

CHROMOSOME SEGREGATION IN *RHODOCOCCUS ERYTHROPOLIS* PR4

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**DEPARTMENT OF BIOCHEMICAL ENGINEERING AND
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by

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DEPARTMENT OF BIOCHEMICAL ENGINEERING AND
BIOTECHNOLOGY

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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.

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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 बेटा 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 जीएफपी-*पारबी* / पारएस 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 उपस्थानीयकरण 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 भुजाओं के अलगाव का सुझाव देता है। कोशिका विभाजन को रोककर क्रोमोसोम अलगाव प्रभावित नहीं हुआ था, लेकिन पारए 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।

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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	<i>Basic local alignment search tool</i>
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
<i>dsz</i>	Desulfurization
DTT	Dithiothreitol
EB	Elution buffer
EDTA	Ethylenediaminetetraacetic acid
EMSA	Electrophoretic mobility shift assay
EtBr	Ethidium bromide
Fig	Figure
FM4-64	<i>N</i> -(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	Immunofluorescence assay
IPTG	Isopropyl- β -D-1-thiogalactopyranoside
Kan ^R	Kanamycin resistance
kb	Kilobase
kDa	Kilodalton
kV	Kilovolt
λ	Lambda
<i>lacZ</i>	β -galactosidase
LA	Luria agar
LB	Luria broth
M	Molar
MALDI	Matrix assisted laser desorption ionization
μ F	Microfarad
μ g	Microgram
μ l	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
<i>oriC</i>	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

<i>seq</i>	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
<i>terC</i>	Terminus (chromosomal)
Tet ^R	Tetracycline resistance
Tris	Tris (hydroxymethyl) amino methane
ts	Temperature sensitive
UV	Ultraviolet
V	Volts
YFP	Yellow fluorescent protein
H ₂ O	Water