DECLARATION

I, Smriti Pradhan, hereby declare that the work embodied in my thesis entitled "**Studies on siderophore producing and arsenic resistant bacteria isolated from agricultural soil and their role in biocontrol and bioremediation**" has been carried out by me under the supervision of Dr. Dipanwita Saha, Professor, Department of Biotechnology, University of North Bengal for the award of the Degree of Doctor of Philosophy in Biotechnology. I also declare that, this thesis or any part thereof has not been submitted for any other degree/diploma either to this or any other university.

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This is to certify that Ms. Smriti Pradhan, M.Sc. has worked under my supervision at the Department of Biotechnology, University of North Bengal for Ph.D. thesis entitled "**Studies on siderophore producing and arsenic resistant bacteria isolated from agricultural soil and their role in biocontrol and bioremediation**". I am forwarding her thesis for the Ph. D. Degree (Science) in Biotechnology of the University of North Bengal. I recommend that she has fulfilled all requirements according to the rules of the University of North Bengal regarding the works embodied in her thesis.

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PREFACE

Global industrialization and agricultural practices have caused extensive accumulation of organic and inorganic pollutants in the environment. Among the inorganic pollutants, heavy metals are considered as the primary contaminant which gets accumulated in the environment due to their non- biodegradable nature. Arsenic is a toxic metalloid which exists everywhere in nature. Arsenic exposure higher than the permissible limit can lead to several health problems with diseases like melanosis, hyperkeratosis, lung disease, peripheral vascular diseases, gangrene, diabetes mellitus as well as cancer. It is estimated that more than 500 million people worldwide are at a risk of excessive levels of arsenic exposure through various sources. Arsenic contaminated drinking water and crops grown in arsenic contaminated agricultural fields are the main route for arsenic entry into human food chain. Indeed, several remediation strategies have been implemented for successful removal of this toxic element from ground water as well from soil. However, these are accompanied with certain limitations including the high costs as well as generation of large amount of secondary chemical sludge which ultimately harms the environment. Therefore, research works are focused on the use of biological methods as ecofriendly and cost effective option for arsenic remediation.

Agriculture plays an important role in the economic development of the country and India is considered as a global powerhouse of agriculture. The world population is expected to reach approximately 9.1 billion by 2050, therefore it is estimated that agricultural food production needs to be increased by around 70%. To meet the growing demands for food, farmers are becoming more dependent on the use of different chemicals in the form of pesticides and fertilizers for controlling the phytopathogen attack and improving plant growth. However, the excessive use of such chemicals lead to several negative impacts to crops, human who consume them as well as to the environment. Therefore, the scientific community should take the responsibility of combating these problems and contribute towards the development of a healthy, ecofriendly and sustainable agriculture practices. At present, the use of biological agents as alternative method for plant disease management has gained a lot of attention in developing a sustainable agriculture for safer food production. Biocontrol agents uses

different mechanisms to kill/inhibit the growth of pathogens which include the direct antagonism by producing antifungal metabolites like hydrolytic enzymes and antibiotics or indirect antagonism by rhizosphere competition as well as inducing systemic resistance. Siderophore production is one of the most studied competitive pathogen inhibition observed in the biocontrol agents where they chelate the iron with the help of siderophore and make it unavailable for the pathogens. Moreover, the role of siderophores in mobilization of different metals from the contaminated sites has also been studied by many researchers.

Considering this background, this work was commenced with the main aim of providing an ecofriendly approach by using rhizosphere bacteria for sustainable agriculture with proper disease management as well as arsenic cleanup from the environment. This thesis initiates with an introduction to the subject of study followed by detailed literature review of the past, and the methods adopted. The results obtained in this study is represented in six major chapters and the overall inference has been documented separately. The additional supportive materials are provided in the appendices at the end of the thesis.

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ABBREVIATIONS

A_r	Absorbance of reference	**dsDNA**	Double stranded DNA
A_s	Absorbance of sample	**EDTA**	Ethylene diamino tetraacetic acid
ANOVA	Analysis of Variance	**et al.**	Et alia
As	Arsenic	**Fig.**	Figure
AsV	Arsenate	**FTIR**	Fourier Transform Infrared
AsIII	Arsenite	**g**	gram
BCA	Biocontrol agent	**GIS**	Geographical Information System
bp	base pair	**h**	hour
BLAST	Basic Local Alignment Search Tool	**HCN**	Hydrogen cyanide
BSA	Bovine Serum Albumin	**HDTMA**	Hexadecyltrimethyl-ammonium bromide
°C	Degree Celcius	**IAA**	Indole-3-acetic acid
CAS	Chrome Azurol Sulphonate	**IARI**	Indian Agricultural Research Institute
cfu	Colony forming unit	**IPTG**	Isopropyl β-D-1-thiogalactopyranoside
cm	Centimeter	**ITCC**	Indian Type Culture Collection
CMC	Carboxy methyl cellulose	**ITS**	Internal transcribed spacers
Cont.	Continued	**kb**	kilobase
CTAB	Cetyl trimethyl ammonium bromide	**kg**	kilogram
dapi	days after pathogen inoculation	**L**	litre
DMRT	Duncan's Multiple Range Test	**LB**	Luria Bertani
DNA	Deoxyribonucleic acid	**LC-ESI-MS**	Liquid chromatography-electrospray ionization mass spectrometer
DNase	Deoxyribonuclease	**MDI**	Mean Disease Index
		MR	Methyl Red
dNTP	Deoxy nucleotide triphosphate	**µg**	microgram

µl	microlitre	**PEDC**	Percent Efficacy of Disease Control
mM	mili molar	**psi**	per square inch
mg	milligram	**psu**	percent siderophore units
ml	millilitre	**PBS**	Phosphate Buffer Saline
mm	millimeter	**PIPES**	Piperazine-1.4-bis (2-ethanesulphonic acid)
mM	Millimolar	**PGPR**	Plant Growth Promoting Rhizobacteria
M9YE	Minimal 9 Yeast extract medium	**PCR**	Polymerase Chain Reaction
MIC	Minimum Inhibitory Concentration	**PDA**	Potato Dextrose Agar
min	Minutes	**PDB**	Potato Dextrose Broth
M	Molar	**PPL**	Pusa Purple Long
MEGA	Molecular Evolutionary Genetics Analysis	**RF**	Retention Factor
nm	Nanometer	**rRNA**	Ribosomal ribonucleic acid
ng	Nanogram	**rpm**	rotation per minute
N	Normal	**SEM**	Scanning Electron Microscopy
No.	Number	**SDS**	Sodium dodecyl sulphate
NA	Nutrient Agar	**SEA**	Soil Extract Agar
NB	Nutrient Broth	**Soln.**	Solution
NCBI	National Center for Biotechnology Information	**sp.**	Species
NMR	Nuclear Magnetic Resonance	**SPSS**	Statistical Package for the Social Sciences
OD	Optical Density	**TLC**	Thin Layer Chromatography
OD_{260}	Absorbance at 260 nm	**TSI**	Triple Sugar Iron
OD_{280}	Absorbance at 280 nm	**TAE**	Tris acetic acid EDTA
’ (prime)	denotes a truncated gene at the indicated side	**TE**	Tris HCl EDTA

TRIS	Tris hydroxymethyl amino methane
TSA	Tryptic Soy Agar/ Tryptone Soy Agar
U	Unit
UV	Ultra violet
v/v	volume by volume
w/v	weight by volume
W.B.	West Bengal
X-gal	5-bromo-4-chloro-3-indoyl-beta-D-galacto-pyranoside
YEM	Yeast Extract Mannitol