ACC DEAMINASE POSITIVE BACTERIA IN STRESSED AGRICULTURAL ENVIRONMENTS

GAUTAM ANAND

DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

APRIL 2019

ACC DEAMINASE POSITIVE BACTERIA IN STRESSED AGRICULTURAL ENVIRONMENTS

by

GAUTAM ANAND

DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY

Submitted

In fulfillment of the requirements of the degree of Doctor of Philosophy

to the

INDIAN INSTITUTE OF TECHNOLOGY DELHI

APRIL 2019

Dedicated

To

My family

ACKNOWLEDGEMENTS

I would take this opportunity to express my deep sense of gratitude for my supervisor, ***Dr. Shilpi Sharma****. There would not be words enough to describe her contribution in my overall development as a researcher and good human being. She has been an epitome of inspiration in all spheres of life and would forever drive me to work harder and diligently to accomplish any task. I would even consider myself to be lucky to have worked under such as genuine human being who leads you by example to excel in any task. She has been instrumental for inculcating discipline, confidence and a rational scientific approach for the completion of this work. Her constant endeavours to provide the best of facilities, scientific environment, freedom to pursue ideas, brainstorming scientific discussions all have been responsible in the completion of this thesis work and would be an encouragement to for my future research work. Even in moments of great turmoil, she had been the saviour. Finally, I would like to say that my PhD journey has been an exciting, adventurous and pleasant experience all thanks to my supervisor.*

I would like to thank ***Prof. V.S. Bisaria*** *for the constant support, guidance and valuable suggestions during the course of my work. His critical and helpful insights have been very constructive for my work. His constant motivation was a driving force that enabled me to complete this work.*

My sincere thanks to my SRC members ***Prof. Saroj Mishra****,* ***Dr. Preeti Srivastava****, and* ***Dr. K. Annapurna*** *for the valuable suggestions and critical review that helped me to improve my work.*

I would like to thank the present Head of Department, ***Prof. Atul Narang*** *for the kind help and support. I would also like to acknowledge our previous Head of Department,* ***Prof. T.R. Sreekrishnan*** *for the motivation and working bench in his esteemed lab.*

I would like to acknowledge the fellowship received from ***Indian Institute of Technology Delhi*** *and* ***Ministry of Human Resource and Development (MHRD)*** *during the course of my PhD.*

A special thanks to ***Dr. Alicia Chroňáková*** *for welcoming me to work in her lab for three months. She was a source of constant support, motivation and encouragement during my stay in the Czech Republic. I would also like to acknowledge all the members of the lab in the Czech Republic for their kind help and support. I would like to acknowledge* ***European Molecular Biology Organization (EMBO)*** *for the short term fellowship awarded which made my visit to the Czech Republic possible.*

The journey could not have been exciting and pleasant without the unwavering support and help provided by my lab mates ***Rashi ma'am, Sunil, Madhu, Jeny, Swati Sharma, Varsha, Richa, Upma, Vijal, Vasu, Shivashankar, Monika, Nidhi, Sakshi ma'am, Abhineet, Atul, Ayushi, Priyanka, Swati Varshney, Shubham and Shivani****. It was wonderful to*

have such kind and generous people as lab mates and friends. I would regard our group as a unified entity that sails through each hardship together and is always there for each other. I would always be grateful to ***Rashi ma'am, Madhu, Sunil*** *and* ***Jeny*** *who helped me in my early days and welcomed me in the group with warmth and a cheerful attitude. I would also like to acknowledge the support and help provided by the working staff especially* ***Shiv bhaiya, Kishan ji, Santram ji and Mukesh bhaiya.***

I would like to thank my batch-mates and friends ***Sonam, Moolchand, Sakshi, Siddhi, Apoorva, Jitendra*** *and* ***Richa*** *for the support and the wonderful outings and birthday celebrations. I would also like to mention my friends* ***Neelam, Parul, Shubhendu, Anees, Aditya, Gaurav, Vidisha, Piyush, Avinash, Atika, Robin, Preeti****, and* ***Ritam*** *for the wonderful time that we have spent together. I would also like to acknowledge the support and help of my school friends* ***Brijendra, Saif, Anupam*** *and* ***Daud****.*

I would finally want to thank my parents and family for their constant support and blessings without which nothing would have been possible. Saying thanks to my best friend and wife ***Sonam*** *would not be enough, she has been a rock-solid support through every twist and turns and a constant source of enthusiasm, inspiration, love and support.*

Finally, it is the will of the almighty GOD that gave me constant strength and inspiration to follow this path and add meaning to it.

Agriculture, the backbone of uprising Indian economy, is under tremendous pressure to meet the requirements of ever-increasing population. Moreover, there is a constant threat to environmental health with the use of chemical amendments. Hence there is an eminent need for an eco-friendly, long term and sustainable boost in agricultural productivity in the presence of emanating climatic adversities and environmental deterioration. While microbes have been in application in agriculture since decades, they are still not considered an economical alternative because of their poor performance and survivability in field due to competition by resident microflora. ***The objective of the present study was to come up with potent bacterial strains that could serve as plant probiotic and stress buster for pigeonpea under adverse conditions. Hence the focus was on selection of indigenous ACC deaminase producing strain with multiple plant growth promoting (PGP) properties and better survivability in vivo. Besides the non-target impact of such an application was to be established to ascertain ecological health.*** ACC deaminase protects the plant from abiotic and biotic stresses by specifically reducing the deleterious stress ethylene levels. Enumeration of ACC deaminase bacteria was done from rhizosphere of pigeonpea from a control experimental field (without salinity stress), as well as from an experiment designed to simulate salinity and drought stress conditions. Isolates were shortlisted on the basis of various PGP properties such as indole acetic acid production, siderophore production, nitrogen fixation, HCN production and salt tolerance. The isolates were then assessed for plant growth promotion and mitigation of salinity stress under controlled conditions, followed by validation in field. Further, to

understand the impact such an application has on resident microbiome, molecular microbiology techniques of denaturing gradient gel electrophoresis and Illumina sequencing were applied. These bioinoculants not only proved to be efficient in mitigating the stress and enhancing plant growth, but also enriched the soil by its non-target impact of shaping the microbiome towards a more beneficial bacterial community. These isolates hold potential for dual benefit, i.e. plant growth promotion and plant protection, under stressed agricultural environments. ***With a comprehensive polyphasic study from lab to field the potency of ACC deaminase producers has been established, which opens avenues for their widespread application to a range of crops and other types of stresses.***

कृषि, बढ़ती भारतीय अर्थव्यवस्था की रीढ़ है, और बढ़ती जनसंख्या की आवश्यकताओं को पूरा करने के लिए बहुत दबाव में है। इसके अलावा रासायनिक संशोधनों के उपयोग से पर्यावरणीय स्वास्थ्य के लिए लगातार खतरा है। इसलिए कृषि उत्पादन को इको-फ्रेंडली, दीर्घावधि और स्थायी रूप से बढ़ावा देने की आवश्यकता है। वह भी बढ़ती जलवायु विकृतियों और पर्यावरणीय गिरावट की उपस्थिति में। सूक्ष्मजीव कृषि में दशकों से उपयोग कर रहे हैं, लेकिन उन्हें अभी भी एक किफायती विकल्प नहीं माना जाता है जिस का कारण है उनकी फील्ड मै खराब प्रदर्शन और निवासी माइक्रोफ्लोरा द्वारा प्रतिस्पर्धा के कारण उनका उत्तरजीविता खराब होना। वर्तमान अध्ययन का उद्देश्य हैं शक्तिशाली जीवाणु उपभेदों को प्राप्त करना का जो की तनाव की स्थिति में कजानस कजान के लिए पौधे की वृद्धि और तनाव नाशक के रूप में कार्य कर सके। इसलिए प्राथमिकता यह थी की निवासी एसीसी डेमिनमिनस जीवाणु का चयन किया जाया जिसमें अनेक पौधों की वृद्धि को बढ़ावा देने (पी.जी.पी) के गुणों और फील्ड में बेहतर उत्तरजीविता हो। इस अध्ययन का उद्देश्य यह भी था की इस तरह के आवेदन के पारिस्थितिक स्वास्थ्य का पता लगाने के लिए गैर-लक्ष्य प्रभाव स्थापित किया जाना था। ए.सी.सी डेमिनमिनस विशेष रूप से एथिलीन के हानिकारक स्तर को कम करके पौधे को सभी अजैविक और जैविक तनावों से बचाता है। ए.सी.सी डेमिनमिनस जीवाणु की गणना कजानस कजान के रहिजोस्फीयर से की गयी थी जिसमे एक नियंत्रण प्रायोगिक क्षेत्र (लवणता तनाव के बिना) के साथ ही लवणता और सूखे तनाव की स्थिति का अनुकरण करने के लिए एक प्रयोग शामिल थे। उपभेदों के विभिन्न पी.जी.पी गुणों जैसे इंडोल एसिटिक एसिड उत्पादन, साइडरोफोर उत्पादन, नाइट्रोजन नियतन, एच.सी.एन उत्पादन और नमक सहिष्णुता के आधार पर शॉर्टलिस्ट

किया गया था। पौधों की वृद्धि को नियंत्रित करने और नियंत्रित परिस्थितियों में लवणता के तनाव को कम करने की आइसोलेट्स की क्षमता का आकलन भी किया गया था। इसके बाद क्षेत्र में सत्यापन की गयी थी। इसके अलावा, इस तरह के एक अनुप्रयोग के प्रभाव को समझने के लिए निवासी माइक्रोबायोम, आणविक माइक्रोबायोलॉजी तकनीकों में डीएन्ट्यूरिंग ग्रेडिएंट जेल वैद्युतकणसंचलन और इलुमिना अनुक्रमण किए गए थे। ये बायोइनोकुलेंट्स न केवल तनाव को कम करने और पौधे के विकास को बढ़ाने में कुशल साबित हुए, बल्कि माइक्रोफ्लोरा में अधिक लाभकारी जीवाणु समुदाय की ओर आकार देने के अपने गैर-लक्ष्य प्रभाव से मिट्टी को समृद्ध किया। इन आइसोलेट्स के दोहरे लाभ है, पौधों के विकास में बढ़ोतरी और पौधों की सभी अजैविक और जैविक तनावों से सुरक्षा। प्रयोगशाला से लेकर ए.सी.सी डेमिनमिनस उत्पादकों की क्षमता को व्यापक पॉलिफ़ैसिक अध्ययन के साथ स्थापित किया गया है, जो फसलों और अन्य प्रकार के तनावों के लिए उनके व्यापक अनुप्रयोग के लिए मार्ग खोलता है।

LIST OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

Elements and Chemicals

Ca^{2+}: Calcium ion

Mg^{+}: Magnesium ion

K^{+}: Potassium ion

Na^{+}: Sodium ion

$(NH_4)_2SO_4$: Ammonium sulphate

H_3BO_3: Boric acid

CTAB: Cetyl trimethyl ammonium bromide

DEPC: Diethyl pyrocarbonate

EDTA: Ethylenediaminetetraacetic acid

H_2O_2: Hydrogen peroxide

MoO_3: Molybdenum trioxide

NBT: Nitro tetrazolium

NaCl: Sodium chloride

NaOH: Sodium hydroxide

Media

D&F: Dworkin & Foster

LB: Luria Bertani

NA: Nutrient agar

NBRIP: National botanical research institute phosphate solubilizing

PAF: Pseudomonas agar F

TSB: Tryptic soya broth

Important terminology

ACC: 1-aminocyclopropane-1-carboxylic acid

acdR: Gene encoding leucine responsive regulatory protein (LRP; negative regulator of *acdS*)

acdS: Gene encoding ACC deaminase enzyme

ACO: ACC oxidase

ACS: ACC synthase enzyme

bp: Base pairs

CAS: Chroma azurole S

CBZ: Carbamazepine

cDNA: complementary DNA

CFU: Colony forming units

CID: Chemically induced drought

CLIN: Clindamycin

DAS: Days after sowing

DGGE: Denaturing gradient gel electrophoresis

EC: Electrical conductivity

FW: Fresh weight

g: Gram

G-ACC: γ glutamyl-ACC

Ha: Hectare

HS: High salinity

IAA: Indole acetic acid

JA-ACC: Jasmonoyl-ACC

kDA: kilodalton

kg: Kilogram

M-ACC: Malonyl-ACC

MB: Mega-byte

Mha: Million hectare

min: Minutes

s: Seconds

MPa: Megapascal

MS: Medium salinity

MSI: Membrane stability index

NID: Naturally induced drought

OTU: Operational taxonomical unit

PGP: Plant growth promoting

PGPR: Plant growth promoting rhizobacteria

POD: Peroxidase

PS: Phosphate solubilization

rpm: Rotations per minute

RT: Reverse transcriptase

RWC: Relative water content

SAM: S-adenosyl-L-methionine

SOD: Superoxide dismutase

TNA: Total nucleic acid

ANOVA: analysis of variance

DMRT: Duncan's multiple range test

FLASH: Fast length adjustment of short reads

QIIME: Quantitative insight into microbial ecology

UPGMA: Unweighted pair group method with arithmetic mean