ISOLATION AND CHARACTERIZATION OF PROTEIN(S) INVOLVED IN REGULATION OF *dsz* OPERON FOR BIODESULFURIZATION OF ORGANOSULFURS

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by

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Submitted

In fulfilment of the requirements of the degree of Doctor of Philosophy

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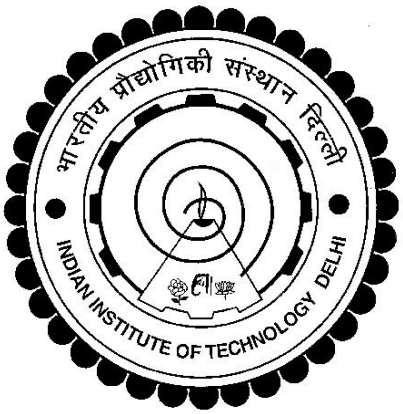

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CERTIFICATE

This is to certify that the thesis entitled "**Isolation and characterization of protein(s) involved in the regulation of the *dsz* operon for biodesulfurization of organosulfurs**" being submitted by **Ms. Pooja Murarka** 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

Combustion of organosulfur compounds in fossil fuels is one of the major sources of air pollution. With increasing concerns of environmental hazards, government has lowered the permissible sulfur levels in fuels. Chemical methods such as hydrodesulfurization have been used for removal of sulfur from fuel but this method is incapable of removing polyaromatic sulfurs. Therefore, there is a need for an alternative method that can overcome the limitations of hydrodesulfurization. Biodesulfurization is an attractive method for removing sulfur from polyaromatic sulfur heterocyclics. Many bacteria have been isolated that can desulfurize different organosulfurs. One such bacterium is *Gordonia* sp. IITR100. It is a broad substrate range bacterium that can desulfurize both aliphatic and aromatic organosulfurs. Similar to other desulfurizing bacteria, in *Gordonia* sp. IITR100, the desulfurization genes are arranged in an operon under the control of a promoter. The bacterium follows 4S pathway of biodesulfurization where DBT is metabolized to 2- hydroxybiphenyl and sulphite. The operon is known to be repressed in the presence of inorganic sulfur and is transcribed in the presence of organosulfurs. However, the regulators and the mechanism of regulation of this operon has not been reported to date. The present study aims at the isolation and identification of proteins that regulate the *dsz* operon from *Gordonia* sp. IITR100. Two *in vitro* methods; pull down assay and preparative gel shift assay followed by MALDI-ToF analysis were used to isolate and identify the proteins that bind to the *dsz* promoter. An *in vivo* method was developed to identify the proteins that bind to a known DNA sequence. Based on the results obtained by all three methods, six putative transcription regulators belonging to the family TetR, LuxR, two XRE, FIS and DNA binding response regulator were studied for their role in biodesulfurization. The TetR family protein was found to activate the *dsz* operon in recombinant

E. coli, *Gordonia* sp. IITR100 and *Rhodococcus erythropolis* IGTS8 at sub optimal inducer concentration. The TetR family protein was purified and characterized. To decipher the mechanism of regulation by this protein, the region of activation in the *dsz* promoter was determined. The binding of the protein to the promoter and the role of bending on activity was studied. The probable mechanism for the regulation of *dsz* operon has also been hypothesized in the study. The results on the regulation of *dsz* operon can help in establishing methods for improvising the rate of biodesulfurization.

सार

जीवाश्म ईंधन में ऑर्गेनोसल्फर यौगिकों का दहन वायु प्रदूषण के प्रमुख स्रोतों में से एक है। पर्यावरणीय खतरों की बढ़ती चिंताओं के साथ, सरकार ने ईंधन में अनुमेय सल्फर के स्तर को कम कर दिया है। सल्फर को ईंधन से निकालने के लिए हाइड्रोडिसल्फराइजेशन जैसे रासायनिक तरीकों का इस्तेमाल किया गया है, लेकिन यह विधि पॉलीएरोमेटिक सल्फर को हटाने में असमर्थ है। इसलिए, एक वैकल्पिक विधि की आवश्यकता है जो हाइड्रोडिसल्फराइजेशन की सीमाओं को पार कर सके। बायोडिसल्फराइजेशन, विषमचक्रीय पॉलीएरोमेटिक सल्फर से सल्फर को हटाने के लिए एक आकर्षक विधि है। कई बैक्टीरिया पाए गए हैं जो विभिन्न ऑर्गेनोसल्फर्स को निष्क्रिय कर सकते हैं। ऐसा ही एक जीवाणु है गॉर्डोनिया प्रजाति आई. आई. टी. आर. १००। यह एक व्यापक सब्सट्रेट रेंज वाला जीवाणु है जो दोनों स्निग्ध और सुगंधित ऑर्गेनोसल्फर्स को अवरूद्ध कर सकता है। गॉर्डोनिया प्रजाति आई. आई. टी. आर. १०० में अन्य डिसल्फराइजिंग बैक्टीरिया के समान, डिसल्फराइजेशन जीन को एक प्रमोटर के नियंत्रण में एक ओपेरॉन में व्यवस्थित किया जाता है। जीवाणु ४एस के बायोडिसल्फराइजेशन के मार्ग का अनुसरण करता है, जहां डीबीटी २- हाइड्रॉक्सीबाईफेनिल और सल्फाइट से चयापचय होता है। ओपेरॉन को अकार्बनिक सल्फर की उपस्थिति में दमित किया जाता है और ऑर्गोसल्फर्स की उपस्थिति में प्रत्यारोपित किया जाता है। हालाँकि, इस ओपेरॉन के नियमन और नियमन के तंत्र की आज तक रिपोर्ट नहीं की गई है। वर्तमान अध्ययन में प्रोटीन के अलगाव और पहचान का लक्ष्य है जो गॉर्डनिया प्रजाति आई. आई. टी. आर १०० से डीएसज़ेड ओपेरॉन को नियंत्रित करता है। इन विट्रो विधियों में दो; माल्डी-टॉफ़ विश्लेषण के बाद परख और प्रारंभिक जेल शिफ्ट परख को अलग करने और डीएसज़ेड प्रमोटर को बांधने वाले प्रोटीन की पहचान करने के लिए उपयोग किया

गया था। एक ज्ञात डीएनए अनुक्रम से बंधने वाले प्रोटीन की पहचान करने के लिए इन विवो विधि विकसित की गई थी। सभी तीन विधियों द्वारा प्राप्त परिणामों के आधार पर, परिवार टेटआर, लक्सआर, दो एक्सआरई, एफ फीस और डीएनए बाइंडिंग रिस्पांस रेगुलेटर से संबंधित छह पुटटीवे ट्रांसक्रिप्शन रेगुलेटर बायोडिसल्फराइजेशन में उनकी भूमिका के लिए अध्ययन किए गए थे। ट्रेटआर परिवार के प्रोटीन को पुनः संयोजक ई कोलाई, गोर्डोनिया प्रजाति में डीएसज़ेड ओपेरॉन को सक्रिय करने के लिए पाया गया। आइ. आइ. टी. आर १०० और रहोदोकॉकस एरिथ्रोपोलिस आइ जी टी एस ८ उप इष्टतम िन्दुसर एकाग्रता में। टेटआर परिवार के प्रोटीन को शुद्ध और विशेषता बनाया गया था। इस प्रोटीन द्वारा विनियमन के तंत्र को समझने के लिए, डीएसज़ेड प्रमोटर में सक्रियण का क्षेत्र निर्धारित किया गया था। प्रमोटर को प्रोटीन के बंधन और गतिविधि पर झुकने की भूमिका का अध्ययन किया गया था। डीएसज़ेड ओपेरॉन के नियमन के लिए संभावित तंत्र को भी अध्ययन में परिकल्पित किया गया है। डीएसज़ेड ओपेरॉन के नियमन पर परिणाम बायोडासल्फराइजेशन की दर को सुधारने के तरीकों को स्थापित करने में मदद कर सकते हैं।

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ABBREVIATIONS

2-HBP	2-Hydroxybiphenyl
3D	Three dimensional
Amp	Ampicillin
APS	Ammonium per sulfate
ATP	Adenosine Tri-Phosphate
BDS	Biodesulfurization
Bp	base pair
BSA	Bovine serum albumin
BNT	Benzonaphthothiophene
BT	Benzothiophene
CD	Circular dichroism
ChIP	Chromatin immunoprecipitation
Cm	Chloramphenicol
DBRR	DNA binding response regulator
DBT	Dibenzothiophene
DBTS	Dibenzothiophene sulfone
DEPC	Diethyl pyrocarbonate
DLS	Dynamic light scattering
DMSO	Dimethyl sulfoxide

dNTP	Deoxy ribose nucleotide triphosphate
Dsz	Desulfurization
DTT	Dithiothreitol
EDTA	Ethylene diamine tetra acetic acid
EMSA	Electro mobility shift assay
Fig	Figure
FMN	Flavin mononucleotide
GFP	Green Fluorescent Protein
Gibbs Reagent	4,6 dichloroquinone-4-chlorimide
Gm	Gram
HCl	Hydrochloric acid
HDS	Hydrodesulfurization
HBPS	2-hydroxybiphenyl-2-sulfinate
HTH	Helix turn helix
IPTG	Isopropyl β-D thio galactopyranoside
IMAC	Immobilized metal ion affinity chromatography
Kan	Kanamycin
Kb	Kilobase pair
KDa	Kilodalton
LA	Luria Bertani Agar
LacZ	β-galactosidase

LB	Luria Broth
LB	Luria Bertani Broth
M	Molar
MALDI	Matrix assisted laser desorption ionization
MCC	Microbial culture collection
Mg	Milligram
Mm	Molar
MW	Molecular weight
NADH	Nicotinamide adenine dinucleotide -Hydrogen
Ni-NTA	Nickel-nitrilotriacetic acid
Nm	Nanometer
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase Chain Reaction
PMSF	Phenylmethane sulfonyl fluoride
ppm	Parts per million
RNase	Ribonuclease
Rpm	Revolution per minute
SDS	Sodium dodecyl sulphate
SILAC	Stable isotope labeling with amino acids in cell culture
SO_x	Sulfur oxide

TBE	Tris-borate-EDTA
TEMED	N,N,N’,N’-Tetramethylethylenediamine
Tet	Tetracycline
Tris	Tris (hydroxymethyl) amino methane
UV	Ultra violet
XRE	Xenobiotic response element