloride

KCl	Potassium chloride
CaCl ₂	Calcium chloride
mAbs	Monoclonal antibodies
MB	Molecular Grade
LB	Luria-Bertani
OD	Optical Density
EDTA	Ethylenediaminetetraacetic acid
SDS	Sodium dodecyl sulfate
T _m	Melting Temperature
SD	Standard deviation
Δ	Deletion
bp	Base pair