CHROMOSOME SEGREGATION IN *RHODOCOCCUS ERYTHROPOLIS* PR4

DIVYA SINGHI

DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

MARCH 2020

CHROMOSOME SEGREGATION IN *RHODOCOCCUS ERYTHROPOLIS* PR4

by

DIVYA SINGHI

DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY

Submitted

In fulfillment of the requirement of the degree of Doctor of Philosophy

to the

Indian Institute of Technology Delhi

March 2020

CERTIFICATE

This is to certify that the thesis entitled **"Chromosome segregation in *Rhodococcus erythropolis* PR4"** being submitted by **Ms. Divya Singhi** to the Indian Institute of Technology delhi, for the award of degree of **Doctor of Philosophy**, is a record bonafide research work carried out by her under my supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology Delhi.

The results presented in this thesis have not been submitted in part or full to any other University or Institute for the award of any other degree or diploma.

Date

Dr. Preeti Srivastava
Associate Professor
Department of Biochemical
Engineering and Biotechnology
Indian Institute of Technology Delhi
New Delhi-110016

ACKNOWLEDGEMENTS

Pursuing PhD is a long journey with various ups that accelerate it and numerous downs which tries to stop it. It is a dream which becomes reality not only through lots of hard work, determination, dedication and patience but also with the support of many people. I was and I am still very fortunate in meeting such people who helped, encouraged, inspired and supported me in achieving my goals. I know this acknowledgement would never be sufficient to appreciate the gratefulness of many who had faith in me throughout this roller coaster ride. But still I take this opportunity to convey my deepest gratitude towards them.

The first person whom I would like to acknowledge is my supervisor Prof. Preeti Srivastava for her invaluable contributions throughout my stay in IITD. Without her constant support and motivation this journey would have never been accomplished. The enthusiasm and dedication she has for science and research is extremely motivating. Her guidance, suggestions, ideas and timely discussions has helped me in improving my work. I thank her for inspiring me to do good research.

Further, I would like to express my sincere gratitude to my research committee members; Prof. Saroj Mishra, Prof. Ritu Kulshreshtha and Prof. Ashok Patel for putting efforts in evaluating my work from time to time. I thank them all for their valuable suggestions and appreciation which motivated me to work even harder.

I would also like to acknowledge and convey my sincere thanks to Prof. Shilpi Sharma for supporting me since beginning and providing her valuable time and suggestions for qPCR analysis. I would like to extend my thanks to the Head of the Department and all the other faculty members of DBEB and Kusuma School of Biological Sciences for imparting knowledge through classroom programs which helped me to excel in my course work and comprehensive exams. I would also like to thank all the members of the non-teaching staff of DBEB for all their support and help.

I feel really happy to thank all the people with whom I shared the most memorable and wonderful time of my life, the members of my lab, RNA-I people. Firstly I would like to thank

my seniors Dr. Pooja Singh, Dr. Anees and Ankur Kumar for their guidance, help and support during my initial days in the lab. Special credits to Dr. Pooja Singh; I had learnt several things from her. Not only for this lab but also in my life the name "Pooja" holds a very precious and respectable place, proved by the presence of Dr. Pooja Murarka. I can't even imagine my journey in this lab without her. She has been one of the most supportive, helpful, and caring person I could ever talk about. Our relation is way beyond professionalism; she has supported and been with me in the most difficult situations. The warmth she has provided me could only be explained when I say she is my family in Delhi.

I would like to extend my thanks to Jananee for being an excellent labmate. She has been a great support throughout and is a friend forever. Special thanks to Rohit, who is not just my friend but actually more like a brother. All the members of RNA-I lab holds a special place in my heart for this unforgetful experience at IITD. I acknowledge the help and thank Arif, Kavita, Sonal, Tanaya, Mansi, Deeksha, Krishna Priya and Priyanka for their support in the lab. Also would like to extend my thanks to Aditi and Aparna for the lively environment in the lab.

I would like to thank all the MTP and BTP students of the lab escpecially Aayushi Jain, Gaurav and Sagar. I had a great start with these three fellows. Aamir, Prashanth, Siddhant Ayan, Dwaraka, Gunjan, Aniruddh, Bintu, Jayaram and Ayushi Agrawal, all of them really made this journey memorable. I would also like to thank Roshinee, Prince, Piyush, Rakesh and Komal for maintaining a friendly environment in the lab. I always shared a special bond with the SURA students, whether it's with Aashima or Gunjan, Mira or Vageesh, Ayushi or Sonakshi or with my Gwalior buddy Saksham. I really had joyful time and have wonderful memories with all of them. Special thanks to Mr. Avneesh (Amit bhaiya), for maintaining clean lab and managing the process of our bills payment.

I would also like to thank Mr. Udit Mangal and Mr. Subhash Tripathi from ThermoFisher Scientific, for helping me to carry out Flow cytometry analysis and Mr. Lalit and Mr. Vibhuti from Nikon for providing me support and assistance regarding microscope whenever needed. Earnest thanks to all those whose names are missing but they are not forgotten. I acknowledge

the infrastructural support from IIT Delhi and I am thankful to DST and CSIR for financial support in the form of research scholarship.

It is impossible for me to thank enough one extremely celebrated person in my life, my best friend Mr. Abhijeet Thakur. I can never acknowledge him for the strength he has given me by always being there in need. His enormous faith and confidence in me even when I was losing hope, made me what I am today. I admire the way he stood beside me dedicated and firm like a mountain. He is truly a blessing in disguise. My heartiest gratitude to him for the way he is and best wishes for his thesis.

Finally, I extend my heartfelt regards to the most wonderful and supportive family. I honestly feel blessed to be a part of it. Taiji, Tauji, Mummy, Papa, Deepti, Jiju and Bhaiyu, they all are the strongest pillars of my life. It is their prayers and wishes which have helped me come so far in my journey. Apart from expressing my deepest thanks to them I even feel sorry for being not there at times when they needed me. I am extremely thankful to Deepti and Bhaiyu, who took most of the family responsibilities upon themselves so that I could just focus on my studies and work. One person who is with God now must be the happiest amongst us all on successful completion of my PhD thesis, Daddy (badepapa) it is all due to his blessings and faith in me. As a child I can never thank my parents for all their efforts and sacrifices, so I don't even want to try it but just want to say that mummy papa you both are my lifeline and I dedicate this thesis to you.

Last but not the least; I thank God, the Almighty for everything. Without his blessings and grace, this journey would not have been possible.

Divya Singhi

ABSTRACT

A bacterial cell cycle consists of various essential processes which run simultaneously in a coupled and coordinated manner. Broadly, these processes include DNA replication, segregation and division to form daughter cells with equal genetic material. Many studies have been conducted on various aspects of these processes in bacteria. Amongst Actinomycetes, chromosome organization and segregation studies have been limited to *Streptomyces coelicolor*, *Corynebacterium glutamicum* and *Mycobacterium* spp. There are differences with respect to chromosome number, organization, replication and segregation pattern in them. To enhance the repertoire and to study the diverse pattern, another member *Rhodococcus erythropolis* PR4, a non-pathogenic bacterium containing 6.5 Mbp completely sequenced circular genome, was used in the study. The present study aimed at understanding the cell cycle and associated processes such as chromosome replication, organization and segregation in *R. erythropolis* PR4 along with the role of partitioning and cytoskeletal proteins. The study was conducted using P1 GFP-ParB/*parS* system as the localizing tool. With the localization studies of small plasmid replicons, it was shown that different plasmids have different spatial position inside the cell. The subcellular localization of replisome revealed that the cocci shaped cells of the bacterium are non-replicating. The origin of replication was identified, and the DNA content in the cell under different growth conditions was determined. The number of origins increased in rich medium, suggesting overlapping replication cycle in this bacterium. Subcellular localization of origin region displayed polar positioning in minimal and rich medium. The terminus that is the last region to be replicated and segregated was found to be localized at the cell center in large cells. The middle markers corresponding to 1.5 Mb and 4.7 Mb loci did not overlap, suggesting discontinuity in the segregation of the two arms of the chromosome. Chromosome segregation

was not affected by inhibiting cell division but deletion of *parA* or *parB* affected chromosome segregation. The study suggests that *R. erythropolis* is different from other members of Actinobacteria; it is monoploid and has a unique chromosome segregation pattern. Transposon mutagenesis performed helped in identification of some of the important genes which might play key role in maintenance of the cell morphology. Till date, there are no such reports on chromosome organization, replication and segregation in *R. erythropolis* PR4. This study emphasizes on the fundamental and extremely significant aspects essential for understanding this bacterium. The study will be useful not only in designing better therapeutics in future for pathogenic strains but also for studying the genome maintenance in strains used for bioremediation.

सार

एक जीवाणु कोशिका चक्र में विभिन्न आवश्यक प्रक्रियाएं होती हैं जो एक साथ युग्मित और समन्वित तरीके से चलती हैं। मोटे तौर पर, इन प्रक्रियाओं में बेटी की कोशिकाओं को समान आनुवंशिक सामग्री के साथ बनाने के लिए डीएनए प्रतिकृति, अलगाव और विभाजन शामिल हैं।बैक्टीरिया में इन प्रक्रियाओं के विभिन्न पहलुओं पर कई अध्ययन किए गए हैं। एक्टिनोमाइसेट्स के बीच, क्रोमोसोम संगठन और अलगाव अध्ययन स्ट्रेप्टोमीस कोलीक्लोरोल, कोरिनेबैक्टेरियम ग्लूटामिकम और मायकोबैक्टीरियम एसपीपी तक सीमित रहे हैं। उनमें गुणसूत्र संख्या, संगठन, प्रतिकृति और अलगाव के संबंध में *विभिन्नता* हैं। प्रदर्शनों की सूची को बढ़ाने और विविध पैटर्न का अध्ययन करने के लिए, एक अन्य सदस्य रोडोकोकस एरिथ्रोपोलिस पीआर ४, एक गैर-रोगजनक जीवाणु, जिसमें ६.५ एमबीपी पूरी तरह से अनुक्रमित परिपत्र जीनोम होता है, का उपयोग अध्ययन में किया गया था। वर्तमान अध्ययन कोशिका विभाजन और संबंधित प्रक्रियाओं जैसे गुणसूत्र प्रतिकृति, संगठन और रोडोकोकस एरिथ्रोपोलिस पीआर ४ में विभाजन और साइटोस्केलेटल प्रोटीन की भूमिका के साथ है। स्थानीयकरण उपकरण के रूप में पी१ जी*एफपी-पारबी* / पारऐस प्रणाली का उपयोग करके अध्ययन किया गया था। छोटे प्लास्मिड प्रतिकृतियों के स्थानीयकरण अध्ययनों के साथ, यह दिखाया गया कि विभिन्न प्लास्मिडों की कोशिका के अंदर अलग-अलग स्थानिक स्थिति होती है। प्रतिकृति के उप-स्थानीयकरण से पता चला कि जीवाणु के गोलाणु आकार की कोशिकाएं गैर-प्रतिकृति हैं। प्रतिकृति की उत्पत्ति की पहचान की गई थी, और विभिन्न विकास

स्थितियों के तहत कोशिका में डीएनए सामग्री निर्धारित की गई थी। इस जीवाणु में प्रतिकृति चक्र को ओवरलैप करने का सुझाव देते हुए, समृद्ध माध्यमों में उत्पत्ति की संख्या में वृद्धि हुई। मूल क्षेत्र के उप-स्थानीयकरण ने न्यूनतम और समृद्ध माध्यम में ध्रुवीय स्थिति प्रदर्शित की। टर्मिनस जो कि दोहराया जाने वाला और अलग होने वाला अंतिम क्षेत्र है, बड़ी कोशिकाओं में कोशिका केंद्र में स्थानीयकृत पाया गया। १.५ एमबी और ४.७ एमबी लोके के मध्य के मार्करों को ओवरलैप नहीं पाया गया था, जो क्रोमोसोम के दो भुजाओ के अलगाव का सुझाव देता है। कोशिका विभाजन को रोककर क्रोमोसोम अलगाव प्रभावित नहीं *हुआ* था, लेकिन पारऐ या *पारबी* को हटा देने से क्रोमोसोम अलगाव को प्रभावित कर दिया था। अध्ययन से पता चलता है कि आर. एरिथ्रोपोलिस एक्टिनोबैक्टीरिया के अन्य सदस्यों से अलग है; यह मोनोप्लॉइड है और इसमें एक अद्वितीय गुणसूत्र अलगाव की प्रक्रिया है। ट्रांसपोसॉन म्यूटाज़ेनेसिस ने प्रदर्शन किया जिससे कुछ महत्वपूर्ण जीनों की पहचान करने में मदद मिली जो कोशिका आकृति विज्ञान के रखरखाव में महत्वपूर्ण भूमिका निभा सकते हैं। अब तक, आर. इरिथ्रोपोलिस पीआर ४ में गुणसूत्र संगठन, प्रतिकृति और अलगाव पर ऐसी कोई रिपोर्ट नहीं है। यह अध्ययन इस जीवाणु को समझने के लिए आवश्यक मूलभूत और अत्यंत महत्वपूर्ण पहलुओं पर जोर देता है। अध्ययन न केवल भविष्य में रोगजनक उपभेदों के लिए बेहतर चिकित्सीय डिजाइन तैयार करने में उपयोगी होगा बल्कि बायोरेमेडिएशन के लिए उपयोग किए जाने वाले उपभेदों में जीनोम रखरखाव का अध्ययन करने के लिए भी उपयोगी होगा।

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS & SYMBOLS

α	Alpha
aa	Amino acids
Amp^R	Ampicillin resistance
APS	Ammonium per sulphate
AT	Adenine-thymine
ATP	Adenosine tri-phosphate
β	Beta
BCD	B, C and D period
BHI	Brain heart infusion
BLAST	*Basic local alignment search tool*
bp	Base pair
BSA	Bovine serum albumin
cDNA	Complimentary DNA
Ceph	Cephalexin
CFP	Cyan fluorescent protein
CFU	Colony forming unit
ChIA-PET	Chromatin interaction analysis with paired-end tag sequencing
ChIP	Chromatin immunoprecipitation
3C	Chromosome conformation capture
4C	Chromosome conformation capture-on-chip
5C	Chromosome conformation capture carbon copy

Cm^R	Chloramphenicol resistance
DAPI	4′,6-diamidino-2-phenylindole
Δ	Delta/deletion
DEPC	Diethyl pyrocarbonate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribo nucleic acid
dNTP	Deoxyribose nucleotide triphosphate
dsz	Desulfurization
DTT	Dithiothreitol
EB	Elution buffer
EDTA	Ethylenediaminetetraacetic acid
EMSA	Electrophoretic mobility shift assay
EtBr	Ethidium bromide
Fig	Figure
FM4-64	*N*-(3-Triethylammoniumpropyl)-4-(6-(4-(Diethylamino) Phenyl) Hexatrienyl) Pyridinium Dibromide)
FROS	Fluorescence repressor operator system
FRT	FLP recombinase target
GC	Guanine-cytosine
GFP	Green fluorescent protein
gm	Gram
HCl	Hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

h	Hour
::	Insertion
IFA	Immunoflurescence assay
IPTG	Isopropyl-β-D-1-thiogalactopyranoside
Kan^R	Kanamycin resistance
kb	Kilobase
kDa	Kilodalton
kV	Kilovolt
λ	Lambda
lacZ	β-galactosidase
LA	Luria agar
LB	Luria broth
M	Molar
MALDI	Matrix assisted laser desorption ionization
µF	Microfarad
µg	Microgram
µl	Microlitre
ml	Millilitre
mM	Milimolar
MM	Minimal medium
min	Minute
MW	Molecular weight
ng	Nanogram

NCBI	National Center for Biotechnology Information
NGS	Next-generation sequencing
Ni-NTA	Nickel-nitrilotriacetic acid
nm	Nanometer
Ω	Ohm
OD	Optical density
oriC	Origin (chromosomal)
PAGE	Polyacrylamide gel electrophoresis
Par	Partitioning
PBSE	Phosphate buffer saline-EDTA
PCR	Polymerase chain reaction
pmol	Picomol
PMSF	Phenylmethane sulfonyl fluoride
qPCR	Quantitative PCR
RFP	Red fluorescent protein
Rif	Rifampicin
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	Revolution per minute
RT	Room temperature
RT-PCR	Real-time PCR
SDS	Sodium dodecyl sulphate
sec	Second

seq	Sequestration
$Strep^R$	Streptomycin resistance
τ	Tau
TAE	Tris-glacial acetic acid-EDTA
TBE	Tris-borate-EDTA
TE	Tris-EDTA
TEMED	N,N,N',N'-tetramethylethane-1,2-diamine
terC	Terminus (chromosomal)
Tet^R	Tetracycline resistance
Tris	Tris (hydroxymethyl) amino methane
ts	Temperature sensitive
UV	Ultraviolet
V	Volts
YFP	Yellow fluorescent protein
H_2O	Water