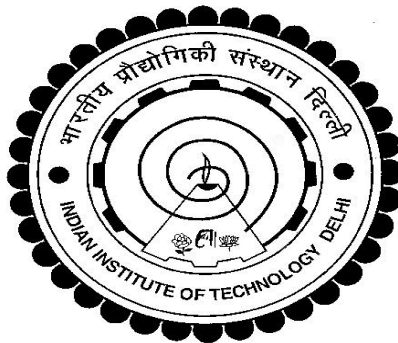


**PROMOTION OF *PANICUM MAXIMUM* JACQ. GROWTH BY
EMPLOYING BENEFICIAL ACC DEAMINASE CONTAINING
RHIZOBACTERIAL INOCULANTS FOR FODDER AND
BIOETHANOL PRODUCTION**

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INDIAN INSTITUTE OF TECHNOLOGY DELHI

JANUARY 2018

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ACC Deaminase Containing Rhizobacterial Inoculants for Fodder and
Bioethanol production**

by

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Submitted

In fulfilment of the requirements of the degree of Doctoral of Philosophy

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CERTIFICATE

This is to certify that the thesis entitled “**Promotion of *Panicum Maximum* Jacq. Growth by Employing Beneficial ACC Deaminase containing Rhizobacterial Inoculants for Fodder and Bioethanol Production**” submitted by **Ms. Garima Tiwari** has been prepared under our guidance with the rules and regulations of Indian Institute of Technology Delhi, India. The research report and results presented in thesis have not been submitted for any degree or diploma in any other institute or university.

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Abstract

All primary production system is depending on land, a non-renewable resource but due to consequences of increases in environmental damage, climatic change and population growth number of wastelands are increased. They are characterized by high soil salinity, infertility, low productivity and limited supply of high quality of water. Extensive research should be carried in developing eco-friendly technology for the reclamation of wasteland. So in view of this Plant growth promoting rhizobacteria who possesses ACC deaminase (ACCd) activity, could be a potential candidate. 1-Aminocyclopropane carboxylic acid (ACC) deaminase is secreted by a wide range of beneficial rhizobacteria which degrades the immediate precursor of ethylene thereby reducing the adverse effect on plants. Furthermore they also known to have other beneficial traits such as, Phosphate solubilisation, Nitrogen fixation, production of IAA and other plant hormone, lytic enzymes, siderophore, antioxidants and volatiles. They induce systemic resistance (ISR) against wide range of insects and pathogens, and induce systemic tolerance (IST) against various abiotic stresses. Different approaches involving inoculation of ACCd⁺ rhizobacteria, and management practices have been studied to enhance biomass yield in several plants against abiotic stress. This thesis consists of four studies aimed at examining (1) Isolation, screening (for abiotic stress), identification and efficacy evaluation of native rhizobacterial strains for biomass quantity and quality improvement (2) Evaluating the potential of selected ACCd⁺ positive rhizobacterial isolates for plant growth and biomass quality improvement under abiotic stress conditions (3) Evaluating the potential of biomass produced for bioethanol and fodder applications.

Plants exposed to abiotic stress exhibits morphological, physiological and biochemical variations which lead to survival of plants by scarifying the overall biomass character. Under stress increased concentration of ethylene in the plant is eventually responsible for the diminished quality and quantity of biomass. 37 ACCd⁺ rhizobacteria were isolated from 25 rhizospheric soils of organically grown *P. maximum* and characterized for their beneficial traits. All bacteria were found positive for root colonization and Indole acetic acid and found improving plant growth under normal conditions. Microscopic analysis, biochemical tests and 16s rRNA gene sequence analysis results revealed that the 73 % of the bacteria belongs to *Bacillus* spp. By considering the importance of each beneficial trait a weightage chart was prepared. Upon applying different weightage to different beneficial traits, 13 bacteria recorded highest total score which were selected for next stage of screening. Further these 13 bacteria

were tested for their potential to enhance the biomass characters (shoot length, root length, leaf area, dry matter, dry/fresh weight ratio, relative water content, ion leakage, total phenol, total chlorophyll, proline, total soluble sugar and total soluble proteins) under salt and drought stress conditions. In comparison with plants growth under normal conditions, all the biomass characters were given weightage and a total score were generated for each bacteria. Among 13 bacteria tested, highest of 1036.3 score was recorded by isolate 22F2 followed by 7D and 20B. The least score was recorded in control plants (695.6). Further based on the overall performance three bacteria (20B, 7D and 22F2) were selected. In last phase of experiment the normal, control and treated plants, with or without drought and salt stress were grown up to 60 days and subjected to various analysis to determine its potential for fodder and fuel application. In control plants, cell wall analysis revealed that upon exposure to stress conditions cellulose and hemicellulose content was significantly ($P \leq 0.05$) reduced and lignin was increased in comparison to normal plants. Isolate 5JD and 20B treatment recorded higher tendency to improve these biomass characters towards normal plants. This was also evident by the significant ($P \leq 0.05$) increase in total reducing sugar and theoretical bioethanol yield obtained from bacterial treatment. On the other hand the fodder value of rhizobacteria treated plants were maintained close to normal plants in comparison to control plants. Fodder quality was improved with enhanced Nutrient Detergent Fibre (NDF) and crude protein in inoculated plants in comparison to control. Whereas ADF values among different treatments, control and normal plants was not significant. In control plants under both stress conditions a significant decrease in total crude protein was observed which was found near half the concentration recorded in normal and treated plants. Similar trend was maintained in plant nutrients where normal plants maintained a higher level of N, P and K and control plants recorded least nutrient characters. Plants treated with rhizobacteria recorded a significant increase in N, P and K accumulation over control and lesser than normal plants.

Morphological, physiological and biochemical parameters analysed for bacteria treated, control and normal plants revealed the potential of ACCd⁺ rhizobacteria in improving biomass character for fodder and fuel applications. From the results, it was evident that along with ACCd other beneficial traits of rhizobacteria synergistically improve the plant growth and health under abiotic stress. The present study gave a new dimension for the applications of ACCd⁺ beneficial rhizobacteria wherein the marginal and degraded land can be brought under cultivation to grow fodder and fuel crops without compromising the quantity and quality of biomass.

सार

सभी प्राथमिक उत्पादन प्रणाली भूमि पर निर्भर करती है, यह एक गैर-नवीकरणीय संसाधन है लेकिन, पर्यावरणीय क्षति, जलवायु परिवर्तन और जनसंख्या वृद्धि में वृद्धि के परिणामों की वजह से बंजर भूमि की संख्या में वृद्धि हुई है। इन भूमि में अधिक मात्रा में लवणता, बांझपन, और कम उत्पादकता पाई जाती है। बंजर भूमि के पुनर्मूल्यांकन के लिए पर्यावरण अनुकूल प्रौद्योगिकी विकसित करने में पर व्यापक शोध होना चाहिए। इस दिशा में ACC डीमिनेश (ACCd⁺) की गतिविधि रखने वाला रेजोबैक्टीरिया एक संभावित उम्मीदवार हो सकता है। लाभकारी रेजोबैक्टीरिया की एक विस्तृत श्रृंखला 1-अमिनोसीक्लोप्रोपेन कार्बोक्जिलिक एसिड (एसीसी) डेमिनेज को स्रावित करती है जो पौधों पर एथिलीन की प्रतिकूल असर को कम करता है। इसके अलावा ये बैक्टीरिया अन्य लाभकारी गुण जैसे कि फास्फेट solubilisation, नाइट्रोजन निर्धारण, आईएए का उत्पादन और अन्य पौधों के हार्मोन, lytic एंजाइम, साइडोरोफोर, एंटीऑक्सिडेंट और वाष्पशील को उत्पन्न करने हेतु भी जाना जाता है। ये बैक्टीरिया पौधे को अबियाटिक तनाव में खड़े रहने की सहनशीलता भी प्रदान करते हैं रहने तनाव की स्थिति में बाइयोमास मात्रा एवम गुणवत्ता सुधार के लिए, ACC +राइजोबैक्टीरिया का विभिन्न तरीकों से अध्ययन किया गया है। इस थीसिस में अध्ययन के उद्देश्य से चार अध्ययन होते हैं (1) अलगाव, स्क्रीनिंग (अबाउटिक तनाव के लिए), देशी की पहचान, बायोमास मात्रा और गुणवत्ता सुधार के लिए राइजोबैक्टीरिया स्ट्रेन्स का प्रभावकारिता मूल्यांकन (2) अबियाटिक तनाव की स्थिति में पौधे की वृद्धि और बायोमास गुणवत्ता सुधार के लिए चयनित एसीसी + पॉजिटिव रेजोबैक्टीरियाल आइसोलेट्स का मूल्यांकन (3) बायोमास की क्षमता का मूल्यांकन बायोएथेनॉल और चारा अनुप्रयोगों के लिए।

जब पौधे अबियाटिक तनाव की स्थिति में आते हैं तो उनके अंदर कई रूपतमक शारीरिक और जैव रसायनिक परिवर्तन आते हैं। ज्यादा तनाव की स्थिति में पौधे के अंदर अधिक मात्रा में एटाइलिन उत्पन्न होता है जिसकी वजह से पौधे की गुणवत्ता में कमी आती है। *P. maximum* की 25 राइजोस्फियरिक मिट्टी से 37 ACC बैक्टीरिया निकले गये और उनके लाभकारी विशेष लक्षणों का मूल्यांकन किया गया। सभी बैक्टीरिया को रूट उपनिवेश और इंडोल एसिटिक एसिड के लिए सकारात्मक पाया गया माइक्रोस्कोपिक विश्लेषण, जैव रासायनिक परीक्षण और 16 आरआरएनए जीन अनुक्रम विश्लेषण के परिणाम बताते हैं कि बैक्टीरिया का 73% हिस्सा बेसिलस spp. का है। प्रत्येक लाभकारी गुण के महत्व को ध्यान में रखते हुए एक वेटेज चार्ट तैयार किया गया। विभिन्न फायदेमंद गुणों के लिए विभिन्न भार लागू करने पर, 13 बैक्टीरिया दर्ज किए गए उच्चतम स्कोर जो स्क्रीनिंग के अगले चरण के लिए चुने गए थे। आगे इन 13 बैक्टीरिया द्वारा नमक और सूखा तनाव की स्थिति में तनाव की स्थिति में बायोमास लक्षण जैसे की (शूट लम्बाई, जड़ की लंबाई, पत्ती क्षेत्र, शुष्क पदार्थ, शुष्क / ताजा वजन अनुपात, सापेक्ष जल सामग्री, आयन रिसाव, कुल फिनोल, कुल क्लोरोफिल, प्रोटीन, कुल घुलनशील चीनी और कुल घुलनशील प्रोटीन) को बढ़ाने की क्षमता का परीक्षण किया गया शर्तों सामान्य परिस्थितियों में पौधों के विकास की तुलना में, सभी बायोमास पात्रों को वेटेज दिया गया था और कुल स्कोर प्रत्येक बैक्टीरिया के लिए उत्पन्न हुए थे परीक्षण के बाद 13 बैक्टीरिया के बीच में अधिकतम स्कोर (1036.3) 22F2 बैक्टीरिया द्वारा दर्ज किया गया था, इसके बाद 7D और 20B द्वारा दर्ज किया गया। कम से कम अंक नियंत्रण पौधों (695.6) में दर्ज किए गए थे। अच्छे प्रदर्शन के आधार पर आगे तीन बैक्टीरिया (20B, 7D और 22F2) का चयन किया गया। प्रयोग के आखिरी चरण में सामान्य, नियंत्रण और इलाज पौधों को, चारा और ईंधन आवेदन की क्षमता निर्धारित करने के लिए सूखा और नमक तनाव के बिना एवम तनाव में 60 दिनों तक बढ़ाया गया, सेल दीवार विश्लेषण से पता चला है कि तनाव की स्थितियों के संपर्क में सेलूलोज और हेमिसेल्यूलोस सामग्री काफी ($p < 0.05$) कम थी और लिग्निन में वृद्धि हुई थी सामान्य पौधों की तुलना में 5JD और 20B द्वारा ट्रीटमेंट करने पर उच्च बायोमास गुणवत्ता पाई गयी

बॅक्टीरिया द्वारा नियंत्रित पौधों में अधिक मात्रा में चीनी ($p < 0.05$) एवम सैद्धांतिक बायोएथेनॉल उत्पादन में महत्वपूर्ण वृद्धि देखी गयी सामान्य पौधों की तुलना राइज़ोबॅक्टीरिया द्वारा ट्रीटेड पौधों में बढ़ी हुई नाट्रेयिंट डिटरजेंट फाइबर (एनडीएफ) एवम क्रूड प्रोटीन की मात्रा के साथ अधिक चारा मुल्य प्राप्त हुआ। वही जबकि आसिड न्यूट्रियेंट फाइबर (एडफ) विभिन्न उपचार, नियंत्रण और सामान्य पौधों में सामान्य और इनॉक्युलेटेड पौधों की तुलना में नियंत्रित पौधे में दोनों तनाव की स्थितियों में कम क्रूड प्रोटीन की मात्रा देखी गयी बॅक्टीरिया ने पौधों में तनाव की स्थिति में भी पोषक तत्वों N, P और K की समान प्रवृत्ति को बनाए रखा। वर्तमान स्टडी ने अग्रिकल्चर की दिशा में एक नायाम दिया कि राइज़ोबॅक्टीरिया के उपयोग से बंजर और कम उत्पादकता वाली भूमि को भी बेहतर व उत्पादक बनाया जा सकता है एवम इनका उपयोग करके बायोमास को चारा और ईंधन के प्रयोग हेतु उगाया जा सकता है।

CONTENTS

ACKNOWLEDGEMENTS	i-ii
ABSTRACT	iii-iv
CONTENTS	v-ix
LIST OF FIGURES	x-xi
LIST OF TABLES	xii-xiii
ABBREVIATION	xiv
CHAPTER 1: INTRODUCTION	1-14
1.1. Scope of work	13
1.2. Study objectives	14
CHAPTER 2: REVIEW OF LITERATURE	15-46
2.1. Grasses for wasteland utilization	16-17
2.2. <i>Panicum maximum</i> (Guinea grass)	17-19
2.3. Abiotic stress	19-22
2.3.1. <i>Drought stress and salt stress</i>	19
2.3.2. <i>Effect of salt & drought stress on plant growth & metabolism</i>	19-22
2.4. Plant adaptation under stress condition	22-25
2.4.1. <i>Rooting characteristics for water uptake</i>	23
2.4.2. <i>Osmolytes and osmoprotectants</i>	23-24
2.4.3. <i>Relative water content</i>	24
2.4.4. <i>Altering antioxidant defense system</i>	24
2.4.5. <i>Ethylene and plants</i>	25
2.5. ACC deaminase: a weapon protection tool to “ethylene”	25-32
2.5.1. <i>Biochemical properties of ACC deaminase</i>	29
2.5.2. <i>ACC deaminase gene regulation</i>	29-31
2.5.3. <i>Ecological significance of ACC deaminase</i>	32
2.5.4. <i>Transgenic Plants with ACC Deaminase Activity & its disadvantages</i>	32

2.6. Plant growth promoting rhizobacteria and their beneficial traits	33-38
2.6.1. <i>Root colonization</i>	34
2.6.2 <i>Phytohormone production</i>	34
2.6.3. <i>Biological nitrogen fixation</i>	35
2.6.4. <i>Phosphate solubilisation</i>	35-37
2.6.5. <i>Biofilm release</i>	37-38
2.6.6. <i>Induced systemic resistance</i>	38-40
2.7. Fodder and energy crisis	40-46
2.7.1 <i>fodder crisis</i>	40-43
2.7.2 <i>Energy crisis</i>	44
2.7.2.1 <i>Ethanol production in India</i>	45-46
CHAPTER 3: MATERIALS AND METHODS	47-68
3.1. Field survey and rhizosphere soil sample collection	47
3.2. Procurement of biological samples	47
3.3. Improvement of qualitative and quantitative beneficial traits of selected grass species using plant growth promoting rhizobacteria	48-55
3.3.1. <i>Isolation of ACCd⁺ bacteria</i>	49
3.3.2. <i>Quantitative assay of ACC deaminase</i>	49-51
3.3.3. <i>Molecular detection of Acds gene in rhizobacteria</i>	51-53
3.3.4. <i>Biochemical characterization</i>	53
3.3.5. <i>Abiotic stress tolerance in ACCd⁺ Rhizobacteria</i>	53-54
3.3.6. <i>Characterization of ACCd⁺ rhizobacteria for beneficial traits</i>	55-55
3.3.6.1. <i>Root colonization</i>	55
3.3.6.2. <i>Plant growth promotion</i>	55
3.3.6.3. <i>IAA production</i>	55-56
3.3.6.4. <i>Biofilm production assay</i>	56
3.3.6.5. <i>Phosphate solubilisation</i>	56-57
3.3.6.6. <i>HCN production</i>	57
3.3.6.7. <i>in vitro Antagonism assay</i>	57-58

3.4. Studies on effect of selected ACCd⁺ rhizobacterial isolates on plant growth and biomass quality improvement under salt and drought stress conditions	58-63
3.4.1. Screening of selected rhizobacteria for inducing drought stress	
tolerance in host plant	58-59
3.4.2. Screening of selected rhizobacteria for inducing salt stress	
tolerance in host plant	59
3.4.3. Plant growth parameter and physiological and biochemical parameters	60-59
3.4.3.1. <i>Relative water content (RWC)</i>	60
3.4.3.2. <i>Electrolyte leakage</i>	60-61
3.4.3.3. <i>Photosynthetic pigment</i>	61
3.4.3.4. <i>Soluble protein content</i>	61
3.4.3.5. <i>Proline content</i>	62
3.4.3.6. <i>Total soluble sugar estimation</i>	62
3.4.3.7. <i>Total phenol activity</i>	62-63
3.5. Evaluation of produced biomass for fodder and bioethanol production	63-65
3.5.1. Chemical Characterization of biomass for fodder value	63
3.5.2. Bioethanol application of enhanced biomass of <i>P. Maximum</i>	64-62
3.5.2.1. <i>Effect of pretreatment on plants inoculated with best identified ACCd⁺ isolates</i>	64
3.5.2.2. <i>Chemical analysis of biomass after pretreatment</i>	64
3.5.2.3. <i>Enzymatic hydrolysis and theoretical ethanol yield</i>	65
3.6. Optimization of pretreatment for enhanced reducing sugar production	65-64
3.6.1. <i>Preliminary pretreatment of P. maximum biomass</i>	65
3.6.2. <i>Optimization of pretreatment by response surface methodology</i>	65-66
3.6.3. <i>Physicochemical characterization of the feedstock</i>	66-64
3.6.3.1. <i>FTIR analysis</i>	66
3.6.3.2. <i>XRD analysis</i>	66-67
3.6.3.3. <i>AFM analysis</i>	67
3.6.3.4. <i>SEM analysis</i>	67

CHAPTER 4: RESULTS AND DISCUSSION**4.1. Isolation and characterization of ACC deaminase producing beneficial rhizobacteria from the rhizosphere of P. Maximum 70-111**

4.1.1. Field survey, procurement of grass seeds and rhizospheric soil	
sample collection	70-71
4.1.2. Isolation, screening and characterization of ACCd ⁺ isolates	71-77
4.1.3. Identification of all 37 ACCd ⁺ isolates	78-79
4.1.3.1. <i>Biochemical characterization of selected isolates</i>	78
4.1.3.2. <i>Molecular characterization of selected isolates</i>	79-82
4.1.4. Abiotic stress tolerance (Salt, temperature and water) in ACCd ⁺ isolates	83-88
4.1.5. Characterization of ACCd ⁺ isolates for beneficial traits	89-109
4.1.5.1. <i>Root colonizing ability</i>	89-90
4.1.5.2. <i>Indole acetic acid (IAA) production</i>	90-92
4.1.5.3. <i>Biofilm production</i>	93-95
4.1.5.4. <i>Phosphate production</i>	96-98
4.1.5.5. <i>Antagonistic activity against Fusarium verticillioides and Aspergillus flavus</i>	98-102
4.1.5.6. <i>HCN production</i>	102-103
4.1.5.7. <i>Plant growth promotion studies</i>	104-108
4.1.6. Score chart for screening of isolates for pot study	109-111

4.2. ACCd⁺ rhizobacterial isolates to induce systemic tolerance against under abiotic stress (Salt and Drought)**4.2.1. Effect of ACCd⁺ on physiological and biochemical parameters**

4.2.1. <i>Growth parameters</i>	113-123
4.2.1.1. <i>Shoot and root length, leaf area</i>	111-117
4.2.1.2. <i>Dry matter</i>	117-119
4.2.1.3. <i>Dry weight / Fresh weight ratio</i>	119-121
4.2.1.4. <i>Relative water content</i>	121-123

4.2.2. <i>Biochemical parameters</i>	123-126
4.2.1.5. <i>Ion leakage</i>	123-125
4.2.1.6. <i>Chlorophyll</i>	125-128
4.2.3. <i>Osmolytes</i>	128-135
4.2.4 <i>Total phenol determination</i>	135-137
4.2.2. Score chart	138-139
4.3. Effect of ACCd⁺ rhizobacterial treatment on cell wall composition of <i>P. maximum</i> grown under drought and salt stress	140-142
4.4. Effect of selected ACCd⁺ rhizobacterial treatment on plant growth and cell wall composition of <i>P. maximum</i> grown under drought and salt stress at harvest period	143-148
4.4.1. <i>Growth Parameters</i>	143-144
4.4.2. <i>Fodder Characteristics</i>	144-147
4.4.3. <i>Effect of selected 3 ACCd⁺ isolates on grass biomass for bioethanol production</i>	147-150
4.5. Standardization of pretreatment for <i>P. maximum</i>	
4.5.1. <i>Screening method of different pretreatment methods</i>	151-152
4.5.2. <i>Evaluation of effect of variables on reducing sugar during pretreatment by response surface methodology</i>	152-164
4.5.2.1. <i>Screening of Factors by PBD</i>	153-156
4.5.2.2. <i>Optimization of parameters</i>	156-161
4.5.2.3. <i>Physicochemical characterization of the feedstock</i>	162-166
4.5.2.3.1. <i>XRD Analysis</i>	162-163
4.5.2.3.2. <i>AFM and SEM Analysis</i>	163-164
4.5.2.3.3. <i>FTIR Spectra</i>	165-166
CHAPTER 5: SUMMARY AND CONCLUSIONS	167-173
REFERENCES	174-201
CURRICULUM VITAE	201-206

LIST OF FIGURES

Figure.1.1	(a) Distribution of wasteland across different states of India; (b) Drought affected areas in India (c) State wise distribution of salt affected soils in India
Figure.1.2	(a) Sources of energy consumed in the world (b) History of the world's annual consumption of energy from various sources. The dashed lines show the average rate of increase from 1970 to 2002 and from 2002 to 2006 (c) Energy emission trends 2005-2011
Figure.1.3	Figure 1.3. Biomass quality and quantity enhancement of <i>Panicum maximum</i> by employing ACCd ⁺ isolates with additional beneficial traits under stressful conditions (Salt and Drought) for bioethanol and fodder application
Figure 2.1	State-wise distribution of degraded land in India
Figure.2.2	Overview of salt and drought stress responses in plants. Salt and drought affect cell physiology and metabolism and as consequence reduce plant growth
Figure.2.3	A brief model of PGPR producing ACC deaminase and mechanism of lowering ethylene level in plant tissue
Figure.2.3	Model of the transcriptional regulation of ACC deaminase expression in <i>Pseudomonas putida</i> UW4
Figure.2.4	Different mechanism of PGPR involved in plant growth and health improvement
Figure.2.5	Various organic/inorganic substances produced by PSB responsible for phosphate solubilisation in soils
Figure.2.6	Role of PGPR against drought, salt and fertility stresses underground
Figure.2.7	Ethanol Production, Supply and Consumption in India
Figure.3.1	Flow chart of work
Figure.3.2	Ninhydrin-ACC assay for screening ACC-utilizing bacteria
Figure.4.1	Field survey and rhizosphere soil sample collection in and around IGFRI, Jhansi and also in some surrounding villages.
Figure.4.2	Isolation and pure cultures of rhizobacterial
Figure.4.3	ACC utilization assay
Figure.4.4	ACC degradation by isolates (Colorimetric ninhydrin assay) and α -ketobutyrate production by isolates (α -ketobutyrate assay)
Figure.4.5	Agarose gel showing the PCR amplified product of Accs gene confirming the ACCd activity in rhizobacterial isolates
Figure.4.6	Occurrence of ACCd ⁺ rhizobacteria in the rhizosphere soil sample of <i>Panicum maximum</i> collected in IGFRI and surrounding fields
Figure.4.7	Biochemical test (a) Gram's test (b) Catalase test (c) KOH test, and (d) Biochemical characters of selected bacteria tested using Himedia test kit.
Figure.4.8	A typical gel showing the amplified product of 16srRNA gene from ACCd ⁺ bacterial isolates.
Figure.4.9	Composition of different ACCd ⁺ rhizobacteria isolated from rhizosphere soil of <i>Panicum maximum</i>
Figure.4.10	Temperature tolerance capability of ACCd ⁺ rhizobacterial isolates
Figure.4.11	Water stress tolerance capability of ACCd ⁺ rhizobacterial isolates
Figure.4.12	Salt tolerance capability of ACCd ⁺ rhizobacterial isolates
Figure.4.13	IAA production by ACCd ⁺ rhizobacterial isolates
Figure.4.14	Indole acetic acid production (μ g/ml) by ACC positive isolates
Figure.4.15	Biofilm production by ACCd ⁺ rhizobacterial isolates

Figure.4.16	Biofilm production (Optical density) by ACC positive isolates
Figure.4.17	Phosphate solubilization by ACCd ⁺ isolates on Pikovskaya's medium
Figure.4.18	<i>in vitro</i> Antagonism assay: inhibition zone by isolates against (a) <i>Fusarium oxysporum</i> and (b) <i>Aspergillus niger</i>
Figure.4.19	HCN production: development of brown color in picric acid saturated strips indicates the HCN production by isolates
Figure.4.20	Effect of ACCd ⁺ rhizobacterial isolate on plant growth promotion of <i>P. maximum</i>
Figure.4.21	Effect of ACCd ⁺ rhizobacterial isolates treatment on (a) shoot length and (b) root length of <i>P. maximum</i> .
Figure.4.22	Effect of ACCd ⁺ rhizobacterial isolates treatment on (a) (d) leaf area (mm ²) and (b) dry matter of <i>P. maximum</i> .
Figure.4.23	Score chart representing total scores achieved by each ACCd ⁺ rhizobacterial isolates
Figure.4.24	Pot study for evaluating the potential of ACCd ⁺ isolates for the growth promotion of <i>P. maximum</i> under abiotic stress condition (Drought and salt)
Figure.4.25	Effect of ACCd ⁺ rhizobacterial isolates on shoot and root length of <i>P. maximum</i> grown under (a) drought and (b) salt stress
Figure.4.26	Effect of ACCd ⁺ rhizobacterial isolates on shoot and root length of <i>P. maximum</i> grown under salt stress conditions
Figure.4.27	Effect of ACCd ⁺ rhizobacterial isolates on leaf area (mm ²) of <i>P. maximum</i> grown under (a) drought and (b) salt stress
Figure.4.28	Effect of ACCd ⁺ rhizobacterial isolates on DM (g/ seedlings) of <i>P. maximum</i> grown under (a) drought and (b) salt stress
Figure.4.29	Effect of ACCd ⁺ rhizobacterial isolates on DW/FW ratio of <i>P. maximum</i> grown under (a) drought and (b) salt stress
Figure.4.30	Effect of ACCd ⁺ rhizobacterial isolates on proline content of <i>P. maximum</i> grown under (a) drought and (b) salt stress conditions
Figure.4.31	Effect of ACCd ⁺ rhizobacterial treatment on total sugar content <i>P. maximum</i> grown under drought (a) and (b) salt stress conditions
Figure.4.32	Effect of ACCd ⁺ rhizobacterial isolates on total soluble protein (mg g ⁻¹ FW) in leaves of <i>P. maximum</i> under drought and (b) salt stress
Figure.4.33	Effect of all 13 selected ACCd ⁺ rhizobacterial isolates on Total phenol content in shoot (a) and in root (b) under salt stress
Figure.4.34	Total score achieved by selected 13 ACCd ⁺ rhizobacterial isolates in improving the health and growth of host plants under drought (a) and salt (b) stress condition
Figure.4.35	Effect of all 13 selected ACCd ⁺ rhizobacterial isolates on lignocellulosic content of <i>P. maximum</i> under (a) drought and (b) salt stress
Figure.4.36	60 day old plants showing effect of best identified isolates under drought and salt stress
Figure.4.37	Normal Plots of Residuals for reducing sugar release by PBD for <i>P. maximum</i>
Figure.4.38	Effect of various selected parameters on reducing sugar production in <i>P. maximum</i> . (a: substrate v/s chemical loading; b: temperature v/s sample loading; c: temperature v/s chemical loading)
Figure.4.39	X-ray diffractogram for a) treated and control b) <i>P. maximum</i>
Figure.4.40	Scanning electron microscopic images of native (a) and treated (b) <i>P. maximum</i>
Figure.4.41	Atomic force microscopy images of native (a) and treated (b) <i>P. maximum</i>
Figure.4.42	FTIR Spectra of A) Native and B) pretreated <i>P. maximum</i>

LIST OF TABLES

Table.2.1.	Detail description about <i>Panicum maximum</i>
Table.2.2.	ACC deaminase production by bacteria to protect plants against abiotic stress
Table.2.3.	ACC deaminase activity of different bacteria
Table.2.4.	Growth promoting substances released by PGPR
Table.2.5.	Growth of Livestock Population and Milk production in India
Table.2.6.	Demand and availability of green and dry fodder in India (Million tonnes)
Table.2.7.	India: Ethanol Used as Fuel and Other Industrial Chemicals (Million Liters)
Table.4.1	List of ACC utilizing rhizobacteria identified and used in this study
Table.4.2	<i>In vitro</i> phosphate solubilisation by ACCd ⁺ bacterial isolate
Table.4.3	<i>in vitro</i> Antagonism assay: inhibition zone by isolates against (a) <i>Fusarium verticilloides</i> and (b) <i>Aspergillus flavus</i>
Table.4.4	Physicochemical chemical characteristics of soil used for the experiments
Table.4.5	Weightage and maximum value obtained for beneficial traits tested for ACCd ⁺ rhizobacteria
Table.4.6	RWC (%) and ion leakage (%) in of <i>P. maximum</i> , treated with ACCd ⁺ rhizobacterial isolates under drought and salt stress conditions
Table.4.7	Chlorophyll a, b and total chlorophyll content of leaf of <i>P. maximum</i> , treated with ACCd ⁺ rhizobacterial isolates under drought stress conditions
Table.4.8	Chlorophyll a, b and total chlorophyll content of leaf of <i>P. maximum</i> , treated with ACCd ⁺ rhizobacterial isolates under salt stress conditions
Table.4.9	Maximum and minimum value obtained for selected characters of plants receiving various treatments grown under drought and salt stress
Table.4.10	Effect of selected ACCd ⁺ bacterial treatment on shoot, root length and dry matter
Table.4.11	Effect of best identified ACCd ⁺ on NDF (%), ADF (%) and crude protein (%)
Table.4.12	Effect of best identified ACCd ⁺ isolates on mineral (N, P, K) and ash content (%)
Table.4.13	Optimized parameters for reducing sugar production by sodium hydroxide (NaOH) pretreatment for <i>P. maximum</i> biomass
Table.4.14	Theoretical ethanol yield after pre-treatment from inoculated and non-inoculated <i>P. maximum</i>
Table.4.15	Preliminary pre-treatments and their effect on cell wall composition and reducing sugar
Table.4.16	Factors with their coded levels
Table.4.17	Plackett burman design for screening of factors for <i>P. maximum</i>
Table.4.18	Regression analysis for Plackett Burman design variables <i>P. maximum</i>
Table.4.19	Box Benkhem Design (BBD) for <i>P. maximum</i>

Table.4.20	ANOVA table for BBD model for <i>P. maximum</i>
Table.4.21	Effect of alkali (Sodium hydroxide) pretreatment on crystallinity

Abbreviations

ACC	1-aminocyclopropane-1-carboxylate
ACCd+	1-aminocyclopropane-1-carboxylate deaminase positive
/	per
G	gram
Mg	milligram
EC	Electrical conductivity
ml	millilitre
SEM	Scanning electron microscope
DF	Dworking and Foster
PCR	Polymerase Chain Reaction
FW	Fresh Weight
SL	Shoot Length
RL	Root Length
DW	Dry Weight
LA	Leaf Area
RWC	Relative Water Content
EC	Electrolyte Concentration
DW/FW	Dry Weight/ Fresh Weight
IAA	Indole Acetic Acid
TW	Turgid Weight
Chl	Chlorophyll
Min	Minute
RS	Reducing Sugar
IAA	Indole-3-acetic acid
SAM	S-adenosyl methionine