MOLECULAR EVENTS RESPONSIBLE FOR SWITCHING OF CELL SHAPE WITH RESPECT TO CELL CYCLE IN *RHODOCOCCUS ERYTHROPOLIS* PR4

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by

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Submitted

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CERTIFICATE

This is to certify that the thesis entitled "**Molecular events responsible for Switching of Cell Shape with respect to Cell Cycle in *Rhodococcus erythropolis* PR4**" being submitted by **Ms. Shabnam Parwin** 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

Bacterial morphogenesis and cell division are closely regulated processes that are influenced by a large number of external and internal factors. Different environmental conditions serve as external factors that regulate bacterial cell shape such as temperature, pH, salt and nutrient availability. Numerous studies have been carried out in different bacteria to see the effect of varying environmental conditions on bacterial morphology. However, these studies are limited to some bacteria only and very few reports are present for cell shape switching in Actinomycetes. To understand the various environmental factors which may affect the morphology in Actinomycetes, one of its members *Rhodococcus erythropolis* PR4 was studied. *R. erythropolis* PR4 is a non-pathogenic bacterium with high bioremediation activity.

The present study also focuses on determining the role of prokaryotic cytoskeleton elements and cell division associated proteins on morphological adaptation in *R. erythropolis* PR4.

R. erythropolis PR4 was grown in different environmental conditions with varying nutrient availability, pH and salt concentration. An altered short rod morphology was observed under nutrient deprived conditions, acidic pH and high salt concentrations.

There are several proteins which modulate the cell wall structure and regulate cell shape. During cell division process, peptidoglycan layer acts as the scaffold for shaping bacterial morphology in which CHAP domain containing proteins play a significant role. CHAP domain containing proteins modify the peptidoglycan layer by mediating their muramidase and peptidase activity during cell division. Deletion of *chap* gene resulted in elongated morphology due to delayed cell division and mislocalisation of the septa ring. The prokaryotic cytoskeleton proteins such as orphan ParA, orphan ParB, RodA and DivIVA play key role in cell shape maintenance. Morphological studies of the deletion mutants of orphan *parA1, parA2, parA3*

and *divIVA-2* genes resulted in elongated rod-shaped morphology due to delayed cell division process. The disruption of *rodA, divIVA-1* and *divIVA-3* genes resulted in short rod-shaped morphology.

The study suggests that *R. erythropolis* PR4 exhibits morphological adaptation in varying environmental conditions. It has multiple copies of *chap*, orphan *parA*, orphan *parB* and *divIVA* genes that are likely to perform unique biological functions. To date there has been no report showing the multiple copies of these genes present in any of the class of Actinomycetes phylum. This research investigation provides deeper insights into the close association between cytoskeleton and cell division proteins which in turn will be useful to develop effective drugs against the pathogenic strain of *R. erythropolis* in future.

अमूर्त

बैक्टीरियल मोर्फोजेनेसिस और कोशिका विभाजन बारीकी से विनियमित प्रक्रियाएं हैं जो बड़ी संख्या में बाहरी और आंतरिक कारकों से प्रभावित होती हैं। विभिन्न पर्यावरणीय विकास स्थितियाँ बाहरी कारकों के रूप में काम करती हैं जो तापमान, पीएच, नमक और पोषक तत्वों की उपलब्धता जैसे बैक्टीरिया कोशिका आकार को नियंत्रित करती हैं। जीवाणु आकृति विज्ञान पर विभिन्न पर्यावरणीय परिस्थितियों के प्रभाव को देखने के लिए विभिन्न जीवाणुओं में कई अध्ययन किए गए हैं। हालाँकि, ये अध्ययन केवल कुछ बैक्टीरिया तक ही सीमित हैं और एक्टिनोमाइसेट्स में कोशिका आकार बदलने की बहुत कम रिपोर्टें मौजूद हैं। विभिन्न पर्यावरणीय कारकों को समझने के लिए जो एक्टिनोमाइसेट्स में आकृति विज्ञान को प्रभावित कर सकते हैं, इसके एक सदस्य रोडोकोकस एरिथ्रोपोलिस पीआर4 का अध्ययन किया गया था। आर. एरिथ्रोपोलिस पीआर4 उच्च बायोरेमेडिएशन गतिविधि वाला एक गैर-रोगजनक जीवाणु है।

वर्तमान अध्ययन आर. एरिथ्रोपोलिस पीआर4 में रूपात्मक अनुकूलन पर प्रोकैरियोटिक साइटोस्केलेटन तत्वों और कोशिका विभाजन से जुड़े प्रोटीन की भूमिका निर्धारित करने पर भी केंद्रित है। .

आर. एरिथ्रोपोलिस पीआर4 को अलग-अलग पोषक तत्वों की उपलब्धता, पीएच और नमक सांद्रता के साथ विभिन्न पर्यावरणीय परिस्थितियों में उगाया गया था। पोषक तत्वों से वंचित परिस्थितियों, अम्लीय पीएच और उच्च नमक सांद्रता के तहत एक परिवर्तित छोटी छड़ आकृति विज्ञान देखा गया।

ऐसे कई प्रोटीन हैं जो कोशिका दीवार की संरचना को व्यवस्थित करते हैं और कोशिका के आकार को नियंत्रित करते हैं। कोशिका विभाजन प्रक्रिया के दौरान, पेप्टिडोग्लाइकन परत जीवाणु आकृति विज्ञान को आकार देने के लिए मचान के रूप में कार्य करती है जिसमें प्रोटीन युक्त CHAP डोमेन एक महत्वपूर्ण भूमिका निभाता है। प्रोटीन युक्त CHAP डोमेन कोशिका विभाजन के दौरान उनकी मुरामिडेज़ और पेप्टिडेज़ गतिविधि में मध्यस्थता करके पेप्टिडोग्लाइकन परत को संशोधित करता है। चैप जीन के विलोपन के परिणामस्वरूप कोशिका विभाजन में देरी और सेप्टा रिंग के गलत स्थानीकरण के कारण लम्बी आकृति विज्ञान हुआ। प्रोकैरियोटिक साइटोस्केलेटन प्रोटीन जैसे ऑर्फ़न ParA, ऑर्फ़न ParB, RodA और

DivIVA कोशिका आकार रखरखाव में महत्वपूर्ण भूमिका निभाते हैं। अनाथ पैरा1, पैरा2, पैरा3 और divIVA-2 जीन के विलोपन उत्परिवर्ती के रूपात्मक अध्ययन के परिणामस्वरूप कोशिका विभाजन प्रक्रिया में देरी के कारण लम्बी छड़ के आकार की आकृति विज्ञान प्राप्त हुआ। रॉडए, डिविवा-1 और डिविवा-3 जीन के विघटन के परिणामस्वरूप छोटी रॉड के आकार की आकृति विज्ञान हुआ।

अध्ययन से पता चलता है कि आर. एरिथ्रोपोलिस पीआर4 अलग-अलग पर्यावरणीय परिस्थितियों में रूपात्मक अनुकूलन प्रदर्शित करता है और एक्टिनोमाइसेट्स के अन्य वर्गों से अद्वितीय है। इसमें चैप, अनाथ पैरा, अनाथ पारबी और डिविवा जीन की कई प्रतियां हैं जो अद्वितीय जैविक कार्य करने की संभावना रखती हैं। आज तक ऐसी कोई रिपोर्ट नहीं आई है जो एक्टिनोमाइसेट्स फाइलम के किसी भी वर्ग में मौजूद इन जीनों की कई प्रतियों को दर्शाती हो।

यह अध्ययन उन मूलभूत और अत्यधिक महत्वपूर्ण तत्वों पर केंद्रित है जो आर. एरिथ्रोपोलिस पीआर4 जीवाणु को समझने के लिए आवश्यक हैं। यह शोध जांच भविष्य में आर. एरिथ्रोपोलिस के रोगजनक तनाव के खिलाफ प्रभावी दवाएं विकसित करने के लिए साइटोस्केलेटन और कोशिका विभाजन प्रोटीन के बीच घनिष्ठ संबंध के बारे में गहन जानकारी प्रदान करेगी।

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Abbreviations & Symbols

α	Alpha
Amp^R	Ampicillin resistance
APS	Ammonium persulfate
aa	Amino acid
ATP	Adenosine triphosphate
β	Beta
BHI	Brain heart infusion
BLAST	Basic logical alignment search tool
bp	Base pair
BSA	Bovine serum albumin
cDNA	Complementary DNA
CFU	Colony forming unit
℃	degree Celsius
cAMP	Cyclic adenosine monophosphate
Δ	Delta/ deletion
DAPI	4’,6-diamidino-2-phenylindole
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DTT	Dithiothreitol
EB	Elution buffer
ECL	Enhanced Chemiluminescence
EDTA	Ethylenediaminetetraacetic acid

EtBr	Ethidium bromide
Fig	Figure
FM-4-64	NN-(3-Triethylammoniumpropyl)-4-(6-(4-(Diethylamino) Phenyl) Hexatrienyl) Pyridinium Dibromide)
FRT	FLP recombinase target
gm	Gram
GFP	Green Fluorescent Protein
hr	Hour
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HCL	Hydrochloric acid
HRP	Horseradish peroxidase
kb	Kilobase
IPTG	Isopropyl-β-D-1-thiogalactopyranoside
kDa	Kilodalton
kV	Kilovolt
Kan^R	Kanamycin resistance
LA	Luria agar
lacZ	β-galactosidase
LB	Luria broth
M	molarity
min	Minute
MW	Molecular weight
MM	Minimal media
mM	Millimole
ml	Millilitre

NCBI	National Centre for Biotechnology Information
ONPG	O-nitrophenyl β-D galactopyranoside
OD	Optical density
Ὼ	Ohm
PAGE	Polyacrylamide gel electrophoresis
Par	Partitioning
PBS	Phosphate buffered saline
PBSE	Phosphate buffered saline EDTA
PCR	Polymerase Chain Reaction
PMSF	Phenylmethane sulfonyl fluoride
PVDF	Polyvinylidene fluoride
qRT-PCR	Quantitative Reverse transcriptase PCR
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	Revolution per minute
RT	Room temperature
sec	Second
$Spec^R$	Spectinomycin resistance
SDS	Sodium dodecyl sulphate
μF	Micro faraday
μm	Micrometre
μl	Microliter
::	Insertion
TAE	Tris-glacial acetic acid-EDTA
TB	Terrific broth

TBST	Tris buffered saline containing Tween-20
TEMED	N,N,N',N'-tetramethylethane-1,2-diamine
Tet	Tetracycline
Tris	Tris (hydroxymethyl) amino methane
UV	Ultraviolet
X-gal	5-Bromo-4-chloro-3-indolyl-β-Dgalactopyranoside