DEVELOPMENT OF ENDOPHYTIC MICROBIAL CONSORTIUM TO MITIGATE MOISTURE STRESS CONDITIONS IN MAIZE CROP (*Zea mays* L.)

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(AGRICULTURAL MICROBIOLOGY)

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BY

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M. Sc.

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2022

DECLARATION

I**, Ms. MOTURU UMA SOWJANYA**, hereby declare that the thesis entitled **"DEVELOPMENT OF ENDOPHYTIC MICROBIAL CONSORTIUM TO MITIGATE MOISTURE STRESS CONDITIONS IN MAIZE CROP (*Zea mays* L.)"** submitted to the **Acharya N.G. Ranga Agricultural University** for the degree of **Doctor of Philosophy in Agriculture** in the major field of Agricultural Microbiology, is the result of original research work done by me. Part of the thesis has been published by me as "Moturu, U.S., Nunna, T., Avula, V.G., Jagarlamudi, V.R., Gutha, R.R. and Thamminana, S. 2021. Evaluation of water stress resilient endophytic bacteria for seed vigor index and antagonistic activity in maize (*Zea mays* L.). *Biological Forum – An International Journal.* 13(3a): 611-619"

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I.D. No: GAD/2018-01

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CERTIFICATE

Ms. MOTURU UMA SOWJANYA has satisfactorily prosecuted the course of research and that thesis entitled **"DEVELOPMENT OF ENDOPHYTIC MICROBIAL CONSORTIUM TO MITIGATE MOISTURE STRESS CONDITIONS IN MAIZE CROP (*Zea mays* L.)"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by her for a degree of any University.

Date:

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CERTIFICATE

This is to certify that the thesis entitled **"DEVELOPMENT OF ENDOPHYTIC MICROBIAL CONSORTIUM TO MITIGATE MOISTURE STRESS CONDITIONS IN MAIZE CROP (*Zea mays L.)*"** submitted in partial fulfillment of the requirements for the degree of **'Doctor of philosophy in Agriculture'** of the Acharya N. G. Ranga Agricultural University, Lam, Guntur is a record of the bonafide original research work carried out by **Ms. MOTURU UMA SOWJANYA** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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Place:

Date: ***(Moturu Uma Sowjanya)***

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LIST OF SYMBOLS AND ABBREVIATIONS

% : per cent
& : and
$(NH_4)_2SO_4$: Ammonium sulphate
@ : at the rate of
+ve : Positive
± : Plus or Minus
× : Multiplication
µ : Micro
µg : Microgram
µg ml^{-1} : Microgram per milli litre
µM : Micromolar
^{o}C : degrees centigrade
a.i : active ingredient
α : Alpha
α kB : α – ketoglutarate
ABA : Abscisic acid
ACC : 1-Aminicyclopropane 1- carboxylate
ANOVA : Analysis of variance
APX : Ascorbate peroxidase
ATP : Adenosine Triphosphate
β : Beta
BCP : Bromo Cresol Purple
BLAST : Basic Local Alignment Search Tool
BOD : Biological Oxygen Demand
BNF : Biological Nitrogen Fixation
BTB : Bromothymol Blue
BTV : Blank Titer Value
$CaCO_3$: Calcium Carbonate
CAS Agar : Chrome Azurol Sulphonate Agar
CAT : Catalase
Cd : Cadmium
CD : Critical Difference
CFU : Colony Forming Unit
cfu ml^{-1} : colony forming units per milliliter

cm : Centimeter
cm^2 : square centimetre
cm^3 : cubic centimeter
CSI : Chlorophyll Stability Index
CRD : Completely Randomized Design
DAI : Days After Inoculation
DAS : Days After Sowing
day $^{-1}$: per day
DDW : Double Distilled Water
df : Degrees of freedom
DNA : Deoxyribonucleic Acid
dS m^{-1} : deci simen per meter
DW : Distilled Water
dw Dry Weight
EC : Electric Conductivity
EPS : Exopolysaccharides
et al. : and others
F- test : Fisher’s test
FAS : Ferrous Ammonium Sulphate
Fig. : Figure
FYM : Farmyard Manure
FW : Fresh Weight
g : Gram
G + C : Guanine and Cytosine
g cc^{-3} : gram per cubic centimeter
g plant^{-1} : grams per plant
g l^{-1} : grams per liter
GC : Gas Chromatography
GDH : Glucose dehydrogenase
h : Hour
H_2SO_4 : Sulphuric Acid
HCl : Hydrochloric Acid
HCN : hydrogen cyanide
Hg : Mercury
hour^{-1} : per hour

HPLC	High Performance liquid chromatography
i.e.,	That is
IAA	Indole Acetic Acid
K	Potassium
$K_2Cr_2O_7$	Potassium Dichromate
K_2SO_4	Potassium Sulphate
KB	King'B medium
KCl	Potassium Chloride
kg	Kilogram
kg ha^{-1}	Kilogram per hectare
$KMnO_4$	Potassium Permanganate
KRB	Potassium Releasing Bacteria
KSE	Potassium Solubilization Efficiency
L	Liter
LB	Luria Broth
m	meter
Ma-35	*Metarrhizium anisopliae*
Max	Maximum
mcg	Micrograms (metric system)
MC	Microbial Consortium
MDA	Malondialdehyde
mg dry wt cells	milligrams dry weight of cells
mg ml^{-1}	Milligram per millilitre
Min	Minimum
min	Minutes
mL	milli liters
ml d^{-1}	milliliter per day
ml lit^{-1}	millilitre per litre
mm	millimeters
mM	Millimolar
Mmol l^{-1}	Millimoles per liter
MOP	Muriate of Potash
MPa	Mega Pascal
MR-VP	Methyl Red- Vogues Prausker
MUB	Modified Universal Buffer

N_2	:	Nitrogen
N	:	Normal
NA	:	Nutrient Agar
NaCl	:	Sodium Chloride
NADH	:	Nicotinamide Adenine Dinucleotide
NaOH	:	Sodium Hydroxide
NCBI	:	National Center for Biotechnology Information
NF	:	Nitrogen Fixer
NFB	:	N Free malate medium with Bromothymol blue
NGS	:	Next Generation Sequencing
nif	:	Nitrogenase Inducing Factor
nm	:	Nanometres
nmol C_2H_4 h^{-1} mg protein^{-1}	:	nanomoles of ethylene per hour and per milligram of protein
nmol ml^{-1}	:	nanomole per milliliter
No.	:	Number
NO_3^-	:	Nitrate
NS	:	Non-significant
number m^{-2}	:	Number per square meter
O_2	:	Oxygen
OC	:	Organic Carbon
OD	:	Optical Density
OF	:	Oflaoxacin
OM	:	Organic Matter
OTU	:	Operational Taxonomic Unit
P	:	Phosphorus
Pen G	:	Penicillin-G
PBZ	:	Pachlobutrazol
PCR	:	Polymerase Chain Reaction
PDA	:	Potato Dextrose Agar
PEG	:	Polyethylene Glycol
PGAP	:	Prokaryotic Genome Annotation Pipeline
PGP	:	Plant Growth Promotion
PGPB	:	Plant Growth Promoting Bacteria
PGPM	:	Plant Growth Promoting Microbes
PGPR	:	Plant Growth Promoting Rhizobacteria

pH	:	Puissance Hydrogen
pNP	:	para Nitrophenol
POD	:	Peroxidase
PPM	:	Parts per million
PSB	:	Phosphate Solubilizing Bacteria
PSBF	:	Phosphate Solubilizing Biofertilizers
PSE	:	Phosphate Solubilization Efficiency
psi	:	pound force for Square Inch
PSM	:	Phosphate Solubilizing Microbes
q	:	Quintal
R1	:	Replication 1
R2	:	Replication 2
R3	:	Replication 3
RBD	:	Randomized Block Design
RDF	:	Recommended Dose of Fertilizer
RDN	:	Recommended Dose of Nitrogen
RNA	:	Ribonucleic Acid
ROS	:	Reactive Oxygen Species
RPM	:	Rotations Per Minute
RWC	:	Relative Water Content
rpm	:	revolutions per minute
rRNA	:	ribosomal Ribonucleic acid
sec	:	Seconds
Sem	:	Standard Error of mean
SEM	:	Scanning Electron Microscopy
SEs	:	Standard Errors
SI	:	Solubilization Index
SIM Agar	:	Sulphide Indole Motility Agar
SO_4^{-2}	:	Sulphate
SOB	:	Sulphur Oxidizing Bacteria
SOD	:	Superoxide Dismutase
Sp.	:	Species
STV	:	Sample Titer Value
t	:	ton
t ha^{-1}	:	ton per hectare

TBA	:	Thiobarbituric Acid
TCP	:	Tri Calcium Phosphate
TPF	:	Triphenyl Formazone
TSB	:	Tryptic Soya Broth
TTC	:	Triphenyltetrazolium Chloride
TW	:	Turgid Weight
UV	:	Ultra Violet
v/v	:	volume by volume
VA	:	Vancomycin
-ve	:	Negative
vis-a-vis	:	in relation to
viz.,	:	namely
w/v	:	weight by volume
WHC	:	Water Holding Capacity
YEMA	:	Yeast Extract Mannitol Agar
YMG Broth	:	Yeast Mannitol Glucose Broth

ABSTRACT

Author : **MOTURU UMA SOWJANYA**

Title of the thesis : **DEVELOPMENT OF ENDOPHYTIC MICROBIAL CONSORTIUM TO MITIGATE MOISTURE STRESS CONDITIONS IN MAIZE CROP (*Zea mays* L.)**

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Maize is the third most vital crop after the rice and wheat in India but it stands first in the context of potential for highest productivity. Maize has been used for multi purposes, for instance as a food for human, feed for livestock and also used as a raw material in many industries. Drought stress has been reported to cause yield reductions of up to 40% in maize around the world and it was also considered as nutrient exhaustive crop. The quality and yield of crop may change due to the association of endophytic microbes that reside in plants asymptomatically and they were known protect plant from abiotic and biotic stress.

The present investigation was aimed to develop an endophytic microbial consortium which is an alternative approach to minimize the use of chemical fertilizers and to mitigate stress conditions in maize without affecting ecosystem. A total of 106 endophytic bacterial morphotypes were isolated from surface sterilized maize plant tissues including roots, stem, leaf and kernels collected from various regions of AP which includes Srikakulam, Vijayanagaram, Visakhapatnam, East Godavari, Guntur, Kurnool and Ananthapuram.

Among all 36 efficient water stress mitigating and plant growth promoting isolates were selected and screened to 16 effective isolates based on quantitative analysis of plant growth promoting attributes. Significant isolates were identified as genera *Gordonia,Microbacterium, Kosakonia, Priestia, Bacillus, Cellulosimicrobium, Pantoea, Pseudomonas, Klebsiella* and *Methylorubrum* by 16S rRNA gene sequencing.

Efficient 7 isolates were selected namely *Kosakonia radicincitans* (NL3E3), *Priestia megaterium* (PdS3E1), *Priestia aryabhattai* (PL3E2) *Bacillus licheniformis* (VaR3E1), *Pseudomonas aeruginosa* (LS3E1), *Klebsiella pneumonia* (LS3E3) and *Methylorubrum populi* (LL3E1) for compatiability and endophytic colonization studies. Based on the obtained results formulated microbial consortium (MC-4)

Bacillus megaterium+ Bacillus licheniformis+ Klebsiella pneumoniae+ Methylorubrum populi was seclected for pot and field experiments.

In pot culture experiment microbial consortium inoculated treatments imposed with (75% WHC) water stress T9 (100% RDF) and T8 (75% RDF) were found to excel in many plant and soil chemical properties when compared to un-inoculated control. For instance chlorophyll stability index T9 (138.67%) T8 (133.33%), relative water content T9 (95.20%), organic carbon ccontent was found highest in T5 (0.39%) and T8 (0.38%), available nitrogen in soil was more in T9 (145.61 Kg ha^{-1}) and T8 (141.65 Kg ha^{-1}), available phosphorous was higher in T8 (67.68 Kg ha^{-1}), available potassium is more in T9 (345.86 Kg ha^{-1}). The N, P and K uptake was found significantly higher in T9 with 13.98 g plant^{-1}, 3.38 g plant^{-1} and 17.29 g plant^{-1} respectively.

Total microbial population in rhizosphere and endosphere was found higher at flowering stage. Total bacterial, fungal and actinomyces were reported highest in T9 with (9.99 Log CFU g^{-1} of soil), T8 (5.66 Log CFU g^{-1} of soil) and T9 (4.24 Log CFU g^{-1} of soil) respectively. At vegetative stage maximum colonization of endophytic bacteria was found in roots followed by stem and least colonization was found in leaves. Contradictory to that at flowering stage maximum endophytic bacterial population was observed in stem followed by roots and leaves. Higher plant biomass was produced by T9 (259.67 g) and grain yield was found highest in T9 (53.56 g) followed by T8 (51.39 g)

In field conditions maximum CSI was observed for T9 (138.67%) and highest RWC was recorded in T7 (95.13%). The production of H_2O_2 was higher in treatments given with lower number of irrigations and so the peroxidase activity. The organic carbon in soil was highest in T7 (0.45%). The available nitrogen in soil was more in T9 (225.29 kg ha^{-1}) and T8 (209.09 kg ha^{-1}), available phosphorous was higher in T9 (70.34 kg ha^{-1}) and T8 (69.92 kg ha^{-1}), available potassium is more in T9 (390.62 kg ha^{-1}), T8 (360.59 kg ha^{-1}). The N, P and K uptake was found significantly higher in T8 (690.03 Kg ha^{-1}), T9 (182.45 Kg ha^{-1}) and T9 (425.61 Kg ha^{-1}) respectively. Total microbial population was found highest at flowering stage and the endophytic microbial population was found highest in root endosphere followed by stem, leaf and kernels.

Significantly higher dehydrogenase activity was found in T9 (58.90 μg of TPF g^{-1} of soil day^{-1}) at flowering stage and maximum phosphatasee activity was found in T8 (94.97 μg pNP g^{-1} of soil h^{-1}). At flowering stage T9 (24054 Kg ha^{-1}) and T8 (22332 Kg ha^{-1}) were found to produce significantly higher amount of plant biomass. Significantly more yields were obtained in T6 (7963 Kg ha^{-1}) followed by T9 (7644 Kg ha^{-1}). Considerably higher net returns were obtained for T6: 5 Irrigations + 100 % RDF + MC (Rs. 113717/- ha^{-1}) followed by T9:7 Irrigations + 100 % RDF + MC (Rs. 106325/- ha^{-1}) and T8:7 Irrigations + 75 % RDF + MC (Rs. 102539/- ha^{-1}).

Thus the current study suggested that significant net returns can be obtained even under water deficit conditions when inoculated with endophytic microbial consortium (*Bacillus megaterium+ Bacillus licheniformis+ Klebsiella pneumoniae+ Methylorubrum populi*) by improved plant growth and yields due to enhanced osmotolerance and mineralization of nutrients. Nearly 120 mm of water (2 irrigations) and 25% of RDF can be minimized for crop production without affecting yield and net returns.

Chapter – I

Introduction

Chapter I

INTRODUCTION

In India among cereal crops maize is the third most vital crop after the rice and wheat but, in the context of potential for highest productivity maize in stands first place. *Zea mays* belongs to the family Gramineae, sub-family Poaceae and it has been used for multi purposes, for instance as a food for human, feed for livestock and also used as a raw material in many industries. Due to this extensive potential maize is termed as a “miracle crop” and it is also entitled as “Queen of cereals”. The production of maize in India has coverage of cultivated in 9.09 M ha producing over 23.29 M tonnes with 2563 kg ha^{-1} productivity. In global production of maize India stands as fifth largest producer by contributing over 3% of production (Suganya *et al.*, 2020).

Literature indicates that the world population is expected to hike 8.6 billion by 2030 and 98 billion by 2050 (Union, 2017). Such an increase will involuntarily require decesive production of additional agricultural products. On the other hand Mooney *et al.* (2009) reported that climate change has became main source of creating stress for foliar growth and emerged as an alarming threat to natural ecosystems. The environmental stress is simply classified as abiotic and biotic stress based on their nature and source of effect. Sometimes abiotic stress may influence the biotic stress and reduces crop productivity (Kumar and Verma, 2018).

Among the environmental stresses, moisture stress has major impact on crop growth and productivity throughout the world. By 2050 more than 50% of arable lands are expected to have negative impact on crop growth because of drought (Vinocur and Altman, 2005). Drought stress has been reported to cause yield reductions of up to 21% in wheat and 40% in maize around the world (Daryanto *et al.*, 2016). On the other hand direct yield losses caused biotic stress including pathogens, animals and weeds will cause yield losses in the range of 20 to 40 % of worldwide agricultural productivity (Savary *et al.*, 2012).

For maize productivity water stress is the major abiotic limitation. Out of the all cultivation area of maize which is about 4.0 million hectares are inclined to dry spell in India because 80% of the India in Kharif season is rainfed. Moisture stress is extremely regular in the regions where maize is transcendently become under rainfed conditions. Whereas disease is one of the major biotic stresses that declines crop yield and furthermore deteriorate the quality of product that ultimately affects the market cost. Estimated crop losses due to major diseases of maize in India is about 13.2% out of which foliar diseases contribute 5% losses while stalk rots, root rots and ear rots causing major yield losses recording 8.2% (Parihar *et al*., 2011).

A protection system is possessed by plants naturally that can tackle adverse stress conditions, even so, plants also interact with a many microorganisms that can alleviate the stress and protects the plant (Marulanda *et al.,* 2006). Plants are more contingent on microorganisms which are capable to enhance their metabolic activity to combat stress (Kavamura *et al.,* 2013). Upon exposure of plants to hostile conditions microbes can pre sensitize the plant cell metabolism, and so microbial treated plants will respond more quickly than untreated plants (Compant *et al.,* 2005). Thus, use of beneficial microbial inoculants as a stress protecting agent for plants may reassure for sustainable and chemical free eco-friendly agriculture.

Plants harbour several microbes, which reside within the cells in intercellular spaces or in vascular system, without affecting the plants are known as endophytes (Sandhya *et al.,* 2017). Bacterial endophytes are beneficial over rhizospheric bacteria as they reside within plant tissue with more intimate contact and they have no competition with rhizosphere microorganisms (Naveed *et al.,* 2014a). Their ability for plant growth and to alleviate the biotic and abiotic stresses is well studied (Chandran *et al.* 2020., Ullah *et al.* 2019 and Sandhya *et al.* 2017). The interaction of plant with endophytic bacteria alleviates the tolerance towards drought stress (Paul and Lade 2014). On the other hand endophytic bacteria have been reported to possess variety of defense mechanisms to control plant pathogen by de novo synthesis of structural compounds, producing antibiotics and antimicrobial products, competing for niche and plant immunity development or induced systemic resistance (Pandey *et al.,* 2019).

The quality and yield of crop may change due to the association of endophytic microbes that reside in plants asymptomatically. It can be said that the endophytic bacteria of the Plant Growth Promoting Bacteria (PGPB) are more favorable than the bacteria inhabiting the rhizosphere. Because endophytes live always in contact with plant tissue and exert a direct beneficial effect. As the endopyte plant association is the result of evolutionary process endophytic bacetria are considered to be more competitive than facultative or non-endophytic microorganisms (Rosenblueth and Martínez- Romero 2006). Many endophytic bacteria, *Klebsiella* (Chelius and Triplett, 2000a), *Pantoea, Bacillus, Herbaspirillum* (Chelius and Triplett, 2000b) were isolated from maize cultivars previously. Maize stem was found to host group of bacterial species *Pseudomonas aeruginosa, P. fluorescens, B. subtilis, Bacillus pumilus* as the relatively more predominant (Rai *et al.,* 2007). Both abiotic and biotic factors may influence the highly diversified endophytic microbial communities residing in plants. Their colonization and plant growth promoting characters are dependent on soil type and the host plant (Grządziel and Gałązka, 2018).

Plant growth promoting bacteria may facilitate essential nutrient acquisition which typically consists of nitrogen, phosphorus, potassium and iron or modulates growth harmones level like auxins, gibberlins and cytokinins within the plant that results in direct growth promotion of plant. In addition, some of PGPB can synthesize an enzyme, ACC deaminase which lowers the level of the phytohormone ethylene by cleaving ACC, the immediate precursor of ethylene in all higher plants (Santoyo *et al.,* 2016). Many studies have isolated endophytic bacteria with the ability to fix atmospheric nitrogen, solubilize inorganic phosphorus, produce iron chelating siderophores and phytoharmone synthesis promoting host plant growth. Moreover, the host plant can adapt to the environment even under stress conditions like salinity, water stress and chilling stress with the activity of endophytic bacteria (Zhu and She, 2018). Therefore, the new endophytic bacteria should be evaluated for their plant growth promoting abilities to get effective and prominent improvement in plant growth and health.

The classical methods of identifying microbes includes morphological and biochemical characterization which are insufficient to differentiate the microbe at species and strains level thus molecular identification by using techniques like sequencing of conserved regions of DNA particularly emphasizing on 16S rRNA has been widely used in the characterization microorganisms (Versalovic *et al.,* 1994). Approximately 3,00,000 plant species that exist on the earth are found to host one or more endophytic microbes. Out of these plants only few have ever been completely studied relative to their endophytic microbial diversity. As a result, the potential for discovering new and beneficial endophytic microorganisms among the diversity of plants in various ecosystems is significant (Ryan *et al.*, 2008).

The cultivation of high-yield genotypes has increased the requirement for chemical fertilizers (Souza *et al.*, 2015a). To maintain proper growth and yield, maize requires an adequate supply of chemical fertilizers. Phosphorus is one of the important macronutrients necessary for the maintenance of physiological processes in the early and late stages of maize crop growth (Onasanya *et al.*, 2009). In the future, excessive use of chemical fertilizers will lead to negative impact on the environment and thus affecting human health. The low solubility of phosphate in the soil and excessive use of nitrogen fertilizers leads to easily runoff in water bodies like lakes, rivers, and oceans causing degradation of water quality, aesthetic value finally leading to cause eutrophication (Kour *et al.*, 2020d). Thus the alternative approach to minimize use of chemical fertilizers without affecting ecosystem is the use of microbial inoculants. Microbial inoculants are primarily made up of living cells of selected microbes that use various mechanisms such as those that convert nutrients to available forms for plant uptake through the root system thereby improving plant fitness and soil fertility to increase crop productivity, yields and also play an important role in nutrient cycling (Mondal *et al.*, 2020).

Identification of bacteria residing in maize crop and their characterization may provide significant information for enhancing the possibility of identifying strategies that may benefit maize cultivation and production by knowing interactions of plant with varied genomic diversity of bacteria. Thus, present investigation was aimed to isolate bacteria from maize endosphere (Root, Stem, Leaf and Kernel) growing in different regions of Andhra Pradesh and screened based on osmotolerance and plant growth promoting traits, further characterized by means of biochemical and molecular methods (16s rRNA gene Sequencing) to develop an efficient stress mitigating and plant growth promoting endophytic microbial consortium for maize crop by considering following objectives.

Objectives:

1. Isolation, screening and biochemical characterization of endophytic bacteria from maize plant samples.

2. Quantitative evaluation of plant growth promoting characters of selected endophytic microorganisms.

3. Screening for compatibility of bacterial isolates under *in vitro* conditions.

4. Molecular characterization of efficient stress mitigating and plant growth promoting endophytic bacterial isolates.

5. To study the influence of developed endophytic microbial consortia / isolates on maize against moisture stress under greenhouse conditions**.**

6. To study the performance of endophytic bacterial consortia / isolates on maize under moisture stress in field conditions.

Chapter – II

Review of Literature

Chapter II

REVIEW OF LITERATURE

The present investigation was carried out for isolation and molecular characterization of stress tolerant plant growth promoting endophytic bacteria from maize and to develop microbial consortia with ability to mitigate stress under field conditions. The literature pertaining to these aspects is reviewed and presented under the following headings, to provide an overview of the current status of the research work.

2.1 PLANT GROWTH PROMOTING BCTERIA (PGPB)

Beneficial bacteria either endophytic or free-living which are concerned with growth stimulation of crops by direct or indirect functions are termed as plant growth promoting bacteria (Shameer and Prasad, 2018). PGPB can stimulate plant growth by several mechanisms (Fig. 2.1) which can range from soil reclamation, direct production of growth promoting substances, suppression of harmful microbes/ pathogens, phosphorus and nitrogen solubilization, nutrient mobilization to the induction of disease resistance and stress tolerance in hosts against a wide array of pathogens. It has been widely reported that these beneficial microbes result in nitrogen fixation and improvement of nitrate uptakes by plant roots, phosphorus solubilization and bioavailability in the rhizosphere, mobilization of nutrients and their uptake, remediation of soils contaminated with heavy metals, soil improvement by multiple activities , release of hormones, vitamins, volatile compounds and other growth regulatory substances , improvement of drought and salinity tolerance suppression of harmful organisms and pathogenic diseases and induction of disease resistance and enhancing immunity responses of hosts. PGPB mediated nutrient bioavailability in soils, biotic and abiotic stress tolerance leads to consequent improved growth, yield and production of plants (Majeed *et al.,* 2018).

Two general types of soil bacteria have been shown to have the capacity to act as PGPB; rhizospheric bacteria, that are typically found around the roots of plants; and endophytic bacteria that are found within the tissues of the plant itself not withstanding the fact that endophytic bacteria may also be found free-living in the soil (Santoyo *et al.,* 2016).

Bacteria that facilitate plant growth may do so either by binding to the plant's outer surface such as the roots (the rhizosphere) or the leaves (the phyllosphere), or they may inhabit the interior surfaces of the plant typically in nodules forming symbiotic relationship (e.g., *Rhizobia* spp. and *Frankia* spp.), or in other plant tissues forming an endophytic relationship (Glick *et al.*, 2014).

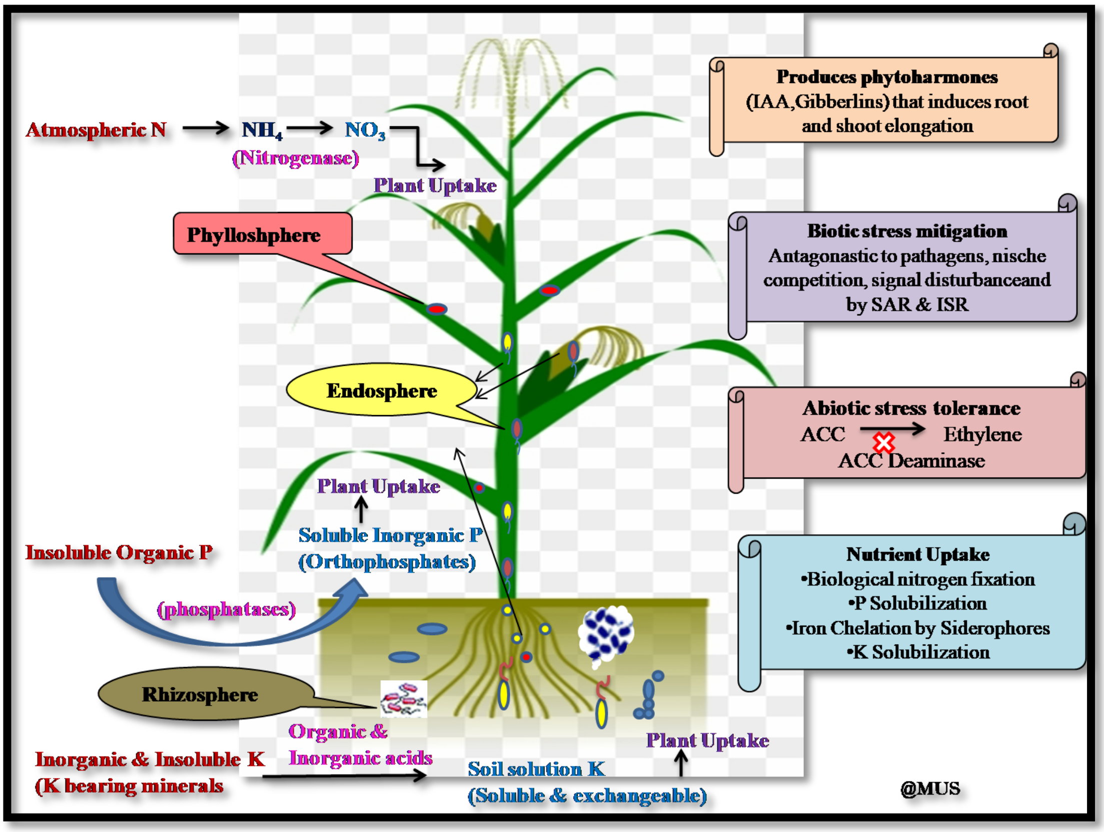


Fig. 2.1. Mechanisms used by PGPB for plant growth promotion and stress mitigation

Microbial endophytes (bacteria and fungi) have been found in every plant species that has been analyzed. According to Partida-Martínez and Heil (2011) an endophyte-free plant is a rare exception to what is typically found in nature. Based on observations of the distribution of rhizospheric PGPB in nature (Timmusk *et al.*, 2011), a plant without endophytes would be less able to cope with pathogens and more susceptible to environmental stress conditions.

Endophytic bacterial diversity can be considered a subset of the rhizosphere and/or root-associated bacterial population (Marquez-Santacruz *et al.*, 2010). Recent estimates suggest that the planet contains about 300,000 species of plants, the vast majority of which contain endophytes (Smith *et al.*, 2008).

Although the presence of bacterial endophytes in plants is variable and, occasionally, transient (Van Overbeek and Van Elsas, 2008), they are often capable of eliciting drastic physiological changes that modulate the growth and development of the plant (Conrath *et al.*, 2006). Often these beneficial effects of endophytes are greater than those of many rhizosphere-colonizing bacteria and they might be exacerbated when the plant is growing under stress conditions (Ait Barka *et al.*, 2006).

Bacterial endophytes may have an advantage over bacteria inhabiting the rhizosphere, since living within a plant's tissues represents an opportunity to always be in contact with the plant's cells and therefore, to more readily exert a direct beneficial effect. Of course, bacteria residing in the rhizosphere might also have the potential to enter and colonize the plant roots. This microecosystem has been widely known as one of the primary sources for endophytic colonization (Hallmann *et al.*, 1997).

2.1.1 Endophytic Bacteria

The word endophyte means "in the plant" (endon = within, phyton = plant). "Endophytes" are most commonly defined as those organisms whose "infections are inconspicuous, the infected host tissues are at least transiently symptomless, and the microbial colonisation can be demonstrated to be internal". Endophytic microorganisms (bacteria or fungi) are a key class of plant symbionts that live inside plant tissues without inducing any disease symptoms (Brader *et al*., 2017).

Some authors have proposed a functional definition of endophytic behavior, considering any bacterium as an endophyte if it can be isolated from surface-disinfested plant tissue or extracted from inside the plant, and if it does not visibly harm the plant. Endophytes are defined as fungi or bacteria, which for all or part of their life cycle invade the tissues of living plants and cause unapparent and asymptomatic infections entirely within plant tissues, but no symptom of disease (Hallman *et al.*, 1997).

In accordance with their life strategies, bacterial endophytes can be classified as 'obligate' or 'facultative'. Obligate endophytes are strictly dependent on the host plant for their growth and survival and transmission to other plants occurs vertically or via vectors. Facultative endophytes have a stage in their life cycle in which they exist outside host plants. Microbes profit from plants because of the enhanced availability of nutrients, whereas plants can receive benefits from bacterial associates by growth enhancement or stress reduction (Hardoim *et al.*, 2008).

Endophytes may be transmitted by seeds, vegetatively (vertical transmission) or by vectors; often they may be recruited from soil (horizontal transmission) and probably require a stage of rhizoplane colonization, for which they need properties conveying ‘‘rhizosphere competence’’. Prominent sites for active ingress into roots are the emergence points of lateral roots and to some extent the zone of differentiation and elongation near the root tip, where slightly disrupted or not completely differentiated tissues may facilitate penetration. Intercellular spaces in the epidermal and cortical regions and lysed plant cells are major sites of colonization, with locally high cell densities of up to 10^{10} cells/cm^3. Vascular tissue and xylem cells may also be invaded, albeit often in lower densities; colonization of vascular tissue is likely to allow systemic spreading into shoots. To localize endophytes and follow the dynamics of their spreading, tagging with constitutively expressed reporter genes encoding fluorescent proteins has proven to be a particularly valuable tool, even for phylogenetically distant bacteria (Reinhold-Hurek and Hurek, 2011)

2.1.2 Colonization of Endophytic Microorganisms in Plants

The endophytic colonization and movement of microorganisms may be greatly influenced by the micro-environment, as well as developmental and environmental factors (Marag and Suman 2018., Deyett and Rolshausen 2019). To achieve a successful colonization, endophytes require a compatible host plant (Miliute *et al.*, 2015)

Plant and endophytic colonization is a complex process that requires the capacity of bacteria to compete in the rhizosphere soil to find a place to communicate and interact with the plant roots. Root exudates are chemically diverse and include those molecules involved in attracting microorganisms to the root, or in the case of endophytes, to be able to colonize the internal plant tissues. To colonize the internal plant tissues, it has been proposed that bacterial endophytes have genomic differences compared to rhizosphere colonizing bacteria, although so far no definitive group of genes has been identified that is responsible for the endophytic lifestyle. However, a list of genes with possible roles in endophytic behavior was recently identified by Ali *et al.* (2014a,b) by comparing the complete genomes of nine Proteobacterial endophytes. At this point, only some of those genes have been experimentally shown to be involved in endophytic colonization.

The endophytic occurrence of particular bacteria is the result of chance factors, determined by the chances of developing roots coming into contact with effective levels of bacteria that can become endophytic, and deterministic factors, determined by the presence of dedicated genetic systems that enable bacterial plant crosstalk and an active endophytic colonization process. The most common way to entry is through the roots, through primary and lateralroot hair cells, root cracks and wounds, as well as hydrolysis of root cells. Other ways sites include stomata, particularly on leaves and young stems; lenticels and germinating radicles as illustrated in fig. 2.2 (Hardoim *et al.*, 2008). Bacterial endophytes were found to transfer from other plant tissues to seeds and suggested as natural biofertilizers early from seed germination for new plantlets (Puente *et al.*, 2009; Ruiza *et al.*, 2011). Low number of endophytic bacteria were found in reproductive organs which seems even the less abundance of endophytic population in seeds might be sufficient for vertical transmission by acting as natural biofertilizer and after establishment of bacterial population in next generation plantlet, further increase in cell density takes place and involve in plant growth promotion (Compant *et al.*, 2010).

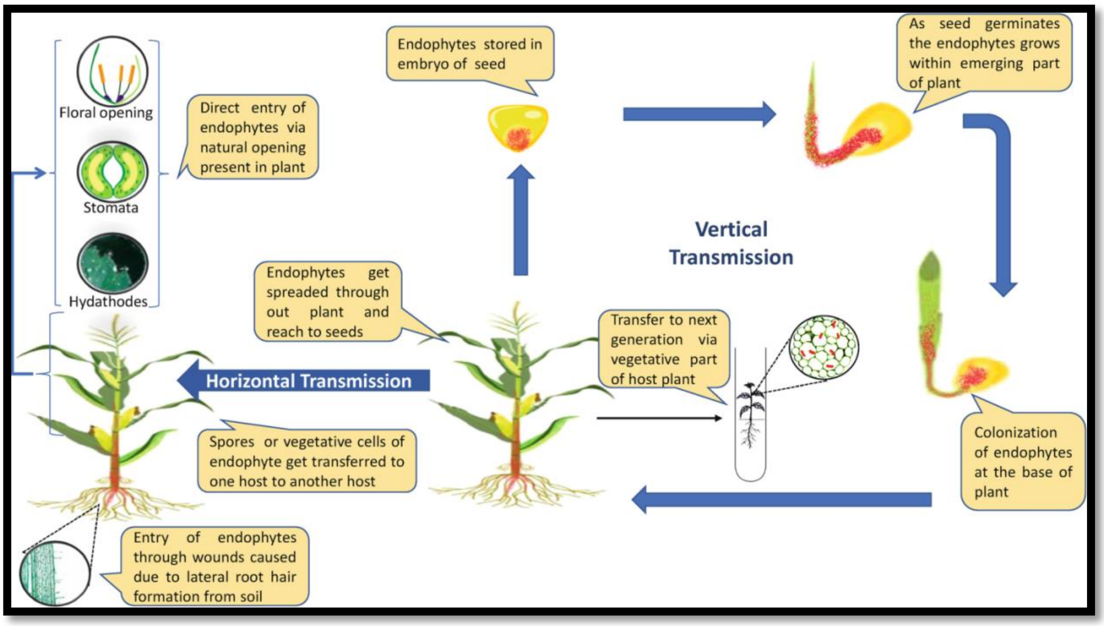


Fig. 2.2. Mechanisms involved in colonization and transmission of endophytic bacteria (Chowdhury *et al.*, 2019)

2.1.3 Isolation of Endophytic Bacteria

Endophytic bacteria were isolated from a hybrid PEEHM-5 and composite PC-4 maize varieties using root, stem and leaf tissues of plants at vegetative, flowering and maturity stages of growth. PEEHM-5 harbored higher endophytic bacterial population than PC-4 at all growth stages, with highest in roots and at flowering stage. Morphologically 188 different endophytic isolates (82 from PEEHM-5, 106 from PC-4) were screened for plant growth promoting attributes. (Marag and Suman, 2018)

Corn sap was screened for the diversity and functionality of culturable bacterial endophytes, and a total of 437 bacterial endophytes were isolated, identified, and characterized for their possible role as biofertilizers and biocontrol agents. The growth promoting traits that were characterized included siderophore production, phosphate and zinc solubilization, nitrogen fixation, indole acetic acid production, and antifungal activity against several plant pathogens (Ali *et al.*, 2018)

Fifty-five endophytic PSB that were isolated from sap, leaves, and roots of maize were tested for their ability to solubilize tricalcium phosphate and produce organic acid. Partial sequencing of the 16S rRNA-encoding gene showed that the isolates were from the genus *Bacillus* and different species of *Enterobacteriaceae* (De Abreu *et al.*, 2017)

Ikeda *et al.* (2013) has established a collection of 217 endophytic bacteria, isolated from roots of four lineages and three hybrid genotypes of maize, and isolated in four different N-free culture media. Biochemical comprising growth in different carbon sources, intrinsic tolerance to antibiotics, and biochemical tests for catalase, nitrate reductase, urease, and growth in N-free media in vitro and genetic characterization by BOX-PCR revealed great variability among the isolates. Both commercial hybrids and homozygous lineages were broadly colonized by endophytes, and sequencing of the 16S rRNA gene revealed the presence of bacteria belonging to the genera *Pantoea*, *Bacillus*, *Burkholderia*, and *Klebsiella*.

Figueiredo *et al.* (2009) have isolated endophytic bacteria from tropical maize and the partial sequencing of 16S ribosomal gene revealed that all isolates are *Bacillus* sp. and among thirteen isolates with similar protein profiles, two were different strains. Among the forty-two isolates identified by rDNA sequencing, *Bacillus subitilis* and *B. pumilus* were the most frequently species (15 and 12 isolates, respectively) followed by *B. licheniformes* (7 isolates), *B. cereus* (5 isolates) and *B. amiloliquefascens* (3 isolates).

2.2 CHARACTERIZATION OF ENDOPHYTIC BACTERIA BASED ON PLANT GROWTH PROMOTING TRAITS

The mechanisms of plant-growth promotion between rhizospheric and endophyte bacteria can be very similar, most of the research work has been done in rhizosphere bacteria, assuming that the mechanism is similar in endophytes. However, the rhizosphere environment is quite different from that of internal plant tissues. For example, the variations in abiotic factors such as temperature, light emission, soil type, pH, as well as the competence for nutrients, the availability of oxygen and the interaction with other organisms in the rhizosphere, can be key factors in the development of different strategies for interaction, lifestyle and survival (once they are) inside the plant. Therefore, it is possible that in the future new mechanisms of plant-growth promotion, unknown in rhizosphere bacteria, may be discovered in bacterial endophytes. (Santayo *et al.*, 2016)

Conceptually, PGPB may affect plant growth either directly or indirectly. Direct promotion of plant growth occurs when a bacterium either facilitates the acquisition of essential nutrients or modulates level of hormones within a plant. Nutrient acquisition facilitated by PGPB typically includes nitrogen, phosphorus and iron. Modulation of hormone levels may entail PGPB synthesizing one or more of the phytohormones auxin, cytokinin and gibberellin. In addition, some PGPB can lower levels of the phytohormone ethylene by synthesizing an enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, that cleaves the compound ACC, the immediate precursor of ethylene in all higher plants (Santoyo *et al.,* 2016). There are a large number of common mechanisms that PGPB use to indirectly promote plant growth including the production of antibiotics, cell wall-degrading enzymes, lowering plant ethylene levels, induced systemic resistance, decreasing the amount of iron available to pathogens, and the synthesis of pathogen-inhibiting volatile compounds (Glick, 2015).

2.2.1 Nitrogen Fixation

Numerous studies have shown that endophytic bacteria are able to promote plant growth by producing a series of nutrients and by facilitating plant nutrient uptake through atmospheric nitrogen fixation and phosphate solubilization (Zhu and She, 2018).

Some endophytic bacteria possess both, nitrogen fixation (e.g., nifH) and denitrification genes. The importance of endophytic bacteria in N cycling is also supported by the evidence that N_2 fixation by foliar endophytic bacteria has occurred in many subalpine conifer species (Moyes *et al.,* 2016).

Klebsiella sp. Br1, *Klebsiella pneumoniae* Fr1, *Bacillus pumilus* S1r1 and *Acinetobacter* sp. S3r2 Showed the highest N_2-fixing capacity of 30.5% (262 mg N_2-fixed plant^{-1}) and 25.5% (304 mg N_2-fixed plant^{-1}) of the total N requirement of maize top at D50 and D65, respectively. It also showed higher ear yield (up to 30.9%) with reduced fertilizer N input (Kuan *et al.,* 2016).

A considerable number of microbial genes involved in N cycling were found in the metagenome of rice roots, which indicates that the rice related nitrification and ammonia oxidation processes might be subjected to the influence of the endophytic root microbiome (Sessitsch *et al.,* 2012).

After isolation, identification and associative nitrogen fixing activity study of endophytic bacteria in maize, the result showed that five bacteria NFR19 (*Pseudomonas geniculata*), NFL28 and NFSt18 (*Klebsiella oxytoca*), NFR7 (*Arthrobacter* sp.), NFR10 (*Cellulosimicrobium cellulans*) were stained with nitrogen-fixing activity. The study found that *Cellulosimicrobium cellulans* could be a nitrogen-fixing active bacterium for the first time. The result also testify that *Arthrobacter* sp.is a nitrogen-fixing active bacterium (Zhang *et al.,* 2010).

The endophytic diazotrophic bacteria have been reported for rice, sugarcane and other cereal crops. *G. diazotrophicus* and *Acetobacter peroxydans* were isolated from four different wetland rice varieties cultivated in the state of Tamilnadu, India (Saravanan *et al.,* 2007). Through biological nitrogen fixation some sugarcane varieties have been found to derive up to 30-60% of their nitrogen need by using endophytic diazotrophs (Suman *et al.,* 2001). According to nitrogen balance studies as high as 150 Kg N ha^{-1} yr^{-1} is contributed mainly by *Gluconoacetobacter diazotrophicus* in sugarcane through BNF (Muthukumarasamy *et al.,* 2005).

Incorporation of ^{15}N experiments showed that sugar cane plants inoculated with *G. diazotrophicus* Pal5 obtained up to 0.6% of total N from BNF over a 24-h period (Sevilla *et al.,* 2001); for rice plants harboring *Herbaspirillum* sp. B501 this value was 0.14% (Elbeltagy *et al.,* 2001), indicating that diazotrophic endophytes can contribute a significant amount of nitrogen to a plant.

Other studies suggested that plants can get up to 70% of the required nitrogen through BNF mediated by endophytic diazotrophs. The best studied endophytic diazotrophs include members of *Azoarcus, Burkholderia, Gluconobacter, Herbaspirillum* and *Klebsiella* (James, 2000).

2.2.2 Phosphate Solubilization

Phosphorus (P) is an essential plant nutrient, after nitrogen and grouped along with nitrogen and potassium as a major plant element. Microorganisms are playing important role in providing phosphorus to the plant through transformation hence vital component of P cycle. However applied phosphorus get rapidly fixed in soil as insoluble forms and become unavailable to the plant (Sharma *et al.,* 2011). The phenomenon of fixation and precipitation of P in soil is pH dependent; in acidic soil Al and Fe phosphate are formed whereas in calcareous soils high concentration of Ca results in pH precipitation. So in this way microorganisms are critical in ransfering of P from the poorly available soil pools (Richardson *et al.,* 2009; Ziadi *et al.,* 2013; Osman, 2013). Organic acids are produced like gluconate, ketogluconate, acetate, lactate, oxalate, tartarate, succinate, citrate and glycolate by most of the bacterial genera for solubilising phosphate (Khan *et al.,* 2009).

The phosphate solubilizing bacteria density in the samples of roots, stems, and leaves of maize plants were found to be 8.3×10^{5} CFU g^{-1}, 5.7×10^{5} CFU g^{-1}, and 4.6×10^{5} CFU g^{-1} respectively (Azizah *et al.* 2020)

The endophytic bacteria belonging to genera *Bacillus, Klebsiella, Microbacterium, Pantoea, Paenibacillus,* and *Pseudomonas,* as endophytes have been reported to solubilize P (Kour *et al.,* 2020 a, b)

Fifty-five endophytic phosphorus solubilizing bacteria that were isolated from sap, leaves, and roots of maize were tested for their ability to solubilize tricalcium phosphate and produce organic acid. The phosphate solubilization index on solid medium and phosphate solubilization in liquid medium varied significantly among the isolates. There was a statistically significant difference ($P \leq 0.05$) for both, the values of phosphate solubilizing activity and pH of the growth medium, among the isolates (De Abreu *et al.*, 2017)

Six plant-associated bacteria were tested for their ability to solubilize tricalcium phosphate ($Ca_3(PO_4)_2$) and their ability to produce gibberelic acid (GA). Five out of the six strains tested were able to solubilize $Ca_3(PO_4)_2$ to the level of >400 mg L^{-1} (Otieno *et al.*, 2015)

Ji *et al.* (2014) reported P- solubilizing endophytic bacteria from 10 rice varieties which were identified as *Bacillus megaterium* and *Klebsiella pneumonia*.

Montanez *et al.* (2012) isolated several endophytic bacteria from 11 maize varieties. They obtained *Burkholderia cepacia* as a seed endophyte from maize variety PAU871. Biochemical characterization revealed that *Burkholderia cepacia* produced indoleacetic acid (40.1 μg ml^{-1}) and phosphate solubilization capability of 2.1 PSI (phosphate solubilizing index).

Castagno *et al.* (2011) characterized fifty phosphate-solubilizing strains and assessed their phosphate-solubilizing pathways. The study identified the strains as *Pantoea*, *Erwinia*, *Pseudomonas*, *Rhizobium* and *Enterobacter*.

2.2.3 Potassium Solubilization

The potassium solubilizing microorganisms solubilized the insoluble potassium (K) to soluble forms of K for plant growth and yield. Major amounts of K containing minerals (muscovite, orthoclase, biotite, feldspar, illite, mica) are present in the soil as a fixed form which is not directly taken up by the plant. The main mechanism of KSMs is acidolysis, chelation, exchange reactions, complexolysis and production of organic acid.

Solubilization of K is performed by numerous bacterial strains like *B. mucilaginosus, B. edaphicus, B. circulans, Pseudomonas, Burkholderia, Acidithiobacillus ferrooxidans*, and *Paenibacillus* spp isolated from stressed environment (Rajawat *et al.*, 2020).

Kour *et al.* (2020a) has reported a wide range of potassium-solubilizing microbes viz., *Acidithiobacillus*, *Agrobacterium*, *Arthrobacter*, *Aspergillus*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pantoea*, *Flectobacillus*, *Klebsiella*, *Microbacterium*, *Myroides*, *Paenibacillus*, *Pseudomonas* and *Stenotrophomonas*.

The biodiversity of wheat associated bacteria was deciphered from the peninsular zone of India. A total of 264 isolated bacteria were analyzed for plant growth promoting traits and 5% of isolates were found as K- solubilizers at high temperature. *Delftia* and *Salmonella* were first time reported as potassium solubilizers at high temperatures. The K-solubilizing bacteria may have use in the amelioration of K-deficient soil in agriculture at high temperatures (Verma *et al.*, 2019)

The last few decades, potassium-solubilizing bacterial genera have been recovered, including *Acidithiobacillus*, *Agrobacterium*, *Aminobacter*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Clostridium*, *Delftia*, *Enterobacter*, *Klebsiella*, *Methylobacterium*, *Microbacterium*, *Myroides*, *Paenibacillus*, *Pseudomonas*, *Rhizobium*, *Salmonella*, and *Sphingomonas* (Verma *et al.*, 2017)

Pirhadi *et al.* (2016) demonstrated the advantage of isolate R-1 (*Enterobacter cloacae*) inoculation on potassium uptake by wheat. Also showed that *Pseudomonas* sp. and *Enterobacter cloacae* have the ability to inhibit the growth of *Fusarium.*

In vitro release of potassium by *Bacillus sp.* and *Pseudomonas sp.* in the range of 0.60 to 1.26 cm at 1 % mica supplementation and 3.23 μg mL^{-1} to 41.20 μg mL^{-1} releases in the broth was observed by Sen *et al.* (2016).

Yuan *et al.* (2015) isolated *Enterobacter* spp. as endophytic bacteria from rhizome, root, leaves and stem of Moso Bamboo with the ability of dissolving potassium. Ghevariya and Deasi, (2014) identified *Pseudomonas* sp. capable of solubilizing potassium from mica. The ability of potassium solubilization by *Enterobacter* spp. has been reported by Zhang and Kong, (2014).

Bagyalakshmi *et al.* (2012) studied the ability of K solubilization by *Bacillus* sp., *Burkholderia* sp., and *Pseudomonas* sp. at different temperatures and carbon sources from tea (*Camellia sinensis*). Among the various types of carbon sources like glucose, fructose, sucrose and starch, the best carbon source for solubilization of K was found to be glucose at 35 ºC temperature.

2.2.4 Indole Acetic Acid Production

Auxins have a range of growth-promoting functions in plants like promotion of root formation, stimulation of cell division, extension, differentiation and regulation of fruit ripening. Auxins can encourage the growth of roots and stems quickly (by increasing cell elongation) or slowly (through cell division and differentiation). The production of such growth regulators by endophytes provides various benefits to the host plant including the facilitation of root system expansion, which enhances the absorption of water and nutrients and improves plant survival. Many studies have described the ability of endophytic bacteria to produce phytohormones and auxins, such as IAA (Hallmann *et al.,* 1997) and other beneficial bacteria such as *Azospirillum, Alcaligenes faecalis, Klebsiella, Enterobacter, Acetobacter diazotrophicus,* and *Herbaspirillum seropedicae* .

Producing phytohormones is a common feature of endophytic bacteria to boost plant growth and increase plant stress tolerance (Pieterse *et al.,* 2009). Genes encoding proteins for biosynthesis of indole acetic acid (IAA) (Zúñiga *et al.,* 2013), cytokinins (CKs) (Bhore *et al.,* 2010) and gibberellins (GAs) (Shahzad *et al.,* 2016) are often present in the metagenome of plant endophytic bacterial communities. The in vitro production of IAA and its possible involvement in PGP has been reported for many other endophytic bacteria (Govindarajan *et al.* 2008., Rothballer *et al.* 2008., Jha and Kumar, 2009 and Malfanova *et al.* 2011).

The bacterial endophytes such as *C. oceanosedimentum* SAK1, *C. luteum* SAK2, *E. ludwigii* SAK5, *B. cereus* SA1, *M. yunnanensis* SA2 and *E. tabaci* SA3 from the plant species *A. princeps, C. ficifolium, O. biennis* and *E. crusgalli* are capable of producing several phytohormones and antioxidants (Khan *et al.,* 2020)

Among the endophytic bacteria-induced growth promoting mechanisms resulting from plant hormones, IAA is the most common and extensively studied phytohormone. Recent studies have shown that more than 40% of all endophytic or rhizospheric bacteria are able to produce IAA (Zhu and She, 2018).

Previous studies reports that IAA production by endophytic bacterial species is useful for plant growth promotion (Damam *et al.* 2016 and Karthik *et al.* 2016). Yu *et* *al.* (2016) reported IAAproducing *Psychrobacillus, Microbacterium, Lysinibacillus,* and *Bacillus* from maize root.

The bacteria with IAA-producing capacity can increase root growth and root length of plants, which leads to the production of a larger root surface area that enables the plant to obtain more nutrients from the soil (Islam *et al.,* 2015).

Maize plant associated microbial genera *Rhanella, Pantoea, Rhizobium, Herbaspirillum, Enterobacter, Brevundimonas, Pseudomona,* and *Burkholderia* were found to produce IAA (Montanez *et al.,* 2012).

The study of Etesami *et al.* (2014a) reported an IAA-producing endophytic bacterium *Pseudomonas putida* CEN7 from berseem clover plants nodules. *Methylobacterium* strains have been reported to produce phytohormones such as cytokinins and auxins (Schauer and Kutschera, 2011). *Pantoea* sp. CCBAU15488 was reported to be able to nodulate soybean and was also recorded for production of IAA on the basis of intensity of red color (Li *et al.,* 2008).

The yeast *Williopsis saturnus* associated with maize roots produces in vitro indole-3-acetic acid (IAA) and indole-3-pyruvic acid (IPYA) which was found to promote maize growth under gnotobiotic and glasshouse conditions (Nassar *et al.,* 2005)

The role of IAA that was synthesized by the PGPB *Pseudomonas putida* GR12-2 in the development of canola roots was studied following the construction of an IAA-deficient mutant of this strain. Seed inoculation with wild-type *P. putida* GR12-2 induced the formation of roots that were 35–50% longer than the roots from seeds treated with the IAA-deficient mutant and the roots from uninoculated seeds (Patten and Glick, 2002).

2.2.5 Siderophores Production

Iron (Fe) is an essential micronutrient for plants and microorganisms, as it is involved in the nitrogen fixation and assimilation process. Iron is also major element required by plant in various vital biological processes, such as photosynthesis, respiration, chlorophyll biosynthesis (Kobayashi and Nishizawa, 2012). Microorganisms have developed dynamic strategies for iron uptake. Bacteria can overcome the nutritional Fe limitation by utilizing chelator agents called siderophores. Siderophores are defined as low-molecular-mass molecules (< 1000 Da) with high specificity and affinity for chelating or binding Fe3+, followed by the transportation and deposition of Fe inside bacterial cells (Krewulak and Vogel, 2008).

Microorganisms growing in aerobic conditions need iron for reduction of oxygen hence ATP synthesis, heme formation and for other metabolic activities. In aerobic conditions, the solubility of iron is very low due to the predominance of ferric (Fe^{3+}) ions. In anaerobic and acidic soils, high concentrations of ferrous (Fe^{2+}) ions generated by the reduction of iron oxides (Fe^{3+}) in flooded soil leading to excessive amount of iron uptake. It was shown that excess amount of iron inhibit plant growth and to cause a decrease in productivity. With respect to plant health, the presence of genes for siderophore production indicates strong biocontrol capacities allowing competition with pathogens for iron, as suspected for some cultured endophytes (Sessitsch *et al.,* 2012).

Plant growth promoting bacteria producing siderophores, effect the plant growth through direct or indirect mechanism under low iron conditions. The excretion of siderophores by bacteria stimulate plant growth, either by improving nutrition (direct mechanism) or by inhibiting the establishment of phytopathogens (indirect mechanism) through the sequestration of Fe from the environment. Unlike microbial pathogens, plants are not affected by bacterial-mediated Fe depletion, and some plants can even capture and utilize Fe^{3+} siderophore bacterial complexes (Souza *et al.*, 2015b).

Upon quantitative analysis, 10 isolates produced siderophore above 65% siderophore units and subsequently characterized for the type of siderophore. Most of them produced both carboxylate and hydroxamate type of siderophores. Production of siderophores is an important attribute of plant growth promoting endophytes and facilitates growth of plants under iron limiting conditions through iron sequestration (Maheshwari *et al.,* 2019).

Santiago *et al.* (2017) studied that siderophore produced by consortium of *Streptomyces* sp. R170 and *Methylibium* sp. R182 which may facilitate the solubility of nutrients such as iron and other metal and utilization of microbial siderophore by potato plant and improve the micronutrient availability. Arruda *et al.* (2013) reported that genus *Bacillus* and *Enterobacter* have ability to produce siderophore, also improve rhizosphere colonization.

Loaces *et al.,* (2011) described the endophytic siderophore-producing bacterial community present in grains, roots, and leaves of *Oryza sativa*. Their population was less than 10% of the heterotrophic bacteria produced siderophores in roots and leaves of young plants, but most of the heterotrophic bacteria were siderophore-producers in mature plants. According to their amplified restriction DNA ribosomal analysis (ARDRA) pattern, 54 of the 109 endophytic siderophore producing bacteria isolated from different plant tissues or growth stages were of genera *Sphingomonas, Pseudomonas, Burkholderia*, and *Enterobacter.* They alternated during plant growth, but the genus Pantoea was predominant in roots at tillering and in leaves at subsequent stages.

In *Jatropha curcus* (L,), co-inoculation of three isolates *Brevibacillus brevis* (MS1), *Bacillus licheniformis* (MS3) and *Acinetobacter calcoaceticus* (MS5) demonstrated distinct plant growth-promoting characteristics, such as siderophore production, and these effects the shoot weight, root weight, total biomass, shoot and root length and grain yield. Therefore siderophores are helpful in plant development and plant disease management for improvement of agriculture production (Patel *et al.,* 2010).

The Fe-pyoverdine complex synthesized by *Pseudomonas fluorescens* C7 was taken up by *Arabidopsis thaliana* plants, leading to an increase of iron inside plant tissues and to improved plant growth (Vansuyt *et al.,* 2007).

Various studies have demonstrated the ability of siderophore producing endophytes in plant growth promotion. Upon inoculation of Mung bean plants with siderophore-producing *Pseudomonas* strain GRP3, chlorotic symptoms were reduced and chlorophyll content increased under iron-limiting environments. A total of 84 endophytic bacteria isolated from nodules and roots of *Cicer arietinum* and *Pisum sativum* plants out of which 14 isolates produced siderophore (Sharma *et al.,* 2003).

2.2.6 ACC deaminase Activity

Plant synthesizes the gaseous hormone ethylene (C_2H_4) in plant tissues from the precursor 1-aminocyclopropane- 1-carboxylic acid (ACC). It is involved in multiple developmental and physiological processes of plants, such as seedling emergence, tissue differentiation, root hair development and elongation, leaf and flower senescence, lateral bud development, anthocyanin synthesis, fruit degreening and ripening, production of volatile compounds responsible for aroma in fruits *etc.*, (Abeles *et al.,* 1992; Frankenberger and Arshad, 1995). Ethylene regulates plant responses to abiotic and biotic stresses. Under ambient conditions, plants produce ethylene in required amount, conferring beneficial effects on plant growth and development; however, in response to abiotic and biotic stresses there is often increase in ethylene production that has an adverse effect on plant growth. Ethylene is thought to be responsible for senescence in all plants (Ali *et al.,* 2012). Ethylene biosynthesis is increased during drought stress that results in reduced root and shoots growth.

The ACC an immediate precursor of ethylene can be utilized by some bacteria cleaving into α-ketogluatarate and ammonia by ACC deaminase activity and relieving plant from stress (Glick, 2007). Under stress conditions generated by salinity, drought, water logging, heavy metals and pathogenicity, the endogenous level of ethylene is significantly increased which negatively affects the overall plant growth.

ACC deaminase positive *Enterobacter*-mediated mitigation of salinity stress, and plant growth promotion of *Cajanus cajan* was studied by Anand *et al.* (2021)

According to Ahmad *et al.* (2016) co-inoculation of ACC deaminase producing *Pseudomonas* sp. with *Mesorhizobium* sp. leads to reduction of ethylene stress level and improved nodule number, nodule dry weight, fresh biomass, grain yield, straw yield and N content in grains of chickpea.

Akhtar *et al.* (2015) studied the interactive effect of biochar and plant growth-promoting endophytic bacterial strains (*Burkholderia phytofirmans* (PsJN) and *Enterobacter* sp. (FD17)) containing 1-aminocyclopropane-1-carboxylate deaminase and exopolysaccharide activity on mitigating salinity stress in maize (*Zea mays* L.).

Verma *et al.* (2015) reported psychrotolerant and drought tolerant endophytic bacteria from wheat showing ACC deaminase activity by different genera of *Arthrobacter, Flavobacterium, Bacillus, Methylobacterium, Providencia, Pseudomonas, Stenotrophomonas* and *Enterobacter.* These bacteria also possess solubilization of phosphorus, potassium, zinc; produced IAA, siderophore, HCN and ammonia and showed antifungal activity against plant pathogens.

Ali *et al.* (2014a) observed that ACC deaminase activity of endophytes played a vital role in relieving salinity stress in plants. In addition to endophytic bacteria, adding biochar (a charcoal-like material) to salt affected soil could also be used an alternative strategy for reducing salinity stress in plants.

Ali *et al.* (2014b) reported *Pseudomonesa fluorescens* and *Pseudomonesa migulae* were tested for the ability of promoting tomato plant growth in the high levels of salt, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity is directly responsible for tomato plants in response to salt stress.

Xu *et al.* (2014) reported plant growth promoting activity of ACC deaminase producing *Bacillus subtilis* (HYT-12-1) on tomato seedlings Isolates exhibited numerous plant growth promoting (PGP) traits: 37% of indole-3-acetic acid production; 37% of phosphate solubilization; 24% of siderophores production; 85 % of potential nitrogen fixation and 6% of 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. Isolate HYT-12-1 was shown to have maximum ACC deaminase activity (112.02 nmol α-ketobutyrate mg^{-1} protein h^{-1}) among the five ACC deamiase producing strains. That was the first study to describe endophytic *Bacillus* communities within tomato seeds, and the results suggest that *B. subtilis* strain HYT-12-1 would have a great potential for industrial application such as biofertilizer.

Tittabutr *et al.* (2013) collected three bacterial isolates containing ACC deaminase, *Enterobacter* sp. ACC1, *Enterobacter* sp. ACC2, and *Chryseobacterium* sp. ACC3 were selected according to their ACC deaminase activity and resistance abilities to drought stress conditions. It coincided with the co-inoculation of plants by *Bradyrhizobium* and rhizobacteria containing ACC deaminase. This co-inoculation obviously alleviated the plants growing under stress conditions by adjusting its expression of ACC deaminase to different levels of stress conditions.

Inoculation with ACC deaminase producing *Bacillus licheniformis* K11 strain enhanced drought tolerance in pepper (Hui and Kim, 2013). Endophytic diazotrophic *Achromobacter xylosoxidans* AUM54 isolated from *Catharanthus roseus* grown in saline soil showed ability to produce ACC deaminase and to reduce ethylene levels (Karthikeyan *et al.*, 2012).

Iqbal *et al.* (2012) described improved nodulation in lentil by adjusting ethylene level with co-inoculation of ACC deaminase containing strains of *Pseudomonas* sp. and *Rhizobium*. Zahir *et al.* (2011) exploited lowering of ethylene concentration by synergestic use of *P. jessenii* and *Rhizobium* improving nodulation, growth and yield of lentil. Similarly, Ahmad *et al.* (2011), studied effect of combined inoculation of PGPR strains and *Rhizobium phaseoli* containing ACC deaminase on growth and yield parameters of mungbean.

Zhang *et al.* (2011b) reported that one hundred Cu-resistant-endophytic bacteria were isolated from Cu-tolerant plants grown on Cu mine wasteland, of which, eight Cu-resistant and 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing endophytic bacteria were obtained based on the ACC deaminase activity of the bacteria and characterized with respect to metal resistance, production of ACC deaminase, indole-3-acetic acid (IAA) as well as siderophores and mineral phosphate solubilization. *Ralstonia* sp. J1-22-2, *Pantoea agglomerans* Jp3-3, and *Pseudomonas thivervalensis* Y1-3-9 with higher ACC deaminase activity (ranging from 213 to 370 μM α-ketobutyrate mg^{-1} h^{-1}) were evaluated for promoting plant growth and Cu uptake of rape grown in quartz sand containing 0, 2.5, and 5 mg kg^{-1} of Cu in pot experiments.

Glick *et al.* (2007) reported that the ACC deaminase producing bacteria sequestered and degraded the ACC produced in the plant cell to supply these with nitrogen (ammonia) and energy (α-ketobutyrate), without disturbing the nutritional balance of the plant. Furthermore, by removing ACC, the bacteria reduce the deleterious effect of excess ethylene, ameliorating plant stress and promoting plant growth.

2.2.7 Exopolysaccharide Production

The production of EPS is an important criterion for the classification of stress tolerant microbes. Most of the bacteria survive under stress condition due to the production of EPS. EPS promotes bacteria survival due to enhancing water retention capacity and regulating diffusion of organic carbon sources. Bacteria also contain high molecular weight lipopolysaccharide–protein (LP) (carbohydrate complexes) and polysaccharide–lipid (PL) complexes that are responsible for desiccation tolerance (Verma *et al.*, 2020)

Minah *et al.* (2015) isolated 74 exopolysaccharide-producing bacteria and identified them as gram-negative bacteria. Of these 34 isolates were of potential producers of exopolysaccharides and 15 isolates produced exopolysaccharides in the range of 0.10 to 2.24 mg ml^{-1}.

Haggag *et al.* (2014) identified the exopolysaccharides from *Pseudomonas fluorescens*, *Bacillus amyloliquefaciens* and *Bacillus polymyxa* have the ability to activate multiple plant defense mechanisms against wheat powdery mildew caused by *Blumeria graminis* and leaf rust caused by *Puccinia reconditata,* under controlled conditions. At the same time, it increased ACC deaminase, indol acetic acid, proline, peroxidase, chitinase and soluble protein contents under saline and normal soil.

Naseem and Bano (2014) isolated three bacterial species *Proteus penneri* (Pp1), *Pseudomonas aeruginosa* (Pa2), *Alcaligenes faecalis* (AF3) and selected them as EPS-producing bacteria based on mucoid colony formation. Consortia of these isolates and their respective EPS showed greater potential for drought tolerance compared to isolate single inoculation.

Exopolysaccharides producing microbes enhanced plant growth under drought stress (Naseem and Bano, 2014). Plants treated with EPS-producing bacteria display increased resistance to water stress. Alami *et al.* (1998) observed a significant increase in root adhering soil 23 per root tissue (RAS/RT) ratio in sunflower rhizosphere inoculated with the EPSproducing rhizobial strain YAS34 under drought conditions

Bacteria like *Pseudomonas* can survive under stress conditions due to the production of exopolysaccharide (EPS), which protects microorganisms from water stress by enhancing water retention and by regulating the diffusion of organic carbon sources (Chenu and Roberson, 1996).

Bacterial exopolysaccharides (EPS) can protect bacteria from various stresses. Production of exopolysaccharides (EPS) is used as criteria for the isolation of stress tolerant microorganisms. A *Pseudomonas* sp. strain increased its EPS production during desiccation (Roberson and Firestone, 1992). The production of EPS possibly enhances water retention in the microbial environment and seems to regulate the diffusion of carbon sources such as glucose. Cellular defenses against desiccation-induced damage include accumulation of compatible solutes, exopolysaccharide production and enzyme synthesis to combat oxidative stress (Leblanc *et al.,* 2008).

2.2.8 HCN Production

A secondary metabolite produced commonly by rhizospheric microorganisms is hydrogen cyanide (HCN), a gas known to negatively affect root metabolism and root growth. It affects sensitive organisms by inhibiting the synthesis of ATP mediated cytochrome oxidase and is a potential and environmentally compatible mechanism for biological control of weeds (Heydari *et al.,* 2008).

Bhutani *et al.* (2019) has isolated endophytic bacteria MJiN9, MJiR4, MJiR9 and MJiR13 from roots and nodules of *Vigna radiata* (Mung bean) were found to produce high levels of HCN.

Etminani *et al.* (2018) isolated 61 endophytic bacteria from the leaves and stems of healthy wild Pistachio trees (*Pistacia atlantica* L.) among all only the isolate Ba66 could produce a low amount of hydrogen cyanide.

Rijavec and Lapanje, (2016) studied HCN as a biocontrol agent, based on its toxicity against plant pathogens by inhibiting the growth of phytopathogens. A new concept was developed, in which HCN does not act as a biocontrol agent, but is involved in geochemical processes in the substrate (e.g., chelation of metals), indirectly increasing the availability of phosphate.

Tashi–Oshnoei *et al.* (2017) demonstrated that the endophytic bacterial strains Sm79 and Pp95 isolated from samples of roots, leaves and stems of healthy oak trees released low concentrations of hydrogen cyanide.

Etesami *et al.* (2014b) has isolated150 strains (endophytic and rhizospheric isolates) from the rhizosphere and inside the roots of canola *(Brassica napus* L.) plants grown in the field from northern Iran and characterized for plant growth promoting (PGP) traits. Of them, one hundred isolates produced indole-3-acetic acid (IAA), whereas 17 isolates solubilized phosphate, 44 isolates produced siderophore, 34 produced 1-aminocyclopropane-1-carboxylate (ACC) deaminase and five produced hydrocyanic acid (HCN).

Karmel *et al*. (2014) isolated *Pseudomonas* sp. and *Bacillus* sp. and evaluated its ability to produce hydrogen cyanide synthesis. Results showed that among these isolates on qualitative analysis of HCN indicated a strong production of HCN in Pf1 and CPf5. In quantitative estimation, Pf1 and CPf5 recorded the maximum OD value of 0.094 and 0.085 respectively but Bs10 recorded least OD value (0.015).

Yogendra *et al*. (2013) evaluated the plant growth promoting characteristic such as production of hydrogen cyanide for three *Rhizobacteria* isolated from rhizosphere of Pigeon pea. HCN production was observed in 33% isolates.

Ghodsalavi *et al*. (2013) isolated 40 colonies of bacteria from the rhizosphere of valerian and tested their ability of bacteria to produce hydrogen cyanide (HCN). Of total, seven isolated bacteria (belong to *Pseudomonas* genus) that showed a high potential of HCN production.

Supraja *et al*. (2011) isolated, identified and characterized fifteen fluorescent *Pseudomonas* from rhizospheric soils of redgram and maize crops in the Rangareddy district. These test isolates were biochemically characterized and screened *in vitro* for their production of Hydrogen cyanide (HCN) and siderophore. Due to production of HCN and siderophores, fluorescent *Pseudomonas* isolates inhibited the growth of *Fusarium moniliforme*.

Maleki *et al.* (2010) out of 144 bacteria from cucumber rhizosphere, eight isolates were identified as *Pseudomonas fluorescens* and selected for root colonization and PGP properties. Among these CV-6 strain produced considerable amounts of siderophore, indole acetic acid and also showed positive reaction for HCN.

2.2.9 Antagonistic Activity

Use of microorganism is one of the important ways for the control of diseases. Biocontrol systems are ecofriendly, cost-efficient and maintaining natural soil flora. The biocontrol agent should remain active under large range of conditions viz., varying pH, temperature and concentrations of different ions to act capably. Biocontrol agents limit the growth of fungal or bacterial pathogens, few nematodes and insects. Such bacteria can limit pathogens directly by producing antagonistic substances, competition for iron, detoxification or degradation of virulence factors; or indirectly by inducing Systemic Resistance (ISR) in plants against certain diseases, signal interference, competition for nutrients and niches and interference with activity, survival, germination and sporulation of the pathogens. Bacterial endophytes colonize an ecological niche similar to that of phytopathogens, which makes them suitable as biocontrol agents (Berg *et al.,* 2005). Indeed, numerous reports have demonstrated that endophytic microorganisms can have the capacity to control plant pathogens.

Gamez *et al.* (2018) studied that *Bacillus* sp. B25 as an effective biocontrol agent against the maize pathogenic fungus *Fusarium verticillioides* (Fv). The genome of B25 was found closely related to the *Bacillus cereus* group and a high percentage (70-75%) of the genetic information was conserved between B25 and related strains, which include most of the genes associated to fungal antagonism. B25 contains genes variety of antagonistic mechanisms including chitinases, glycoside hydrolases, siderophores, antibiotics, and biofilm production that help in root colonization.

The antimicrobial compounds produced by endophytic bacteria represent a promising alternative protection to plants against phytopathogens. Bacterial endophytes have been shown to prevent disease development through endophyte-mediated *de novo* synthesis of novel bioactive compounds. Importantly, lipopeptides can curb fungal and bacterial pathogens by planting in cell membranes and producing pores that make cells leaky and prevent cellular growth or metabolic activity (Bacon *et al.,* 2015).

Gond *et al.* (2015) isolated *Bacillus amyloliquifaciens* or *Bacillus subtilis* from seeds of several varieties of maize. Lipopeptide extracts from *Bacillus* spp. isolated from Indian popcorn and yellow dent corn showed inhibitory activity against *Fusarium moniliforme*. Using MALDI-TOF mass spectrometry the presence of antifungal iturin A, fengycin and bacillomycin in these isolates was detected. *B. subtilis* (SG JW.03) isolated from Indian popcorn showed strong inhibition of Arabidopsis seed mycoflora and enhanced seedling growth. Based on their study of maize endophytes, they hypothesize that, bacterial endophytes that naturally occur in many maize varieties may function to protect hosts by secreting antifungal lipopeptides that inhibit pathogens as well as inducing the up-regulation of pathogenesis-related genes of host plants (systemic acquired resistance).

Pantoea polymyxa strains produced the previously characterized anti-Fusarium compound, fusaricidin (Mousa *et al.* 2015). Certain bacterial endophytes can produce a suit of lipopeptides of antifungal and antibacterial, including iturins, bacillomycins, fengycins, and surfactins (Jr *et al.,* 2014).

Sarvani and Reddy (2013) identified thirty bacterial isolates as Bacillus sp. based on their colony morphology, cell morphology and biochemical characteristics. These were antagonistic to soil-borne phytopathogens viz., *Sclerotium rolfsii, Rhizoctonia solani* and *Fusarium solani*.

Some toxic metabolites such as bacillopeptidase, subtillin E and a lactamase from the endophytic *Bacillus* spp., and production of indole acetic acid and their regulation to produce ammonia by endophytic *Methylobacterium* spp. were also found to be very effective in nematode control (Vetrivelkalai *et al.,* 2010).

Acremonium zeae, an endophyte of maize, has a protective mechanism for maize seeds which display a significant antifungal activity against *Aspergillus flavus* and *Fusarium verticillioides* (Donald *et al.,* 2005).

Pseudomycins, antifungal compounds, are produced by plant-associated *pseudomonads Pseudomonas viridiflava*, a fluorescent bacteria located within the leaf tissues of many grass species, produces ecomycins which represent a family of novel lipopeptides. The structure of these lipopeptides involves common amino acids along with some unusual amino acids such as homoserine and β-hydroxyaspartic acid, which are active against fungal pathogen such as *Cryptococcus neoformans* and *C. albicans* (Miller *et al.*, 1998).

2.2.10 Antibiotic Sensitivity

Painkra *et al.* (2019) has characterize and screen different native isolates of phosphate solubilising bacteria and *Azotobacter*. All the isolates were taken for their antibiotic susceptibility study. Some isolates were found susceptible for tetracycline (30 mcg) and streptomycin (10 mcg).

The 10 promising endophytic bacterial isolates tested for their ability to resist growth inhibition by the antibiotics showed that strain FTR was resistant to eight antibiotics out of 19 tested. This result makes FTR as most potential endophytes to compete against other microbes. Antibiotic resistant strains have the ability to compete with other strains (Sandhya *et al.*, 2017).

Yaish *et al.* (2015) has isolated endophytic bacteria from date palm (*Phoenix dactylifera* L.) seedling roots, characterized and tested for their ability to help plants grow under saline conditions. Molecular characterization showed that the majority of these strains belonged to the genera *Bacillus* and *Enterobacter* and had different degrees of resistance to various antibiotics.

Liasi *et al.* (2009) antimicrobial sensitivity test to 18 different types of antibiotic were evaluated using the disc diffusion method. Inhibition zone diameter was measured and calculated from the means of five determinations and expressed in terms of resistance or susceptibility. All the LAB isolates were resistant to colestin sulphate, streptomycin, amikacin, norfloxacin, nalidixic acid, mecillinam, sulphanethoxazole/ trimethoprim, kanamycin, neomycin, bacitracin and gentamycin but susceptible to erythromycin, penicillin G, chloramphenicol, tetracycline, ampicillin and nitrofurantion.

2.2.11 Induced systemic resistance (ISR) / Systemic acquired resistance (SAR)

The endophytic bacteria triggers a defense mechanism known as induced systemic resistance (ISR), which is phenotypically similar to systemic-acquired resistance (SAR) but SAR develops when plants successfully activate their defense mechanism in response to primary infection by a pathogen (Ryan *et al.*, 2008).

The ISR induces the defense mechanisms and also guards an un-attacked portion of plants from future attack of plant pathogens. Besides this, numerous endophytic bacteria have been confirmed to induce salicylic acid along with jasmonic acid (JA)- and ethylene (ET)-mediated ISR regulation. The cytosol-specific transcription co-regulator NPR1 and transcription factors MYB72 and MYC2 have been well studied for the induction of defense genes in the JA- and ET-mediated ISR regulation (Miliute *et al.*, 2015).

A wheat endophytic bacterium *Pseudomonas aeruginosa* PW09 was reported to trigger an induced systemic resistance in cucumber plants challenged with biotic stress of fungal pathogen *Sclerotium rolfsii* (Pandey *et al.*, 2012).

2.3 ENDOPHYTIC BACTERIA MEDIATED STRESS TOLERANCE

2.3.1 Water stress – Osmotolerance

Endophytic bacteria which survive within plant tissues are among the most appropriate technologies improving plant growth and yield under drought conditions. These endophytic bacteria live within plant tissues and release various phytochemicals that assist plant to withstand in harsh environmental conditions, *i.e.*, drought stress. Their plant growth-promoting characteristics include nitrogen fixation, phosphate solubilization, mineral uptake, and the production of siderophore, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and various phytohormones. These plant growth promoting characteristics of endophytic bacteria improve root length and density, which lead to the enhance drought tolerance. In addition, plant-endophytic bacteria assist plant to withstand against drought stress by producing drought-tolerant substances, for instance, abscisic acid, indole-3-acetic acid, ACC deaminase, and various volatile compounds. Indirectly, endophytic bacteria also improve osmotic adjustment, relative water content, and antioxidant activity of inoculated plants. Altogether, these bacterial-mediated drought tolerance and plant growth-promoting processes continue even under severe drought conditions which lead to enhanced plant growth promotion and yield (Fig 2.3) (Ullah *et al.*, 2019).

Khan *et al.* (2016) evaluated a consortium with 10 endophytic strains selected based on their previous research, by inoculating on commercial hardwood cuttings of *Populus deltoides* × *Populus nigra* clone OP-367. Khan *et al.* (2016) showed that *Populus deltoides* inoculated with endophytes consortia (including *Rhodotorula graminis, Burkholderia vietnamiensis* and *Rhizobium tropici*) under critical drought stress conditions.

The bacterial endophyte *Bacillus subtilis* strain B26 was assumed to improve timothy (*Phleum pretense* L.) growth under drought stress through the modification of osmolyte accumulation in roots and shoots as the notable increase in levels of sugars particularly sucrose, fructose and key amino acids namely asparagine, glutamic acid and glutamine were recorded in colonized plants (Bourque *et al.,* 2016).

Naveed *et al.* (2014b) has evaluated the potential of two endophytic bacterial strains, *Burkholderia phytofirmans* strain PsJN and *Enterobactersp*. FD17, for improving physiology and growth of maize under drought stress. That bacterial inoculation minimized the drought stress-imposed effects significantly increasing shoot biomass, root biomass, leaf area, chlorophyll content, photosynthesis, and photochem-ical efficiency of PSII. Similarly, bacterized seedlings showed higher leaf relative water content (30%) compared to control, whereas 43% higher leaf damage in terms of relative membrane permeability was observed in non-inoculated plants under drought stress.

B. phytofirmans is one of the most studied bacterial endophyte was used to investigate the potential to ameliorate the effects of drought stress on growth, physiology and yield of wheat (*Triticum aestivum* L.) by Naveed *et al.* (2013), the inoculation modulated biochemical and physiological parameters of wheat seedlings under drought stress conditions. Based on their results the application of PsJN is effective to improve physiology, relative water content and biomass of wheat under reduced irrigation. The improved plant physiological and antioxidant activity ultimately leads to enhanced crop yield and quality.

The water stressed plants were observed with reduced damage by reactive oxygen species (ROS) in consortia treated plants indicating a possible mechanism for symbiosis-mediated drought. In addition to, results showed that pepper plants treated with *Bacillus licheniformis* K11 and exposed to drought stress had 50 % higher biomass than non-treated plants (Lim and Kim, 2013)

B. phytofirmans PsJN is among the best studied plant growth promoting endophytes. It colonizes the rhizosphere and endosphere, and promotes growth, and enhances abiotic and biotic stress tolerance in a variety of crops and vegetables (Mitter *et al.*, 2013).

Under drought stress condition maize (*Zea mays* L) was inoculated with *Azospirillum* spp. a plant growth promoting bacteria (PGPB). *Azospirillum lipoferum* enhanced growth of maize by the production of phytohormones such as ABA, IAA, and GAs. The results suggest that both ABA and GAs produced by endophytic *Azospirillum* contribute to drought stress alleviation of host plants (Cohen *et al.*, 2009).

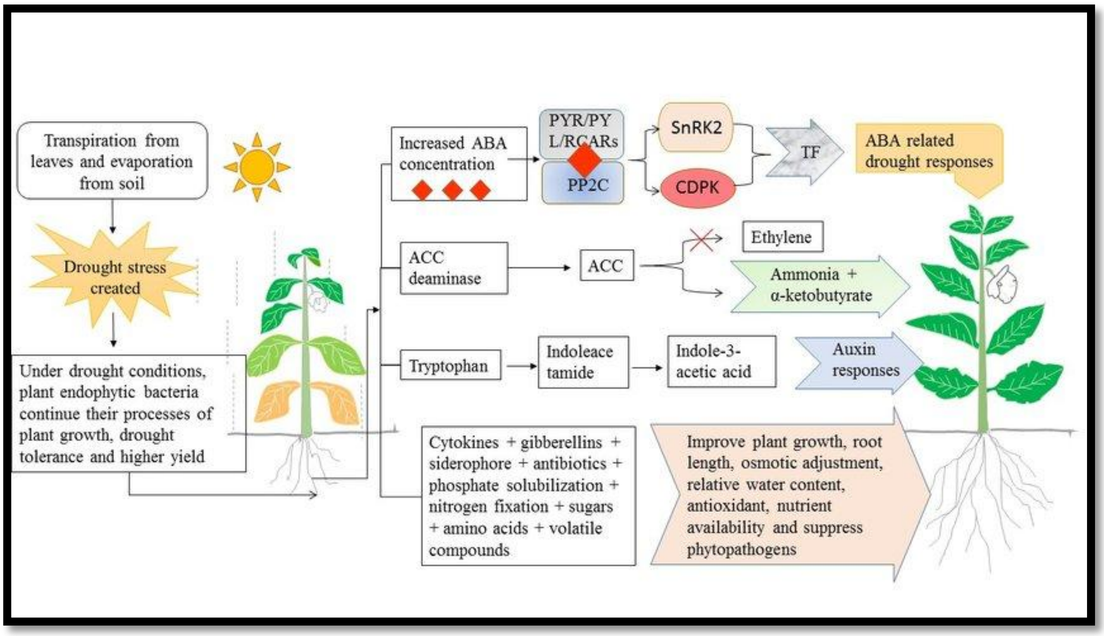


Fig. 2.3. Endophytic bacteria mediated drought tolerance (Ullah *et al.*, 2019)

2.3.2 Salinity Stress

Soldan *et al.* (2019) mentioned that bacterial endophytes (*Gordonia terrae* KMP456-M40) play significant contribution in the early establishment of mangrove propagules and reported to enhance barley ear weight by 65%. Additionally, they also observed that under axenic conditions, endophytic bacteria were able to enhance the root length of mangrove seedlings and the biomass of salt-stressed rice by 65% and 62%, respectively.

Afridi *et al.* (2019) observed the endophytic action of *Kocuria rhizophila* and *Cronobacter sakazakii* on the enhancement of salinity (80–160 mM NaCl) tolerance level in wheat genotypes (cv. Pasban 90 and Khirman) through the production of antioxidant enzymes and ACC deaminase.

Abd-Allah *et al.* (2018) documented that the inoculation of endophytic *B. subtilis* BERA 71 enhanced the dry weight (28.7%) of the chickpea (cv. Giza 1) shoot compared to the un-inoculated control plants under salt stress (200 mM NaCl).

Egamberdieva *et al.* (2017b) showed *Mesorhizobium ciceri* and *Bacillus subtilis* could rescue chickpea from salt stress by decreasing H_2O_2 concentrations and improving proline contents

Li *et al.* (2016) has reported that the endophytic bacteria (*Sphingomonas* sp. pp01, *Bacillus* sp. pp02, *Pantoea* sp. pp04 and *E.ludwigii* sp. pp06) isolated from elephant grass (*Pennisetum purpureum*) enhanced plant growth and biomass yield of hybrid *Pennisetum* under salt stress conditions.

Akhtar *et al.* (2015) indicated that salinity significantly decreased the growth of maize, whereas both biochar and inoculation of endophytic bacteria mitigated the negative effects of salinity on maize performance either by decreasing the xylem Na^+ concentration ([Na^+] xylem) uptake or by maintaining nutrient balance within the plant, especially when the two treatments were applied in combination.

Yaish *et al.* (2015) also revealed that pre-treatment of canola seeds with endophytic *Paenibacillus xylanexedens* PD-R6 and *Enterobacter cloacae* PD-P6 isolated from a date palm tree (*Phoenix dactylifera*) resulted in 27% enhancement in root elongation at 100 mM NaCl when compared to un-inoculated control plants.

Qin *et al.* (2014) documented endophytic *Isoptericola dokdonensis* KLBMP 4942, *Streptomyces pactum* KLBMP 5084, *Arthrobacter soli* KLBMP 5180 and *B. flexus* KLBMP 4941 from *Limonium sinense* halophyte as seed germination and growth booster under salt stress conditions.

2.3.3 Biotic Stress

Zhao *et al.* (2015) reported that *Bacillus* and *Paenibacillus* from the medicinal plant *Lonicera japonica* exhibiting inhibitory activity against phytopathogenic fungi, which are useful model organisms for studying various aspects of host-pathogen interactions.

Zhang *et al.* (2011a) has demonstrated that the rice seedlings inoculated with rSJ-10 an endophytic bacterium, characterized as E*nterobacter cloacae* by morphological, physiological, biochemical and 16s Rdna characteristics, expressing a anti-pest plant lectin that is Pinellia ternate agglutinin (PTA) protein were endowed with the anti-pest activity against White backed plant hopper (WBPH).

Bacon and Hinton, (2011) studied the effect of bacterial endophyte *Bacillus mojavensis* in reduction of maize seedling stalk lesions was evaluated. Mutant strains of *B. mojavensis* that tolerate fusaric acid were used in a growth room study and determined the development of stalk lesions, by co-inoculationing with a wild-type strain of *Fusarium verticillioides* that produces fusaric acid and with non-fusaric acid producing mutants of *F. verticillioides.* The results indicated that *B. mojavensis* mutant reduced stalk lesions, suggesting an in planta role for this substance as an antibiotic

Correlation between plant growth promotion and induction of disease resistance by endophytes of potato, and the putative mechanisms of endophytes interacting with the plant during resistance induction was studied by Pavlo *et al.* (2011). A *Pseudomonas* sp. strain was selected due to antagonism towards bacterial pathogens and a *Methylobacterium* sp. Strain because of efficient plant colonization, both tested strains promoted growth of potato shoots but only the *Pseudomonas* sp. increased potato resistance towards the soft rot disease. Induction of disease resistance by the *Methylobacterium* sp. was inversely proportional to the size of bacterial population used for inoculation. The plant antioxidant system was moderately activated during the induction of resistance by the biocontrol strains. qPCR data on expression of marker genes of induced systemic resistance and acquired systemic resistance in endophyte-infected *Arabidopsis* plants showed activation of both salicylic acid and jasmonate/ethylene-dependent pathways after challenge inoculation with the pathogen.

Senthilkumar *et al.* (2009) isolated endophytic bacteria *Paenibacillus* sp. HKA-15 (HKA-15) and *Bacillus* sp. HKA-121 (HKA-121) from surface sterilized root, stem, and nodule tissues of soybean and were reported as potential candidates for charcoal rot biocontrol as well as soybean plant growth promotion.

Yasuda *et al.* (2009) analysed the effects of a bacterial endophyte, *Azospirillum* sp. B510, on disease resistance in host rice plants. Rice plants were inoculated with B510 exhibited enhanced resistance against diseases caused by the virulent rice blast fungus *Magnapoerthe oryzae* and by the virulent bacterial pathogen *Xanthomonas oryzae*. In rice plants neither salicylic acid (SA) accumulation nor expression of pathogenesis related (PR) genes were induced by interaction with this bacterium, except for slight induction of pathogenesis related gene PBZ1. These results indicated the possibility that strain B510 is able to induce disease resistance in rice by activating a noval type resistance mechanism independent of SA- mediated defense signaling.

Rajendran *et al*, (2007) suggested that the endophytic bacterial strains *Bacillus* strains (EPCO 102 and EPCO 16) and *Pseudomonas fluorescens* strain Pf1 when applied either as a suspension culture or a talc-based formulation with chitin reduced American bollworm in cotton through ISR by inducing defence enzymes. In addition, endophytic bacterial bioformulation with chitin induced more and timely activities of chitinase, b-1, 3-glucanase, peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase and phenol in cotton plants infested with *Helicoverpa armigera.*

2.4 MOLECULAR CHARACTERIZATION OF ENDOPHYTIC BACTERIA

The classical methods of identifying microbes includes morphological and biochemical characterization which are insufficient to differentiate the microbe at species and strains level whereas, the molecular techniques such as BOX-PCR and sequencing of conserved regions of bacterial DNA, with an emphasis on the 16S rRNA have been broadly used in the characterization that results in reliable microbial identification (Versalovic *et al.,* 1994).

A detailed analysis of endophytic bacteria composition, using reference sequences from the 16S rRNA gene, revealed that the majority of bacteria identified in the study of Woźniak *et al.* (2018) belonged to the phylum Proteobacteria.

For the development of areas like microbiology, biotechnology and medicine using molecular techniques for identification of bacterial isolates is decisive. Molecular methods used in microbiology have enabled more comprehensive studies of the abundance and community composition of endophytic bacteria (Santoyo *et al.,* 2016)

Molecular characterization of bacteria is the first step in the development of the bio-stimulators. Most microorganisms are identified using the 16S rRNA gene sequencing technique. Next, in order to screen and select the most efficient strains that reveal plant growth promoting activities, a wide range of in vitro biochemical analyses are used (Szilagyi-Zecchin *et al.,* 2014)

Ten promising endophytic bacteria isolated from maize namely FTR, NFTR, FMZR9, FMZR2, MZ30V92, MRC12, MRC31, MRC33, MRC41 and MRR2 were selected and identified using biochemical and 16S rDNA gene sequencing as *Pseudomonas aeruginosa* (strains FTR and NFTR), *Pseudomonas monteilii* (strain

FMZR2), *Pseudomonas putida* (strain FMZR9), *Acitenobacter brumalii* (strain MZ30V92), *Enterobacter asburiae* (strain MRC12), *Sinorhizobium meliloti* (strain MRC31), *Pseudomonas thivervalensis* (strain MRC33), *Pseudomonas fulva* (strain MRC41), and *Pseudomonas lini* (strain MRR2) (Sandhya *et al.*, 2017)

Johnston-Monje and Raizada (2011) has studied endophytic bacteria of ten different varieties of maize by culturing, cloning, and DNA fingerprinting using terminal restriction fragment length polymorphism (TRFLP) of 16S rDNA. The research concluded the presence of the same bacterial genera across several genotypes of maize seeds (showed *Enterobacter* sp. and *Pantoea* sp. as the most common genera) along with some differences in the composition of the endophytic community.

Ibanez *et al.* (2009) isolated several bacterial isolates from surface-sterilized root nodules of *Arachis hypogaea* L. (peanut) plants growing in soils from Córdoba, Argentina. The 16S rDNA sequences of seven fast-growing strains were obtained and the phylogenetic analysis showed that these isolates belonged to the Phylum Proteobacteria, Class Gammaproteobacteria, and included *Pseudomonas* spp., *Enterobacter* spp., and *Klebsiella* spp.

Tak *et al.* (2009) isolated 21 isolates from *Tephrosia purpurea* and ten isolates from *Tephrosia villosa* growing in Jodhpur, Rajasthan. Based on ARDRA, all the 31 isolates from *T. purpurea* and *T. villosa* were placed into three and two groups, respectively. The 16S rRNA gene sequencing results revealed that the various isolates belonged to phylogenetically distant groups such as *Rhizobium, Ensifer, Bradyrhizobium, Ochrobactrum and Methylobacterium* from alpha proteobacteria and *Pseudomonas* from gamma proteobacteria.

Li *et al.* (2008) classified a total of 98 non-symbiotic endophytic bacterial isolates obtained from soybean root nodules and classified into eight rDNA types in ARDRA analysis and 21 BOX types in BOX-PCR. The phylogenetic analysis of 16S rDNA identified these strains as *Pantoea, Serratia, Acinetobacter, Bacillus, Agrobacterium*, and *Burkholderia*. Limited genetic diversity was revealed among these bacteria since most of the strains (85.7%) were found in three very similar rDNA types corresponding to *Pantoea agglomerans*, and many strains shared the same BOX-PCR patterns.

Rajendran *et al.* (2008) isolated non-rhizobial endophytes from the surface sterilized root nodules of pigeon pea (*Cajanus cajan*). Three of these non-rhizobial isolates showed plant growth promotion. All the three isolates were gram positive rods with two showing endospore formation and formed one single cluster in Amplified Ribosomal DNA Restriction Analysis (ARDRA). Partial sequences of 16S rRNA genes of two isolates showed 97% similarity to *Bacillus megaterium.*

2.5 FORMULATION OF MICROBIAL CONSORTIUM

2.5.1 Compatiability among the bacterial isolates

Based on a large number of studies, multistrain plant growth promoting microorganisms mixtures appear to have greater efficacy on improvement of plant growth and/or biological control than single strains. According to the current trend, prerequisites for successful construction of artificial microbial mixtures are: 1) use of diverse microorganisms that can promote plant growth and protect plants from biotic or abiotic stress, 2) efficacy of seed, leaf or root colonization, 3) compatibility among strains in the mixture, 4) use of microorganisms with different modes of action, 5) human and environmental safety, 6) easy application and 7) easy incorporation in an existing management system (Thomloudi *et al.,* 2019).

The plate assay method by Nagpal *et al.* (2019) revealed no mutual inhibition between *Mesorhizobium* LGR4 and all endophytes (NE6, NE8, RB-1 andRE8). However, *Mesorhizobium* LGR-5 was incompatible with nodule endophyte NE6 due to formation of zone of inhibition. Endophytic bacterial isolates NE6 and RB-1 showed mutual inhibition with *Mesorhizobium* isolate LGR-12…

The issue of compatibility among microbial components of a plant growth promoting multistrain mixture is gaining ground and is considered a basic requirement in the engineering of synthetic microbial mixtures applied to plants. According to the established literature, the microbial components of a PGPM mixture are considered to be compatible when they have no growth suppressive effect on each other during their in vitro co-culture, either in contact or in proximity, or during the plant rhizosphere colonization competition assayy (Santiago *et al.,* 2017; Liu *et al.*, 2018).

The outcome of the in vitro co-culture compatibility tests reflects the actual nature of the interaction to some extent (Prasad and Subramanian, 2017). For example, competitive colonization assays under controlled, greenhouse or field conditions demonstrated that in vitro compatible bacterial and/or fungal stains are also compatible in the rhizosphere; root population levels reached by each strain in the mixture were not significantly different from those obtained when strains were applied individually (Castanheira *et al.,* 2017; Molina-Romero *et al.,* 2017; Santiago *et al.,* 2017).

Deepa and Mathew, (2017) carried compatibility studies of different microorganisms to prepare a consortium. The five bacterial isolates selected from *in planta* experiment were subjected to mutual compatibility test by cross streak method. No lysis was observed at the juncture of TRB-1 x VSB-1, TRB-1 x EkRB-1 and VSB-1 x EkRB-1 combination, which indicated the compatibility among the isolates.

Subramanian *et al.* (2015) reported the compatibility of *Bacillus megaterium* LNL6 and *Methylobacterium oryzae* CBMB20 for co-inoculation with *Bradyrhizobium japonicum* with no zone of inhibition due to synergistic potential in the cultures.

Mansotra *et al.* (2015b) studied compatibility of *Piriformospora indica* with different PGPRs (LPGPR1, LPGPR2 and LK884) along with *Mesorhizobium ciceri* (MR), and reported better compatibility of *Piriformospora indica* and *Mesorhizobium* with LPGPR1 and LK884 in comparison to LPGPR2.

Sundaramoorthy *et al.* (2012) evaluated the protective effects of compatible endophytic bacterial strains (*Bacillus subtilis*; EPCO16 and EPC5) and rhizobacterial strain (*Pseudomonas fluorescens*; Pf1) against chilli wilt disease caused by *Fusarium solani* and results showed that *B. subtilis* (EPCO16 and EPC5) and *P. fluorescens* (Pf1) were compatible and effectively inhibited the growth of the *F. solani*.

2.5.2 Construction of microbial consortium and its influence on plant growth over single inoculants

It has become increasingly recognized that programming interactions between multiple populations represents a new frontier in synthetic biology, in terms of technical challenge and the potential implications of the resulting systems. Such multipopulation systems, often termed consortia, have some potential advantages over systems consisting of one population. First, a well-established consortium, such as a biofilm, is likely to be more resistant to invasion by other microbial species. Second, a properly defined mixed population can survive transient nutrient deprivation better when different subpopulations share essential metabolites. Third, by implementing cooperating circuits in two populations, a task that has a large metabolic burden can be shared, thus dividing the labor between populations (Smith *et al.*, 2013). Moreover, application of microbes in a consortium may improve efficacy, reliability and consistency of the microbes under diverse soil and environmental conditions (Stockwell *et al.*, 2011).

Brzezinska *et al.* (2021) has examined compatibility among the three isolates (*P. grimontii* B14, *S. kitahiroshimense* B16, and *M. oxydans* B19). When the antagonism between strains was not observed, consortium of these strains was used to determine its effect on rape (*Brassica napus* L. var. *napus*) growth. Interestingly, the consortium was more effective in promoting rape growth than single inoculant treatments, significantly increased chlorophyll content, number of live leaves and length of shoots.

Nagpal *et al.* (2020) demonstrated that dual bio-inoculant LGR2 + LCNE8 improved the grain yield by 6.5% over captan as the control and single inoculants. These results suggest that bio-inoculants of *Mesorhizobium* sp. and endophytic bacteria under a competitive environment can be explored as bio-fertilizers/bio-protectants for improving productivity and suppression of *Fusarium* wilt in chickpea.

Kumawat *et al.* (2019) assessed the scope of native potential endophyte *Pseudomonas aeruginosa* (LSE-2) strain (KX925973) with recommended *Bradyrhizobium* sp. (LSBR-3) (KF906140) for synergistic effect to develop as consortium biofertilizer of soybean. . Compatible of LSBR-3 and LSE-2 enhanced IAA, P-solubilization, 1-aminocyclopropane-carboxylate deaminase and biofilm formation over the single inoculant treatment. Improvement in growth parameters were recorded with dual inoculant LSBR-3+LSE-2 as compared to LSBR-3 alone and un-inoculated control treatments. Significantly ($p \geq 0.05$) high symbiotic and soil quality parameters (phosphatase and soil dehydrogenase activity) was recorded with

LSBR-3+LSE-2 at vegetative and flowering stage as compared to LSBR-3 alone and un-inoculated control treatments.

Emami *et al.* (2019) demonstrated that co-inoculation of eight bacteria from different taxa (*Bacillus, Microbacterium, Nocardia, Pseudomonas, Serratia*, and *Stenotrophomonas*) increased plant growth compared to single bacterial inoculation.

In another experiment, when PGPR *Pseudomonas fluorescens* FAP2 and *Bacillus licheniformis* B642 were co-inoculated, plant growth parameters increased compare to control (Ansari and Ahmad 2019).

Harish and Jagadeesh (2016) stated that when microbes were introduced to soil as consortium interact with a host plant, partially mimic the natural soil conditions. To improve stability of the released microbes in different agricultural fields, use of microbial consortia is advocated. The microbial consortium activates the antioxidant enzyme activities and the phenylpropanoid pathway leading to accumulation of total phenolics, proline, and pathogenesis related (PR) proteins.

Sarma *et al.* (2015) suggested that combining an antagonist bioagent may facilitate disease free growth of the plants. Therefore, applying microbes as a consortium has great potentiality particularly in modern agriculture where minimization of chemical fertilizers and pesticides is one of the priorities.

Singh *et al.* (2013) investigated microbial consortium activity on *Cicer arietinum* against *Sclerotium rolfsii.* The organisms used in consortia preparation were *Pseudomonas aeruginosa* (PHU094), *Trichoderma harzianum* (THU0816), *Mesorhizobium* sp. (RL091). The reasons for the best action of microbial consortia compared to individual inoculation were due to activation of phenylpropanoid pathway, lignin deposition antioxidant mechanisms.

Lakshmi (2013) conducted experiment to develop a formulation containing *Rhizobium, Bacillus megaterium* and *Pseudomonas fluorescens*. Seven different consortial combinations were prepared in lignite and urban compost based *Rhizobium, Bacillus megaterium* and *Pseudomonas fluorescens* were used in this study as nitrogen fixer, phosphorus solubilizer and plant growth promoter respectively. Cow pea receiving triple inoculant consortium of urban compost based formulation has resulted in higher plant height (45.17 cm) and number of leaves (44.33/plant) compared to other treatments.

Marimuthu *et al.* (2013) selected AZ204 as N fixer and P solubilizer, Pf1 as biocontrol agent and prepared a consortium to apply on *Gossypium hirsutum. Azospirillum* sp. AZ204 and *Pseudomonas fluorescens* Pf1 performed better and excluded *Rhizoctonia bataticola* more effectively compared to their individual inoculation. The consortium showed better plant growth promoting characteristics when applied as consortium so performed well.

Development of strain mixtures with non-competitive nature of the bacterial strains will have an additive effect in increasing the yield and growth. Strain mixtures of *Pseudomonads* in combinations with other bacteria were found effective than the application of individual organisms (Duijff *et al*., 1999).

2.6 INFLUENCE OF ENDOPHYTIC MICROBIAL CONSORTIA ON PLANT GROWTH PROMOTION AND STRESS MITIGATION IN POT AND FIELD CONDITIONS

2.6.1 Influence on Plant Biochemical Traits

Rana *et al.* (2021) reported three efficient endophytic plant growth promoting strains EU-A2SK1, EU-M4ARAct, and EU-E1RT3-1 were identified as *Pseudomonas brenneri, Ewingella americana, and Pantoea agglomerans*, possessing the capacity for P and K solubilization, and IAA production successfully improved plant growth. The isolates efficiently increased the shoot length and enhanced anthocyanin, chlorophyll content, physiological available iron, and total protein content when compared to untreated control maize plants at 60 days of maize plant growth.

Curá *et al.* (2017) showed that *A. brasilense* and *H. seropedicae* effectively colonized the corn seedlings in plants grown in both the well watered and drought conditions. Thus, bacterial growth was not significantly affected by the drought condition. The RWC was higher in inoculated plants in both the WW and D conditions. It is possible that the integrity of the plasma membrane was better due to the beneficial effects of inoculation, which may have mitigated the damage by the ROS produced in stressful conditions.

Ma *et al.* (2017) isolated an endophytic bacteria, *Pseudomonas azotoformans* from the leaves of *Alyssum serpyllifolium* and tested for enhanced drought tolerance. The isolated bacterial strain showed PGP characteristics such as atmospheric nitrogen fixation, phosphate solubilization, and production of IAA, ammonia, and siderophore. Inoculation of *Trifolium arvense* with *Pseudomonas azotoformans* increased leaf RWC in the absence as well as presence of drought stress. Conclusively, endophytic PGP bacterial strains improved drought tolerance by increasing RWC in the plants. Higher RWC may assist plants to cope osmotic and oxidative stresses triggered by drought stress.

Khan *et al.* (2016) demonstrated that 7 endophytic bacteria, i.e., *Burkholderia vietnameinsis, Rhizobium tropici, Acinetobacter calcoaceticus, Rhanella sp., Burkholderia sp., Enterobacter asburiae, Sphingomonas yanoikuyae, Pseudomonas* sp., and *Curtobacterium* sp., were isolated from willow and poplar stem. In order to evaluate these isolated bacteria for drought tolerance, poplar plants were inoculated with these bacteria and the resulted plants showed decreased ROS activity, in addition to other drought tolerance-enhancing parameters.

Sarma *et al.* (2015) suggested that proper selection of microbes for developing of consortium may lead to enhanced antioxidant activities in plants that can not only reduce pathogen damages but also protect plants from potential oxidative damages due to pathogen attack. These microbes are able to do their work individually.

Khan *et al.* (2014) isolated a drought-resistant endophytic bacteria *Pseudomonas azotoformans* ASS1 from *Alyssum serpyllifolium* leaves. The inoculation of plant with *Pseudomonas azotoformans* ASS1 enhanced plant chlorophyll content, superoxide dismutase, peroxidase, catalase, and proline content, where malondialdehyde concentration decreased.

In the study of Grover *et al.* (2014), sorghum plants treated with plant growth promoting *Bacillus* sp. strain KB 129 under drought stress showed a 24% increase in RWC

Pandey *et al.*, (2012) evaluated the endophytic bacteria to alleviate the stress by assessing the plant mortality due to pathogen infection as well as the physiological state through phenylpropanoid metabolism, antioxidant activities and proline accumulation. The endophyte was found to reduce the seedling mortality by 60%, accumulate higher proline (1.3-fold), total phenolics (1.2- fold), polyphenol oxidase (4.3-fold), phenylalanine ammonia lyase (1.29-fold) and superoxide dismutase (2.5-fold) in *S. rolfsii*-stressed plant. Their effectiveness indicates their potentiality in alleviating biotic stress in cucumber.

Vardharajula *et al.* (2011) has reported that the increase in sugar content was attributed to hydrolysis of starch for providing sugar for osmotic adjustment to negate the effect of drought stress. Maize plants inoculated with *Bacillus* spp. identified as B. *amyloliquefaciens, B. licheniformis, B. thuringenesis, P. favisporus, B. subtilis* on the basis of 16S rDNA gene sequence analysis displayed higher levels of proline, sugars and free amino acids, thus increasing plant biomass, relative water content, leaf water potential and root adhering soil/root tissue ratio.

Sandhya *et al.* (2010) demonstrated that adverse effects of drought stress on plant growth under uninoculated condition might be attributed to declining sugar levels and their enhanced accumulation probably led to increased tolerance to drought.

Sandhya *et al*, (2010) also reported that maize plants inoculated with five drought tolerant plant growth promoting *Pseudomonas* spp. strains namely *P. entomophila*, *P. stutzeri*, *P. putida, P. syringae,* and *P. montelli* were subjected to drought stress and inoculated plants showed significantly lower activity of antioxidant enzymes plants as compared to uninoculated plants indicating a lowering of stress in the plants.

Sandhya *et al.* (2009) reported that *Pseudomonas putida* strain GAP-P45, which has the ability to produce exopolyschharides (EPS), alleviated drought stress in sunfower (*Helianthus annuus* L.) seedlings by activating the host plant’s antioxidant enzyme machinery.

Plant associated bacteria may also exudate osmolytes such as proline, glycine betaine, and trehalose in response to the stress, which along with other PGP attributes can possibly act synergistically with plant-produced osmolytes and stimulate the plant growth even under stressed conditions (Paul and Nair, 2008).

Yoshiba *et al.* (1997) demonstrated that the Plant growth promoting *pseudomonas* spp. strains inoculation also increased proline content under drought stress, which may be due to up regulation of proline biosynthesis pathway to keep proline in high levels, which helps in maintaining cell water status, protects membranes and proteins from stress.

2.6.2 Influence on Soil Chemical Properties

Mukherjee *et al.* (2021) reported inoculating seed endophytic bacteria in chickpea has not shown any significant change in pH of pot and field trials. The pH of pot and field soil was alkaline and the range varied from 7.09 to 8.08 and 8.01 to 8.52, respectively. Other physiochemical property such as EC ranged from 15.04 to 19.12 (μScm-1) and 25.00 to 28.14 (μScm-1) in pot and field soils, respectively, and the significant change was showed in all three treatments. Consortium (T3) > *Enterobacter cloacae* BHUJPCS-21 (T2) > *Enterobacter hormaechei* BHUJPCS-15 (T1) in comparison to untreated control in both pot and field recorded significant EC in soils. The significant increase in % OM and % OC were observed in treatment of consortium followed by *E. cloacae* BHUJPCS-21 as compared to control and *E. hormaechei* BHUJPCS-15.

Decrease in soil pH was recorded due to inoculation of bacterial endophytes GBPI_TWL and GBPI_Twr (Adhikari and Pandey, 2020).

Ren *et al.* (2020) suggested that the electrical conductivity is the measurement of the ability to transport electric charge. The EC of soil depends on salts and sodium content level. Inoculated PGPR treatments increased concentrations of soil NO^{3-}, inorganic N, electrical conductivity (EC) and soil water content (SWC) over control by the production of acids and acid forming compounds that reacted with soluble salts present in the soil.

Raklami *et al.* (2019) conducted soil analyses of microbial consortium treated experiments and observed a linear correlation with the increased plant dry weight and length, seeds production, pigments synthesis in microbial consortium treatments with an increase in available soil N, P, and K content.

Sood *et al.* (2018) demonstrated that soil properties in the terms of available N, P and potassium, microbial biomass carbon, soil enzyme activities and population of phosphate-solubilising bacteria in the wheat crop were significantly increased by the combined application of bacterial inoculants with 80% RFD of NP in both years over the uninoculated control.

Posso *et al.* (2017) suggested that application of the bacterial bioformulations influenced the physicochemical soil parameters. Acidification of soil along with increase in phosphorus content may be attributed to the production of organic acids in soil by application of the endophytic bacteria leading to the conversion of non-available phosphorus to available form.

Laxman *et al.* (2017) observed the comparable results in both acid and alkaline phosphatase activites showed increased activities high at flowering stage of crop by applying microbial consortia on soil and along with foliar spray. Similar results were confirmed by Khati *et al.* (2017) their results shown that significant increment (one to two folds) in the alkaline phosphatase activity was found in all treatments inoculated with PGPR strain in comparison to untreated soil. Similarly Khati *et al.* (2017) results were shown that significant increase in dehydrogenase activity was observed in all treatments inoculated with PGPR strain in comparison to untreated soil.

Anusha *et al.* (2017) observed with they carried out their work on influence of biofertilizers on uptake of NPK in soil. They concluded that combined application of biofertilizers and recommended dose of chemical fertilizers increased the available nitrogen by 63 % in soil.

Islam *et al*. (2016) has reported *B. cereus* Pb25 inoculation not only increased microbial biomass carbon (20.86 µg g^{-1}) but also increased the contents of available phosphorus (0.51 µg g^{-1}) in saline stress soil. Statistically significant difference among soil enzymes (dehydrogenase and phosphatase) could also be observed. The maximum value of dehydrogenase enzymes was found in the sole inoculation of PGPR inoculated soil (0.41 µg g^{-1}) followed by the PGPR inoculated saline stress soil (0.28 µg g^{-1}).

Mishra *et al.* (2015) reported that the soil microorganisms like *Pseudomonas* spp., *Azotobacter* spp., phosphate solubilizing bacteria and *Azospirillum* spp. have enhanced capacity by promoting the secondary growth of roots by increased organic carbon (1.52 %) content and organic matter in the rhizosphere. Inoculation with potential bacteria is an important procedure to improve yield.

Mathivanan *et al.* (2014) reported that application of biofertilizers increased the soil EC from 0.40 in control to 0.99 in PGPR treated one. This may be due to increased solubility of many nutrients.

Cao *et al.* (2014) also claimed higher dehydrogenase enzyme activity under bacterial inoculation and suggested that the soil microbial biomass is correlated with dehydrogenase enzyme because it is an intracellular enzyme which may be involved in microbial oxidoreductase metabolism.

2.6.3 Influence on Uptake of Nutrients by Plants

Under field condition, maize endophytic bacteria BS-290 increased the nitrogen and phosphorus concentrations in shoot dry matter, phosphorus concentration in dry soil and the total number of bacteria in the soil, compared to the control. Under field condition, isolate BS-290 increased the nitrogen and phosphorus concentrations in shoot dry matter, phosphorus concentration in dry soil and the total number of bacteria in the soil, compared to the control (Lobo *et al.*, 2019).

Ghetiya *et al.* (2019) concluded that seed inoculation and soil application of PSB and KSB enhanced microbial count in soil which is capable for solubilizing insoluble form of nutrient and enhanced nutrient uptake and saving of 25% fertilizer dose of phosphorus and potash.

Okur, (2018) suggested that applying plant growth promoting bio-fertilizers, Potassium solubilizing bacteria, sulphur oxidizing bacteria, nitrogen fixing bacteria to increased uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil.

Hauka *et al*. (2017) isolated PSB, KRB and *Azotobacter* and were evaluated for plant growth promoting efficiency and used as microbial inoculants to improved the nutrient uptake in corn, wheat and rice.

Kuan *et al.* (2016) suggested that the increase in plant-N uptake and dry biomass of microbes inoculated plants was observed due to plant growth promoting abilities such as BNF, phosphate solubilisation and root promoting phytohormone production namely IAA, cytokinin and gibberellins. Inoculation with *Bacillus pumilus* S1r1 significantly increased the total N uptake in plant top by 55.1% and 50.1%, followed by *Klebsiella pneumoniae* Fr1 with similar increments of 61.4% and 48.4% at D50 and D65, respectively, in comparison to uninoculated control.

Sarma *et al.* (2015) suggested that when compatible strains of these microbes are applied together as a consortium, the crop plants are expected to get a combined benefit of high N and P availabilities for uptake leading to better plant health and yield. Similar results were also reported in that with respect to nutrient uptake in different crops due to microbial inoculants application (Abdullahi and Sheriff 2013).

Inoculation with N_2-fixing bacteria *Pseudomonas* sp. RFNB3 (Islam *et al.*, 2013) and *Acinetobacter johnsonii* strain 3-1 (Shi *et al.*, 2011) had resulted in significantly greater uptake of N, P, K, shoot and root biomass production.

Jarak *et al.* (2012) also reported the ability of different strains viz., *Pseudomonas* sp, *Bacillus* sp and *Azotobacter chrocooccum* strain to enhance maize growth (*Zea mays* L.) and yield by increasing nutrient uptake in maize plants.

Basak and Biswas, (2010) stated that the higher phosphorus and potassium uptake may be attributable to the increased mobilization of nutrients and root biomass from soil because of the secretion of organic acids mediated by soil microorganisms.

2.6.4 Influence on Stress Tolerance and Yield

Adhikari and Pandey, (2020) reported the positive results of inoculating endophytic bacterial isolates GBPI_TWr and GBPI_TWL on test crops (rice and black soybean) were found statistically significant. In both the treated crops, the seedlings showed enhancement in total biomass (g) and length (cm) in comparison to the control plants. Root and shoot biomass of *Oryza sativa* increased by 13 and 24 %, respectively. Root, shoot, leaf, and nodule biomass of *Glycine max* increased by 11, 37, 24, and 9 %, respectively, as compared to the control plants under net house conditions.

Joshi *et al.* (2020) reported that under the drought stress conditions, *Bacillus* spp. consortium inoculation showed a considerable difference in plant biomass and grain yield as compared to un-inoculated control plants. The effect of inoculation of selected bacterial isolates had a more significant positive effect on grain yield under drought stress (by 13% over un-inoculated control plants) than under non-stressed conditions (by 7% more than un-inoculated control plants). The significant reduction of antioxidant enzyme activity was observed in the plants treated with the consortium T3 and T4 respectively, as compared to single bacterial strain inoculation (T1 and T2) under drought stress conditions.

Chowdhury *et al.* (2019) suggested that selected endophytic microbe of maize seed may be applied for crop improvement. Biotechnological approaches and genetic engineering may help to utilize these endophytic microbes for plant growth promotion in host as well as non host crop plant.

Several studies have tried to identify the appropriate biofertilizers utilized on bacterial species which belong to *Bacillus, Azospirillum, Pseudomonas, Rhizobium, Ralstonia, Burkholderia and Klebsiella* genera in order to ameliorate the effects of abiotic stresses in many plant species, including rice, corn, tomato and wheat, since many of these species acts as growth promoters and stress tolerance enhancers (Bokhari *et al.*, 2019 and Dong *et al.*, 2019).

Emami *et al.* (2018) demonstrated that co-inoculation of endophytic and rhizospheric bacteria improved the protein of grain by 11.3 % (Marvdasht) and 9 % (Roshan). Microbial inoculation had a positive effect on the concentration of P, Zn, and Fe in wheat grains. While inoculating wheat genotypes with a single rhizospheric strain increased dehydrogenase activity by 53.4 %, this effect became stronger with increasing endophytic strain: up to 72 % increase in soil dehydrogenase activity was observed in bacterial consortium treatment.

Sood *et al.* (2018) has reported combined application of 80% RDF of NP with *Serratia* sp. significantly increased wheat yield by 9.4%, number of tillers per plant by 28.03%, grain number per spike by 19.61 %, 1000-grain weight by 10.5 %, and biomass by 9.2 % relative to the uninoculated control with 100 % RFD.

Lata *et al.* (2018) suggested that the endophytic community travels from one generation to another generation of maize via systemic infection in seed. These endophytic microbes protect plants not only against pathogens but also under abiotic stresses

Sandhya *et al.* (2017) demonstrated that out of 39 endophytic bacteria isolated from maize, 32 could show drought tolerance up to –1.02 matric potential (MPa) and exhibited most of the plant growth promoting traits. But, only five isolates could show antagonistic activity against plant fungal pathogens. Further, at –1.02 MPa all the 10 isolates showed PGP traits, and 3 isolates (FTR, NFTR and MRC12) showed antifungal activity. Thus, indicating that drought tolerant plant growth promoting antagonistic endophytic bacteria (PGPAE) helps in plant growth and disease management under drought stress.

Egamberdieva *et al.* (2017a) stated that plants inoculated with plant growth promoting endophytic bacteria might sustain (or nearly sustain) shoot growth and enhance yield under drought stress conditions.

Many of these mutualistic organisms can act as biofertilizers, increasing the efficiency of nutrient absorption by the plant and producing substances that promote growth. It is estimated that biofertilizers could reduce the use of common fertilizers by 50 % with no yield losses (Pereg and McMillan 2015, Suman *et al.,* 2016).

Naveed *et al.* (2014a) studied the inoculation of maize plants with the *Burkholderia phytofirmans* improved shoot growth. The inoculated plants showed higher root and shoot biomass compared to non-inoculated plants under drought stress. In addition, higher yield of inoculated plants was also recorded.

Hussain *et al.* (2013) investigated five promising strains of PSB [PS-01 (*Burkholderia* sp.), PS-12 (*Bacillus* sp.), PS-32 (*Pseudomonas* sp.), PS-41 (*Flavobacterium* sp.), and PS-51 (*Pseudomonas* sp.)] and found that they significantly increased plant height, root length, shoot dry weight, root dry weight, and grain yield up to 16, 11, 42, 29, and 33 % respectively, over the un-inoculated control.

Marulanda *et al.* (2009) reported an increase in IAA production by *P. putida* and *B. megaterium* at a PEG concentration of 60 % is an indication of bacterial resistance to drought. Their inoculation increased shoot and root biomass and water content under drought conditions. Bacterial IAA production under stressed conditions may explain their effectiveness in promoting plant growth and shoot water content increasing plant drought tolerance.

Sandhya *et al.* (2009) reported that inoculation of *Pseudomonas* sp. strain GAP-P45 increased the survival, plant biomass, and root adhering soil/root tissue ratio of sunflower seedlings subjected to drought stress.

Zahir *et al.* (2008) stated that ACC deaminase producing *Pseudomonas* spp. increased root length in *Pisum*, leading to enhanced uptake of water under drought stress conditions.

Singh *et al.* (2006) has isolated three novel endophytic rhizobial strains (RRE3, RRE5, and RRE6) from naturally growing surface sterilized rice roots. These isolates had the ability to nodulate common bean (*Phaseolus vulgaris*). Polymerase chain reaction–restriction fragment length polymorphism and sequencing of 16S rDNA of these isolates revealed that RRE3 and RRE5 are phylogenetically very close to *Burkholderia cepacia* complex, whereas RRE6 has affinity with *Rhizobium leguminosarum* bv. *phaseoli.* Plant infection test using gusA reporter gene tagged construct of these isolates indicated that bacterial cells can go inside and colonize the rice root interiors. A significant increase in biomass and grain yield was also recorded in greenhouse-grown rice plants inoculated with these isolates.

Wu *et al.* (2005) reported that the co-inoculation of *Bacillus megaterium*, *Azotobacter chroococcum* and *Bacillus mucilaginous* significantly increased maize biomass and plant height equivalent to half of the chemical fertilizer inputs.

Chapter – III

Material and Methods

Chapter III

MATERIAL AND METHODS

The present study was carried out at the Department of Agricultural Microbiology, Advanced Post Graduate Center, Lam and Regional Agricultural Research Station, Anakapalle. Isolated endophytic bacteria from the maize plant samples collected from different regions of Andhra Pradesh. Isolates were screened based on plant growth promoting traits and their growth in varied water potentials. The efficient plant growth promoting and stress mitigating endophytic bacteria were identified molecularly using 16S rRNA sequencing. Endophytic colonization of identified endophytic bacteria was confirmed by reisolation of inoculated cultures in pot experiment and compatiability of confirmed bacteria was tested to make consortium. Influence of developed endophytic microbial consortium was evaluated against water stress and limited nutrient availability under both pot and field conditions. The material used and methods employed in the investigation are outlined below.

The general laboratory techniques followed in the present study were those described by Cappuccino and Sherman (1992), Nene and Thapliyal (1993) and Aneja (2001) for preparation of media, sterilization, isolation and maintenance of bacterial cultures, with slight modifications wherever necessary.

3.1 ISOLATION, MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF ENDOPHYTIC BACTERIA FROM MAIZE PLANT SAMPLES

In Andhra Pradesh major growing areas of maize crop were selected and plant samples were collected from different regions of AP which includes Srikakulam, Vijayanagaram, Visakhapatnam, East Godavari, Guntur, Kurnool and Ananthapuram in the Kharif and Rabi seasons of 2019 (Plate 3.1).

3.1.1 Glassware

Petriplates, test tubes, microscopic slides, conical flasks of different capacities *i.e.*, 1000mL, 500mL, 250mL, pipettes of 1, 2.2, 5, 10 and beakers and measuring cylinders of 50,100,500 and 1000mL ,eppendorf tubes of 1.5mL PCR tubes, eppendorf micropipettes of 0.5-10µL,10-100µL,100-1000µL were used. All the glassware used was of Borosil make.

3.1.2 Cleaning of glassware

Glassware were first washed with a detergent, then cleaned with tap water and finally placed in the chromic acid solution prepared with following composition:

Potassium dichromate : 60 g
Conc.H_2SO_4 : 60mL
Distilled water : 1000mL

The glassware were kept in the cleaning solution for 24 h and then thoroughly washed with running tap water before its final cleaning with distilled water and dried.

3.1.3 Sterilization of glassware

Glassware was wrapped in butter paper and sterilized in hot air oven at 160 ºC for 2 h before use. Media, distilled water, *etc.*, were sterilized in an autoclave at 15lb psi (121 ºC) for 20 min.

3.1.4 Precaution to avoid contamination

The inoculation work of microbial cultures were carried out in Laminar air flow. The Laminar bench and air flow was disinfected using U.V lamp prior to commencement of work

3.1.5 Equipment and apparatus used

Hot air oven and autoclaves were used for sterilization of heat stable and media respectively. BOD incubators were used for incubating cultures at different temperatures. Cultures were stored and maintained in a refrigerator. The pH was measured by using digital pH meter. Vortex was used for homogenization during serial dilution. Plate mixer was used for spread plate technique. Centrifuge was used for making cell-free cultures and DNA pellet formatting protocols. Eppendorf thermocycler (PCR), gelelectrophoresis, Bio rad UV trans illuminator. Hi-media zonal scale was used to measure the zone around the colonies during phosphate solubilization and biocontrol activity. Samples were weighed using a single pan electric balance. Compound electron microscope was used to observe the morphology of bacterial cultures.

Anantapur
14.8007 ◦N 77.5982 ◦E

ARS, Peddapuram
17.0712 ◦N 82.1479 ◦E

Visakhapatnam
17.6896◦N 83.0024◦E

Srikakulam
18.3030 ◦N 83.7063◦E

Vizianagaram
18.3014◦N 23.1649◦E

Guntur
16.2129◦N 80.2847◦E

Plate.3.1. Collection of maize plant samples from different regions of Andhra Pradesh

3.1.6 Isolation of endophytic bacteria

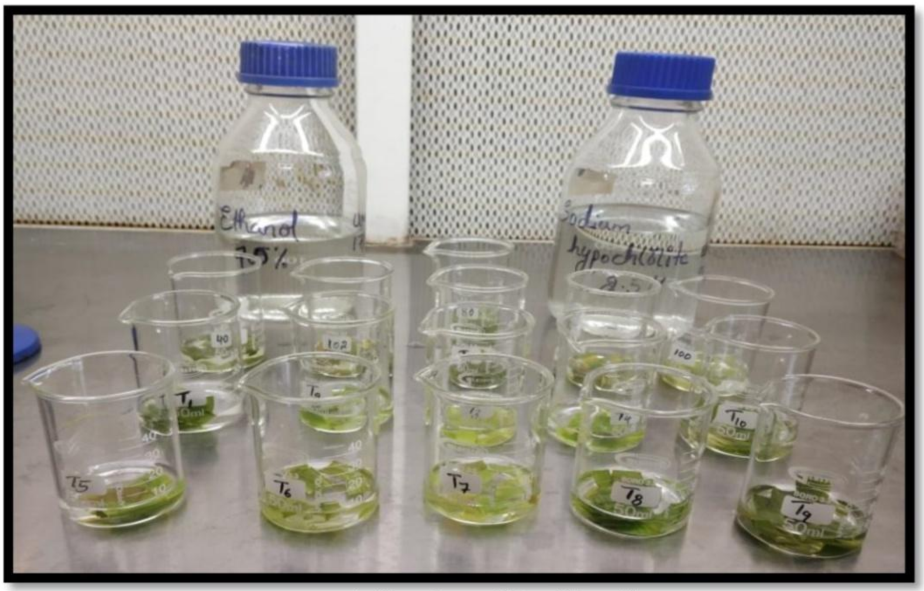
Soil from the roots was removed by vigorous tapping and the remaining adhered soil particles were removed by washing the roots with sterilized water. Each plant was separated into 3 subsamples of different type of tissues viz. root, stem and leaves (kernels as 4th sub sample if any) by sterilized knife and scissors. For isolation of endophytic bacteria, different tissues were soaked separately in sterile phosphate buffered saline (PBS) solution of pH 7.0 for 15 min for equilibrating osmotic pressure and halting any passive diffusion of sterilizing agent into the roots (Suman *et al.*, 2001). From each plant root, stem, leaves and kernels were surface sterilized separately by the method given by Barzanti *et al.* (2007). Plant roots (0.5 g) were dissected into ca. 1-cm pieces and surface-sterilized for 40 s with 70 % ethanol and 20 min with 2.5 % sodium hypochlorite. Plant leaves and stems (0.5 g) were surface-sterilized for 20 s with 70 % ethanol and 10 min with 2.5 % sodium hypochlorite (Plate 3.2a). To remove the disinfectant, sections were rinsed three to four times in sterile distilled water; samples were then dried with sterile filter paper and subsequently ground with 1 mL 0.9 % sodium chloride with a sterile mortar. Samples (100 μL) of tissue extracts and their different dilutions were spread plated on two different media (Nutrient agar and Triptic Soya Agar) in triplicate. Aliquots (100 μL) of the last washing water were plated as sterility controls, which never showed to be contaminated (Plate 3.2b) and incubated at 28 $^{\circ}$C for 72 hrs. Pure cultures were obtained by the streak plate method. These pure cultures of different bacterial isolates were preserved on agar slants (Plate 3.2c) and used for further analysis.

3.1.7 Morphological Characterization Bacterial Isolates

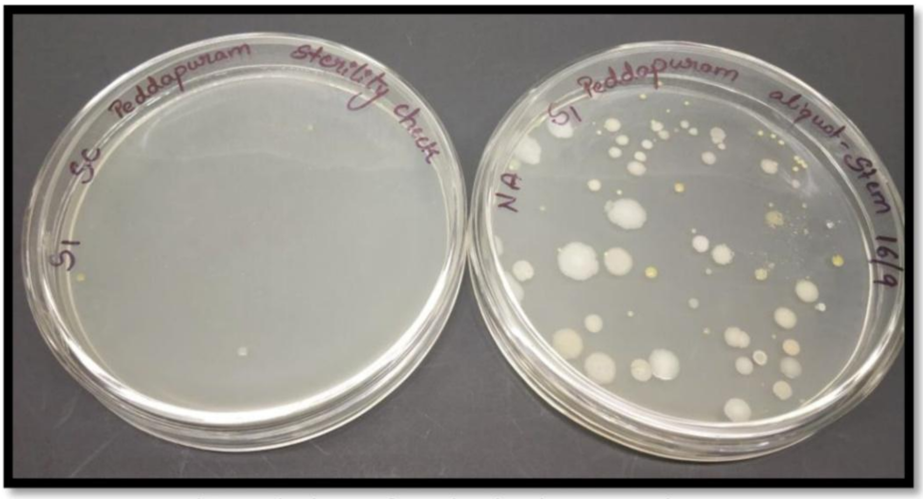
All the isolates were checked for their purity and then studied for the colony morphology and pigmentation. The cell shape and gram's reaction were also recorded as per the standard procedures given by Barthalomew and Mittewar (1950)

3.1.7.1 Colony morphology :

Morphological characteristics of the colony of each isolate were examined on Nutrient agar. Cultural characterization of isolates observed by different characteristics of colonies such as shape, size, elevation, surface, margin, color, pigmentation, motility, etc were recorded.



a) Surface Sterilization



b) Isolation of Endophytic Bacteria

c) Pure Cultures of Endophytic Bacteria

Plate 3.2. Isolation of endophytic bacteria from maize

3.1.7.2 Gram's staining : A drop of sterile distilled water was placed in the center of glass slide. A loopful of inoculm from young culture was taken, mixed with water, and placed in the center of the slide.

The suspension was spread out on slide using the tip of inoculation needle to make a thin suspension. The smear was dried in air and fixed through mild heating by passing the slide 3 to 4 times over the flame. The smear was then flooded with crystal violet solution for 1 min and washed gently with flow of tap water. Then the slide was flooded with iodine solution. After incubation at room temperature for 1 min, iodine solution was drained out followed by washing with 95 % ethanol. After that, it was washed with water within 15 to 30 sec and blot carefully. The smear was incubated with safranin solution for 1 min. The slide was washed gently in flow of tap water and dried in air. The slide was examined under microscope at 100X power with oil immersion and data were recorded.

3.1.7.3 Motility test : Each isolate was spot-inoculated on the centre of semi-solid nutrient agar plates (0.2 % agar) and incubated at 30 ^{o}C. The diffusion of colony was observed and recorded at 24 hours (Elbeltagy *et al.,* 2000).

3.1.8 Biochemical and Physiological Characterization

Different biochemical tests performed and the protocols followed are briefly outlined below. Biochemical based characterization was done as per Bergey's Manual of systematic Bacteriology (Holt *et al.,* 1994).

3.1.8.1 Starch hydrolysis (MacFaddin, 2000) : Sterile starch agar plates were spotted with 10 μL overnight broth cultures of the isolates and incubated at 28 ±2^{o} C for 24-48 h. After incubation, the plates were flooded with iodine solution. The formation of a transparent zone around the colony was taken as positive reaction for the test.

3.1.8.2 Hydrogen sulfide test (Beishir, 1991) : Sterilized Hydrogen sulfide- Indole-Motility agar (SIM agar-Appendix I) stabs were inoculated along the wall of the tubes with overnight cultures of the isolates and incubated for 48 h at 28 ±2 ^{o}C. Visualization of black colour along the line of inoculation indicated a positive reaction for the test.

3.1.8.3 Indole production (Isenberg and Sundheim, 1958) : Sterilized SIM agar slants were inoculated with the overnight cultures of the isolates and incubated for 48 h at 28 ±2 ^{o}C. Following incubation, 10 drops of Kovac's indole reagent were added to each tube. The isolates showing production of red colour were recorded as positive for indole production.

3.1.8.4 Catalase test (Rangaswami and Bagyaraj, 1993) : This test was performed to study the presence of catalase enzyme in bacterial colonies. Fresh cultures of pure isolates were taken on glass slides and one drop of H_2O_2 (30 %) was added. Appearance of gas bubble indicated the presence of catalase enzyme.

3.1.8.5 Oxidase test (Collins and Lyne, 1970) : The overnight cultures of the test isolates were spotted on plates poured with sterile trypicase soy agar (TSA-Appendix I) and the plates were incubated for 24 h at 28 ±2 ^{o}C. After incubation, 2-3 drops of N, N, N', N'- tetramethyl- p-phenylenediamine dihydrochloride (Wurster's reagent) were added onto the surface of growth of each test organism. The isolates showing change of colour to maroon were noted as oxidase positive.

3.1.8.6 Gelatin liquefaction (MacFaddin, 2000) : The overnight cultures of the test isolates were inoculated to sterilized nutrient gelatin (Appendix I) deep tubes and incubated for 24 h at 28 ±2 ^{o}C. Then the tubes were kept in the refrigerator for 30 minutes at 4 ^{o}C. The isolates showing liquefied gelatin were taken as positive and those which resulted in solidification of gelatin on refrigeration were recorded as negative for the test.

3.1.8.7 Methyl Red test (Crown and Gen, 1998) : Sterilized glucose- phosphate broth tubes were inoculated with the test culture and incubated at 28±2 °C for 48h. After incubation five drops of methyl red indicator was added to each tube and gently shaken. Red colour production was taken as positive and yellow colour production was taken as negative for the test.

3.1.8.8 Voges Prausker's test (MacFaddin, 2000) : To the presterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37 °C for 48h. After incubation ten drops of Baritt's reagent A was added and gently shaken followed by addition of 10 drops of Baritt's reagent B. Development of pink colour in the broth was taken as positive for the test.

3.1.8.9 Citrate utilization (MacFaddin, 2000) : Isolates were streaked on Simmon's citrate agar slants and incubated at 28±2 °C for 24h. Change in colour from green to blue indicates the positive reaction for citrate utilization.

3.1.8.10 Ammonia production (Juanda, 2005) : The isolates were tested for ammonia production by inoculating the isolates into 10 mL of pre-sterilized peptone water in test tubes. The tubes were incubated for 48-72 hours at 36 ± 2 oC. After that Nessler's reagent (0.5 mL) was added in each tube. Change in color of the medium from brown to yellow color was taken as a positive test for ammonia production.

3.1.8.11 Carbohydrate utilization (MacFaddin, 2000): All pure bacterial isolates were screened for the carbohydrate fermentation abilities using 4 different carbohydrates (lactose, sucrose, dextrose and mannitol) in Peptone broth medium. Bacterial isolates were inoculated in broth containing specific carbohydrate. The change in colour of Peptone broth was observed for utilization of particular carbohydrate present in broth.

3.2 EVALUATION OF OSMOTOLERANCE AND PLANT GROWTH PROMOTING CHARACTERS OF ENDOPHYTIC BACTERIAL ISOLATES

To evaluate PGP traits bacterial suspensions were generated, all isolates were incubated in nutrient broth medium (3.0 g of beef extract, 10.0 g of protease peptone, and 5.0 g of NaCl in 1 L of distilled water, pH 7.2) for 18 h at 30 °C with shaking at 150 rev min^{-1}. Next, the bacterial cells were pelleted by centrifugation (8000g, 10 min) and then resuspended in a 0.85 % saline solution to a final OD600 of 1.0 (approximately 10^8 CFU mL^{-1}) for subsequent assays.

3.2.1 Nitrogen fixation

Bacterial isolates (106) were inoculated in nitrogen free semi-solid agar medium containing trace amount of BTB (Bromo Thymol Blue) as pH indicator (N-free broth : 0.1 g of dipotassium hydrogen phosphate (K_2HPO_4), 0.4 g of KH_2PO_4, 0.2 g of $MgSO_4$, 0.1 g of sodium chloride (NaCl), 0.02 g of $CaCl_2$, 0.01 g of ferric chloride ($FeCl_3$), 0.002 g of sodium molybdate [$NaMoO_4$], and 10 g of glucose in 1000 mL of distilled water) (Döbereiner *et al.*, 1972) incubated for 24 h at 30°C

3.2.2. Phosphate solubilisation

Sterilized Pikovskaya's medium was poured as a thin layer on to the sterilized petri plates and incubated for 24h. The agar plates were spot inoculated with isolates and incubated at 28±1°C for 4-5 days. Formation of a clear zone around the colonies was considered as positive result for phosphate solubilization.

PSI (Phosphate Solubilization Index) = Z / C x 100

Z- Clearance zone including bacterial growth

C- Colony diameter

All the bacterial isolates were tested for their ability to solubilize insoluble inorganic phosphate on Pikovskaya's agar plates by the procedure suggested by Pikovskaya (1948). Quantitative estimation of solubilized P by bacterial isolate was done by the vanadomolybdophosphoric yellow color method (Subbarao, 1988) in pikovskaya's broth containing 5000 mg/mL tricalcium phosphate. The phosphate solubilizing ability of each isolate was tested using insoluble tricalcium phosphate [$Ca_3 (PO_4)_2$] as sole P source in Pikovskaya's medium. 10 mL of pikovskaya's broth containing 5000 mg/mL P in the form of tricalcium phosphate was inoculated with 0.1 mL of bacterial culture (inoculums adjusted to ~$2X10^8$ CFU/mL) at 28 $^{\circ}$C± 2 $^{\circ}$C up to 8 days. After incubation, 1 mL of the supernatant was taken out on 8th day. The supernatant was obtained by centrifugation at 10,000 rpm for 20 min and then 0.1 mL of the supernatant (filtered) was mixed with 0.25 mL of Barton's reagent and volume was made up to 5 mL with double distilled water (ddw). After 10 min, the intensity of yellow color was read on spectrophotometer (UV− VIS Spectrophotometer) at 430 nm and the amount of P-solubilized was extrapolated from the standard curve.

A standard curve was prepared by dissolving 0.02195 gm of potassium dihydrogen orthophosphate/Monopotassium phosphate (dried at 60 $^{\circ}$C for 1 h and then cooled in desiccators) in 100 mL of double distilled water (ddw) and labeled as stock P solution. A further dilution of 15 mL of stock solution was taken and volume was made up to 25 mL with ddw and labeled as working solution. Aliquots of 0.2, 0.3, 0.5, 0.8, 1.0, 1.5, 2.0, 2.5, 3.0 mL of working solution and 0.25 mL of Barton's reagent were added to each flask and the volume was made up to 5 mL with distilled water. After 10 min, the intensity of yellow color developed was read at 430 nm spectrophotometrically. Standard curve was prepared by plotting absorbance at 430 nm vs concentration of P.

3.2.3 Potassium solubilization

The bacterial strains were evaluated for their potassium solubilizing ability by spot inoculation on Aleksandrov agar medium plates (Hu *et al.*, 2006). The plates were incubated at 30°C for 5-7 days and observation of clearing zone around bacterial colony indicates K-solubilization and calculated potassium solubilization efficiency by using formula.

Quantitative evaluation of potassium alumino silicate solubilization was carried out using flame photometer. The bacterial strains are inoculated in Aleksandrov broth and placed on orbital shaking incubator (120 rpm) at 28±2 °C for 1 week. The bacterial cells are harvested using centrifuge at 10,000 rpm for 30 min. The supernatant (1 mL) was diluted and analyzed by flame photometer (Singh *et al.*, 2020).

3.2.4 Indole Acetic Acid (IAA) production

Indole acetic acid production was tested according to Gordon and Weber (1951). The active culture of each test isolate was raised in 5ml respective broth tubes and incubated at determined temperature and time. After incubation these cultures were centrifuged at recommended rpm and time. Two drops of O- phosphoric acid was added to 2 mL of supernatant and incubated for 30 min to develop the color. Development of pink color considered as positive for IAA production.

Quantitative production of indole acetic acid was determined by the capacity of the isolates to produce IAA and similar molecules using 0.5 mg mL^{-1} L-tryptophan in peptone water medium (10.0 g protease peptone and 5.0 g NaCl in 1 L of distilled water, pH 7.2) (Glickmann and Dessaux 1995). Briefly, 1 mL of each bacterial cell suspension was transferred into 20 mL of peptone water medium as described above, and incubated for 24 h at 30 °C. Subsequently, 1 mL of the cell-free extracts was mixed with 2 mL of Salkowski reagent (Gordon and Weber, 1951) at room temperature for 20 min, after which the absorbance was determined at 530 nm using pure IAA as a standard.

3.2.5 Siderophore production

Siderophore production was estimated qualitatively using Chrome Azurol S (CAS) Agar medium (Schwyn and Neilands, 1987). For the detection of siderophores, each isolate was grown in synthetic medium, containing 0.5 µM of iron and incubated for 24 hours on a rotary shaker at room temperature. Chrome Azurol S (CAS) assay was used to detect the siderophores. The CAS plates were used to check the culture supernatant for the presence of siderophores. Culture supernatant was added to the wells made on the CAS agar plates (mannitol, 10.0 g; sodium glutamate, 2.0 g; K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.2 g; NaCl, 0.1 g; distilled water, 1000 mL, pH- 6.8-7.2) and incubated at room temperature for 24 h. Formation of yellow to orange colored zone around the well indicates siderophore production.

Quantitative evaluation of siderophore production was carried out by mixing supernatant (0.5 mL) of each bacterial culture grown in nutrient broth medium with CAS reagent (0.5 mL) and taking optical density at 630 nm after 20 minutes of incubation (Hu and Xu, 2011). Percentage of siderophores production was measured according to the formula given by Payne (1993).

$$\% \text{ Siderophores Production} = (Ar - As) \times 100/Ar$$

A_r = Absorbance of reference (CAS solution and un-inoculated broth)

A_s = Absorbance of sample (CAS solution and cell-free supernatant of sample).

3.2.6 Exopolysaccharide production

The pre-inoculum was prepared by growing bacterial isolates in YMG broth for 24 hours at 25 ^oC and 200 µL of this broth was inoculated into YMG broth (50 mL) of and incubated for 5 days in orbital shaking incubator (120 rpm) at 25 ^oC. Bacterial cell free extracts were obtained by centrifugation (10,000xg for 20 minutes) and then three volumes of ethanol to the culture supernatant was mixed by adding slowly along the side wall of the conical flask and kept to stand at 4 ^oC for 20 minutes so as to get precipitated exopolysaccharides. The EPS production was measured by taking weight of the precipitate after drying at 80 ^oC for three days (Kumar *et al.*, 2011)

3.2.7 ACC deaminase activity

ACC deaminase activity was checked for their ability to utilize 1-Aminocyclopropane -1- carboxylate (ACC) as sole nitrogen source. It was done on MDF (Modified Dworkin and Foster medium) agar plates supplemented with 0.3 g of ACC per liter of growth medium by spot plate assay. Bacterial isolates were also spotted on plates of MDF medium containing 0.3 g L^{-1} ammonium sulphate as positive control and on plain MDF agar plate as negative control. Positive growth after incubation at 30 °C for 72 h was taken as positive for ACC deaminase activity (Jacobson *et al.,* 1994).

3.2.8 HCN production

Method of Bakker and Schipper, (1987) was followed for testing hydrogen cyanide (HCN) production. The overnight raised bacterial cultures were streaked on King's B agar plates amended with 4.4 g L^{-1} of glycine. Whatman no. 1 filter paper disc soaked in 0.5 % picric acid (in 2 % sodium carbonate) was placed inside the lid of Petri plate. The plates were sealed with parafilm and incubated at 28 °C for 4 days. Development of deep orange to dark brown color indicated production of HCN.

3.2.9 Antagonistic activity

Antagonistic activity of the isolated endophytic bacteria was assayed against maize pathogens, *Exserohilum turcicum* (Turcicum leaf blight), *Rhizoctonia solani* (root and stalk rot) and *Fusarium oxysporium* following the dual culture technique (Dennis and Webster, 1971). On a Petri dish containing potato dextrose medium bacterial isolate was streaked on one side and perpendicular to it on the opposite side a mycelial disc of 8 mm diameter taken from a 7-day-old culture of the fungal pathogen was placed. Incubated the plates at 28± 2 °C for seven days. Isolates with antagonistic activity inhibited the growth of the fungus when it grew towards the bacterial colony on PDA. The level of antagonism is calculated using the formula (Mugiastuti *et al.*, 2020).

$$I = \frac{C - T}{C} \times 100\%$$

I: The level of inhibition of antagonist (%)

C: The radius of pathogen colony opposite to antagonist

T: The radius of the colony of pathogen towards antagonist

3.2.10 Determining bacterial growth under varied water potential

Isolated strains were cultivated in flasks containing 50 mL nutrient broth medium supplemented with polyethylene glycol (PEG) 6000 at various concentrations (0, 10, 20, 30, 40 %) to generate water potentials of -0.05, -0.65, -1.57, -2.17 and -2.70 MPa, respectively (Busse and Bottomley, 1989). Flasks were incubated in an orbital shaker (100 rpm) and maintained at 30°C. Bacterial growth was monitored by changes in optical density (OD) at 600 nm after 3 days of inoculation and calculated the percent reduction in growth in comparison to that obtained under control conditions (without PEG 6000). Cultures showing lesser reduction in growth in presence of PEG 6000 were selected for further studies.

3.2.11 Seed germination test

Seeds were surface sterilized with 0.02% sodium hypocholoride for 2 min and washed with distilled water for 4-5 min. Then the seeds were soaked for 20 - 30 min in 48h old bacterial broth cultures containing at least 10^8 cells mL^{-1}. Bacterized and control treatment seeds were used for a germination test by the paper towel method (Karthik *et al.*, 2017). The germination paper was soaked in distilled water, and then 50 bacterized seeds and untreated seeds were placed on paper towels, rolled and incubated at 30 ºC. After 15 days, the towels were unrolled and the number of seeds that had germinated was counted. On the same day, seedling vigor was analyzed. The lengths of roots and hypocotyls of all the individual seedlings were measured. The vigor index (VI) was calculated using the formula

VI = (mean root length + mean shoot length) × % germination.

3.3 DEVELOPMENT OF STRESS MITIGATING AND PLANT GROWTH PROMOTING MICROBIAL CONSORTIUM

Bacterial isolates showing all the plant growth promoting characters significantly like N fixation, K release, IAA production, ACC deaminase activity, HCN production, exopolysaccharide production along with the capability of growing under higher water potential were selected as plant growth promoting and efficient stress mitigating endophytic bacterial isolates. Compatiability of selected isolates was carried so as to develop microbial consortia.

3.3.1 Screening for compatiability of bacterial isolates

Plant growth promoting rhizobacterial strains were tested for their compatibility by cross streak assay on nutrient agar medium to be used in multi-strain inoculation (Raja *et al.,* 2006). One bacterial strain was streaked on the solidified nutrient agar plate and incubated at 28±2 °C for 24 h. The counter bacterial strain was streaked vertically to the growth of already streaked bacterial strain. The Petri plates were observed for bacterial growth at 28±2 °C for 48 h. The growth suppression of counter bacterial strain by already streaked bacterial strain were considered as noncompatible and mixing of bacterial growth of both strains were considered as compatible. All dual combinations of selected PGPR strains were evaluated for their compatibility to be used in multi-strain inoculation.

The synergism/antagonism between selected isolates was assessed. For synergism/antagonism assays, the selected bacterial isolates were cultured at 28±2 °C for 24 h in nutrient broth. The aliquots bacterial cultures (10 µL) were spot inoculated/ streaked horizontally on nutrient agar (NA) plates pre-seeded with tested bacterial strain (100 µL). The growth of bacteria was observed on Petri plates after 48 h of incubation at 28±2 °C and formation of any clear zones of inhibition were observed (Khan *et al.*, 2017).

3.3.2 Construction of bacterial consortium

Based on the compatibility tests, the isolates were confirmed to be compatible with one another. Isolates were cultured individually, using specific media then mixed and grown together in conical flask @ 10 mL / isolate. The population of each isolate in the consortia was monitored regularly in the conical flask.

Fresh inoculum of these selected strains were prepared by taking 100 mL of sterilized nutrient broth in four conical flasks having 250 mL volume and sterilized at 15 psi pressure and 121 ºC temperature for 20 minutes. Each sterilized conical flask was inoculated with a strain (along with an un−inoculated control) and incubated at

28 ± 1 ºC in a shaking incubator at 100 rpm. After gaining proper population (10^7−10^8 CFU mL^{-1}) of each strain in the flasks, the consortium was prepared by mixing respective inoculum in equal proportion (100 mL each) in separate sterilized conical flasks (Shahzad *et al.,* 2017).

3.3.3 Primary screening of formulated endophytic microbial consortia and characterization of endophytic colonization

Pot experiment was conducted to study the endophytic colonization of inoculated isolates and to screen the efficient isolate or consortium for further experimentation.

3.3.3.1 Seed priming and sowing : Maize seeds were surface sterilized with 4% sodium hypochlorite (NaOCl) for 5 min followed by 5 washings of sterile distilled water. Soak the seeds in nutrient broth of 48hrs grown selected endophytic isolates individually and in consortia. Non treated seeds were soaked in uninoculated nutrient broth taken as control. Bacterized seeds were air dried and sowing was done in pots filled with sterilized finely sieved soil.

3.3.3.2 Inoculation of plants with bacterial endophytes : Bacteria were grown to the mid-log phase, pelleted by centrifugation, washed twice, suspended in phosphate buffer and maintained inoculation density of 10^8 per mL. Maize seeds were surface sterilized with 70 % ethanol for 40 seconds and 2.5 % NaOCl for 20 minutes followed by four washings with sterile distilled water. Surface sterilized seeds were primed with bacterial isolates by soaking in individual bacterial suspensions for about 2 hrs. Treated seeds were air dried and sown in triplicates in pots filled with sterilized soil to avoid indigenous soil microorganisms.

Plants were inoculated in triplicate when a minimum height of 7.6 cm was reached and the stalks were at least 0.5 cm in diameter, which corresponded to 7 to 14 days after seed germination. A 26-gauge needle attached to a tuberculin syringe containing a bacterial suspension was passed horizontally through the seedling stem approximately 1 cm above the crown of the plant. The plant was rotated 90°, the needle was reintroduced perpendicular to the first wound channel, and the inoculation procedure was repeated. After 15 days of germination bacterial isolates were applied to soil at the rate of 1mL (10^8) of suspension near the root zone (Zinniel *et al.*, 2002).

Table 3.1. Details of pot experiment for consortium screening and characterization of endophytic colonization

Location	RARS, Anakapalle
Design	CRD
Variety	PIONEER 3396
Treatments	10
Replications	3
Date of sowing	24-09-2020
Re-isolation of endophytic bacteria	45-50 DAS
Treatments details	T1: FYM+EB1 (*Kosakonia radicintcitus*) T2: FYM+EB2 (*Priestia megaterium*) T3: FYM+EB3 (*Priestia aryabhattai*) T4: FYM+EB4 (*Bacillus licheniformis*) T5: FYM+EB5 (*Pseudomonas aeruginosa*) T6: FYM+EB6 (*Klebsiella pneumoniae*) T7: FYM+EB7 (*Methylorubrum populi*) T8: No Inoculum T9: FYM+EB1+ EB3+ EB6 (*MC-1) T10: FYM+EB1+ EB5+ EB6 (*MC-2) T11: FYM+EB1+ EB2+ EB4+ EB6 (*MC-3) T12: FYM+EB2+ EB4+ EB6+ EB7 (*MC-4) *MC- Microbial Consortium

3.3.3.3 Re-isolation of endophytic bacteria from experimentally inoculated plants: Maize plants were uprooted 45-50 days after sowing. All the tissues (roots, stem and leaves) were cut, surface sterilized and endophytic bacteria were isolated from the serially diluted aliquots of maize tissues (Sec 3.1.7)

3.3.3.4 Parameters to confirm endophytic colonization of inoculated isolates

3.3.3.4.1 Colony Morphology : The colony morphology, pigmentation, grams reaction of isolated bacteria were compared with the inoculated isolates. The phenotypically similar isolates were considered for further testing.

3.3.3.4.2 Antibiotic sensitivity : Use of antibiotic resistance markers for identifying inoculated isolates is a key parameter. The antibiotic sensitivity of re isolated bacteria from inoculated plants was assayed and compared with the antibiotic sensitivity of inoculated isolates.

3.3.3.4.3 Carbohydrate utilization : All the bacterial isolates obtained from inoculated plants were tested for the carbohydrate fermentation abilities using four different carbohydrates (lactose, sucrose, dextrose and mannitol) in the peptone broth medium. Bacterial isolates were inoculated in broth containing specific carbohydrate. The change in colour of peptone broth was observed for the utilization of a particular carbohydrate present in broth and results were compared with the carbohydrate utilization characters of inoculated isolates.

3.3.3.4.4 Molecular tracking by 16S rRNA gene sequencing : The reisolated bacteria which were confirmed as inoculated ones by the above tested parameters, further confirmed by sequencing of 16S rRNA gene and blasting with NCBI gene bank (Sec 3.4).

3.4 MOLECULAR CHARACTERIZATION OF EFFICIENT STRESS MITIGATING AND PLANT GROWTH PROMOTING BACTERIAL ISOLATES BY USING 16S RRNA

Molecular characterization of most efficient bacterial isolates was done by sequencing of their 16S rRNA gene.

3.4.1 Genomic DNA extraction

The genomic DNA from 16 endophytic bacteria was isolated using the standard protocol of hexadecyl-trimethyl ammonium bromide (CTAB) method with minor modifications. The isolates were grown in nutrient broth and 96 h old culture of 10 mL quantity was centrifuged at 6,000 rpm for 5 min at 4 °C. The supernatant was discarded; the pellet was suspended in 1 mL TE buffer, added with 0.5 mL of 1-butanol, vortexed well to mix with the cells followed by centrifuged at 5000 rpm for 5 min at 4 °C. The supernatant was discarded and the pellet was resuspended in 2 mL of TE buffer and centrifuged again to remove all traces of butanol. The pellet was resuspended in 1 mL TE buffer added with 100 µL of lysozyme (10 mg ml^{-1} freshly prepared) and incubated at 28 ± 2 °C for 5min. After incubation, 100 µL of 10 % of SDS and 25µL of 100µg mL^{-1} proteinase K was added, mixed well and incubated at 37 °C for 1 h. To this, 200 µL of 5 M NaCl was added and mixed well. CTAB (10 per cent CTAB in 4.1 per cent NaCl solution) at 150 µL was added, mixed well and incubated at 65° C for 10 min. The mixture was extracted with 1 mL of phenol:

chloroform mixture and centrifuged at 6000 rpm for 15 min at 4° C. The aqueous layer was transferred carefully to a new 2 ml microfuge tube and DNA was precipitated by adding 0.6 volume of ice-cold isopropanol, incubated for 1 h to overnight at -20 °C. The DNA was pelletized by centrifugation at 12000 rpm for 15 min at 4 °C and the pellet was washed with 70 per cent ethanol, dried under vacuum for 10 min and resuspended in 50 μL of TE buffer. One μL of DNAse free RNAse (10 mg mL^{-1}) was added by swirling and incubated at 37 °C for 30 min. The DNA was stored at -20 °C for further use.

3.4.2 PCR amplification of 16S rRNA gene

The 16S rRNA gene of the target bacterial isolates was amplified by using universal eubacteria primers as reported by Heddi *et al.* (1998). These were custom synthesized and brought by barcode biosciences, Pvt. Ltd, India.

3.4.3 Base sequences of 16S rRNA gene primers

Name of gene	**Primer's**	**Sequence (5' to 3')**
16S rRNA	27 F	AGAGTTTGATCCTGGCTCAG
	1492 R	TACGGTTACCTTGTTACGACTT

The PCR amplification was carried out in 0.2 mL PCR tubes with 25 μL reaction volume consisting of the following components.

Reaction mixture	**Quantity (μL)**
Buffer 10 X	2.5
$MgCl_2$ (25 mM)	1.5
dNTPs mix (10 mM each)	2.0
Taq DNA Polymerase (5 U μL^{-1})	0.2
Primer forward 20 p mol	1.0
Primer reverse 20 p mol	1.0
Water (SDW)	14.8
DNA (10 ng μL^{-1})	2.0
Total Volume	25.0

The reaction mixture was vortexed and centrifuged in a refrigerated centrifuge (Eppendorf). Amplifications were performed using a thermal cycler (Eppendorf PCR Systems) with the following temperature transitions.

Steps	Temperature	Time
1. Initial Denaturation	94 ^{o}C	5 minutes
2. Denaturation	94 ^{o}C	45 Seconds
3. Annealing	53 ^{o}C	45 Seconds
4. Elongation	72 ^{o}C	30 Seconds

The thermal cycler was programmed for 30 cycles with one cycle of initial denaturation and steps 2-4 were repeated 30 times and a final extension at 72 ^{o}C for 30 seconds using fastest ramp time between the temperature transitions.

3.4.4 Agarose gel electrophoresis of PCR products

The amplified PCR products were resolved by electrophoresis using 1% agarose gel in 1X Tris- acetate EDTA buffer (2 M Tris Base, 57.10 mL Acetic acid and 0.5 M EDTA, Ph 8.0, 50X, Appendix-II). Agarose gel was mixed with ethidium bromide (0.5 μgmL^{-1}) before pouring, 1kb DNA ladder (Banglore genie) was used as a marker. The gel was run at 120 V for 45 minutes using banglore genie powerpack system. The ethidium bromide stained gel was viewed and the image captured using a gel documentation system.

3.4.5 Sequensing and data analysis

PCR products of 16S rRNA gene of 16 efficient bacterial isolates obtained through amplification with specific primer were freeze dried in a Lyophilizer and sent for custom sequencing using same upstream and downstream primers used for the amplifications of 16S rRNA gene (Barcode Biosciences, Pvt. Ltd, India).

3.4.6 Nucleotide sequence analysis

The 16S rRNA sequences of different bacterial isolates were BLAST (Basic Local Alignment Search Tool) searched against the sequences of 16S rRNA of bacterial isolates available in the NCBI Gene Bank Nucleotide Database (www.ncbi.nlm.nih.gov). Based on maximum identity score sequences were selected and aligned using multiple alignment software programme Clustal W (http..//clustalw.genome.jp/). Distance matrix was generated using RDP Data base and the Phylogenetic tree was constructed using MEGA X (Tamura *et al.,* 2007) (http..//megasoftware.net)

3.5 TO STUDY THE INFLUENCE OF DEVELOPED ENDOPHYTIC MICROBIAL CONSORTIA ON MAIZE AGAINST WATER STRESS IN POT CULTURE EXPERIMENT

A pot experiment was conducted to investigate the effect of efficient endophytic microbial consortia on soil health, growth and yield of maize under varied moisture stress in green house conditions.

3.5.1 Location of the experiment

The pot culture experiment was carried out at RARS, Anakapalle (Plate 3.3).

3.5.2 Physical and chemical properties of the soil

The soil of the experimental site was loamy soil with good drainage and fine bed. Representative soil sample was collected from the field prior to pot filling and analysed for the physical, chemical and biological properties by adopting standard procedures at Department of Agricultural Microbiology, APGC, Lam and Department of Soil Science and Agricultural Chemistry, RARS, Anakapalle.

3.5.3 Microbiological properties of the experimental site

Viable population of bacteria, fungi, Actinomycets, were analyzed by the standard serial dilution plate count method (Vlassak *et al*., 1992). The microbial colonies appearing after the stipulated time period of incubation were counted as colony forming units (CFU) g^{-1} weight of the soil sample. The microbial populations were expressed as number of colony forming units per gram of soil.

3.5.4 Soil Preparation

The soil used in this experiment was loamy field soil which was sieved to remove stones and any stubbles to get fine soil. Soil was sterilized at 121°C for 15 minutes at 15 lb pressure and about 12 kg of sterilized soil was filled into each pot.

Table 3.2. Chemical and microbial properties of initial soil used for pot experiment

pH	7.9
EC (dS m^{-1})	0.18
OC (%)	0.123
N (kg ha^{-1})	80.23
P (kg ha^{-1})	36.67
K (kg ha^{-1})	153.91
Alkaline phosphatase activity (µg pNP g^{-1}soil h^{-1})	12.63
Dehydrogenase activity (µg of TPF g^{-1} of soil day^{-1})	4.23
Bacterial population (Log CFU g^{-1} of soil)	5.23
Fungal population (Log CFU g^{-1} of soil)	4.11
Actinomycetes population (Log CFU g^{-1} of soil)	3.66

3.5.5 Sowing and treatment details

Maize hybrid PIONEER 3396 was selected and sown at the rate of 4 seeds/pot then thinning was done and only 2 plants per pot were maintained.

Table 3.3. Treatment details of pot experiment

Location	RARS, Anakapalle
Design	CRD
Variety	PIONEER 3396
Treatments	10
Replications	3
Date of sowing	28-12-2020
Date of harvesting	12-03-2021
Treatments details	T_1 :25 % WHC + 50 % RDF + MC T_2 :25 % WHC + 75 % RDF + MC T_3 :25 % WHC+ 100 % RDF + MC T_4 :50 % WHC+ 50 % RDF + MC T_5 :50 % WHC+ 75 % RDF + MC T_6 :50 % WHC+ 100 % RDF + MC T_7 :75 % WHC+ 50 % RDF + MC T_8 :75 % WHC+ 75 % RDF + MC T_9 :75 % WHC+ 100 % RDF + MC T_{10}:100% WHC+ 100 % RDF (Control) (WHC: Water holding capacity; RDF: Recommended dose of fertilizer; MC: Microbial consortium/ inoculants)

Plate 3.3 a. General view of the pot experiment

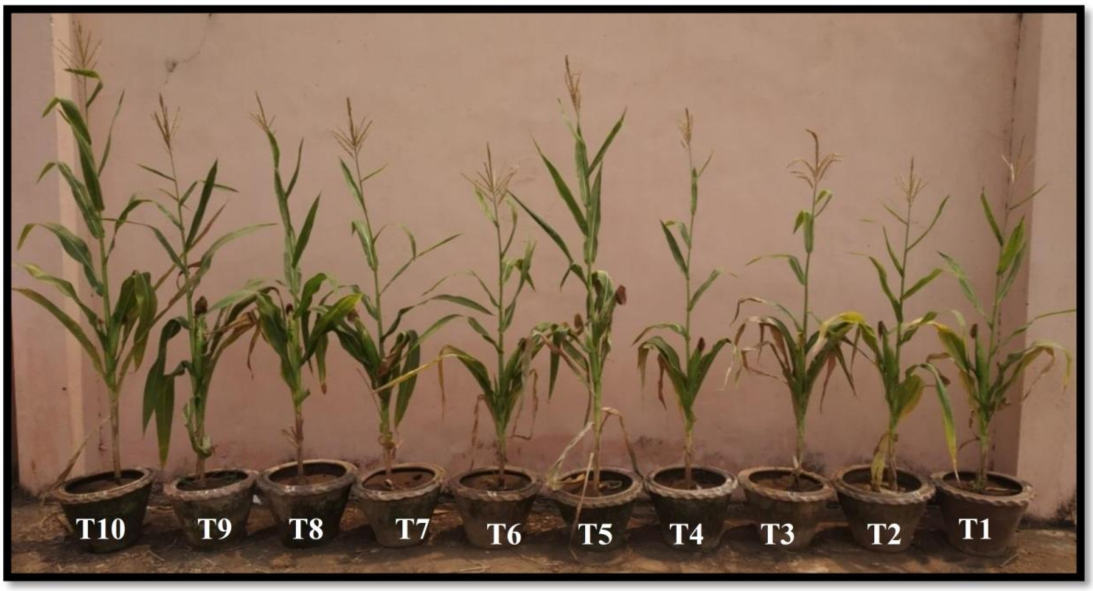


Plate 3.3 b. Influence of endophytic microbial consortia on maize plant growth under moisture stress in pot culture

3.5.6 Plant Biochemical Analysis

3.5.6.1 Chlorophyll stability index : The total chlorophyll content was estimated by the method of Arnon (1949) by soaking 0.1 g of fresh leaf sample in 10 mL of dimethyl sulphoxide (DMSO) and incubated for 24 hours in the dark. The light absorbance values were recorded at 645 nm and 663 nm using UV 2450 Visible spectrophotometer. The chlorophyll content was calculated by using formula given by Arnon (1949) and chlorophyll stability index (CSI) was estimated by using the method of Murthy and Majumdar (1962)

Chlorophyll a (mg g^{-1} fr.wt) = 12.7 (D 663) - 2.69 (D 645) X $\frac{V}{100 \times W}$

Chlorophyll b (mg g^{-1} fr.wt) = 22.9 (D 645) – 4.68 (D 663) X $\frac{V}{100 \times W}$

Total Chlorophyll (mg g^{-1} fr.wt) = 20.2 (D 645) + 8.02 (D 663) X $\frac{V}{100 \times W}$

Where,V= Volume made up; W= Weight of leaf sample

$$\mathrm{CSI} = \frac{\text{Total chlorophyll content of the treated leaves}}{\text{Total chlorophyll content of untreated leaves}} \times 100$$

3.5.6.2 Relative water content : Leaf RWC was measured in control and stressed seedlings. Fully expanded leaves were excised and fresh weight (FW) was immediately recorded from control and stressed plants, then the leaves were soaked for four hours in distilled water at room temperature under constant light and turgid weight (TW) was recorded. After drying for 24 hours at 80 $^{\circ}$C total dry weight (DW) was recorded. RWC was calculated according to the formula:

$$\mathrm{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

3.5.6.3 Estimation of H_2O_2 : Macerate 4 g of leaf tissue in 5 mL of ice cold 0.01M phosphate buffer (pH 7.0), centrifuge at 8000X g for 10min. and use the supernatant for estimating H_2O_2 content. Add 5 µL of sample to 1.95 mL of 0.01 M potassium phosphate buffer (pH 7.0). Add 2 mL of 5 % potassium dichromate and glacial acetic acid (1:3 v/v) to the mixture. Keep the tube in boiling water bath for 10 minutes and then cool. Read the absorbance at 570 nm against the reagent blank without sample extract and determine the quantity to H_2O_2 from a standard calibration curve (10 to 160 µmol of H_2O_2) (Patterson *et al.*, 1984).

3.5.6.4 Estimation of proline : Proline content was determined by the method of Bates *et al.* (1973). Extract 500 mg of fresh plant material was homogenized in a mortar and pestle with 10 mL of 3 % aqueous sulfosalicylic acid. The homogenate was filtered through Whatman No.1 filter paper and the residue was re-extracted. The extracts were pooled and made up to 20 mL with aqueous sulfosalicylic acid and used for the estimation.

The 2 mL of leaf extract, 2 mL of acid ninhydrin and 2mL glacial acetic acid were added in a test tube. The mixture was incubated for 1hour at 100 °C in boiling water bath. Then, the tubes were transferred to a water bath to terminate the reaction. Then, 4mL toluene was added and mixed vigorously using a test tube stirrer for 15 to 20 seconds and the toluene containing the chromophore was separated with the help of separating funnel and the absorbance was measured at 520 nm in a spectrophotometer against an appropriate blank. The proline content was determined from a standard curve prepared with proline and expressed in mgg^{-1}dw. For the preparation of acid ninhydrin reagent, 1.25 g of ninhydrin was dissolved in 30 mL warm glacial acetic acids and to it 20 mL of 6 M phosphoric acid was added with agitation.

3.5.6.5 Estimation of peroxidase : Peroxidase was estimated as per the method given by Harde *et al*. (2019). Enzyme extract was prepared by taking one gram of fresh leaf sample and ground with 3 mL of sodium phosphate buffer. The grounded sample was subjected to centrifugation at 18000 rpm for 15 minutes at 5 °C. After centrifugation the supernatant was collected and 0.1 mL of this supernatant was added to a test tube containing 3mL of sodium phosphate buffer, 50 µL of guaiacol solution and 30 µL of hydrogen peroxide solution. Similarly, blank is maintained without the enzyme extract and absorbance was recorded at 436 nm and expressed in units $g^{-1}min^{-1}$. One unit is defined as a change in absorbance per gram fresh weight per minute.

3.5.6.6 Plant nitrogen content: Nitrogen content (%) in leaf was estimated by the micro kjeldhal method (Piper, 1960). Approximately 0.1 g of raw material was hydrolyzed with 2.5 mL concentrated sulfuric acid (H_2SO_4) containing 1 g of two copper catalyst mixture in a heat block (Kjeltec system 2020 digestor) at 420 °C for 2

h. The tubes are cooled to room temperature after digestion. The digest solution was ensured to be totally clear and the tubes were attached to KELPLUS automatic nitrogen distillation unit. In 150 mL conical flask took 20 mL of 4 % boric acid containing mixed indicator and placed at receiver tube. After the completion of distillation for 9 minutes conical flask from the receiver tube was removed and the distillate was titrated against 0.02N H_2SO_4 till the pinkish shade appeared. The blank was run simultaneously without sample. The nitrogen content in sample was estimated by using the formula

$$\% \mathrm{N} = \frac{\mathrm{TV} \times 0.00028 \times 100}{\mathrm{W}}$$

Where,

TV= Titration value (Sample titration value-Blank titration value)

W= Weight of sample

3.5.6.7 Plant phosphorus content: The plant samples were digested with triacid mixture consisting of HNO_3: H_2SO_4: $HClO_4$ (9:4:1). The digest was made up to 100 mL. The phosphorus content in the triacid digest was determined by vanadomolybdo phosphoric acid yellow color method (Piper, 1960). The intensity of yellow color was measured by using spectrophotometer at 420 nm wave length. Percent of phosphorus (% P) in plant sample was calculated by using following formula

$$\% \text{ Phosphorus} = \mathrm{P} \times \frac{\text{Final Volume (50 mL)} \times 100 \times 100}{\text{Weight of sample} \times \text{Volume of aliquot} \times 1000000}$$

Where, P= Phosphorus concentration (ppm) obtained from standard curve

Weight of sample= 0.5g Volume of aliquot= 5 mL

3.5.6.8 Plant potassium content: The potassium content in the triacid digest was determined by using ELICO Flame photometer (Piper, 1960). Percent of potassium (% K) in plant sample was calculated by using following formula

$$\% \text{ Potassium} = \mathrm{R} \times \frac{100 \times 100}{\text{Weight of sample} \times 1000000}$$

Where, R= Concentration of potassium (K) in ppm

Weight of the sample = 0.5g

3.5.6.9 Nutrient (N, P, K) uptake : The nutrient uptake was calculated using the following formula and expressed in g plant^{-1}.

$$\text{Nutrient uptake } \left(\frac{\text{g}}{\text{plant}}\right) = \frac{\text{Nutrient content (\%)} \times \text{Dry matter production (g/plant)}}{100}$$

3.5.7 Soil chemical analysis

3.5.7.1 pH in the soil samples : pH of the slurry samples was determined in 1:2.5 substrate water suspension by using digital pH meter (Systronics pH system 361) (Jackson, 1973).

3.5.7.2 Electrical conductivity (EC) in the soil samples : The electrical conductivity of slurry samples was determined in saturation extract by using Elico CM 180 conductivity meter (Jackson, 1973).

3.5.7.3 Organic carbon content : Soil organic-carbon was estimated following the method described by Walkley and Black (1934). One gram of soil was placed in a 500 mL conical flask. 10 mL of 1N Potassium dichromate ($K_2Cr_2O_7$) solution was pipette into the conical flask. 20 mL of concentrated sulphuric acid was added and the mixture was allowed to stand for 30 minutes. A blank was also run in the same manner. The contents were then diluted to 200 mL with distilled water. Further 10 ml of 85% Ortho Phosphoric acid and 1mL of Diphenylamine indicator was added to each flask. The solution was titrated with 0.5 N Ferrous sulphate till a turbid blue color changed to brilliant green. Soil organic carbon was calculated as given below and expressed in per cent.

$$\text{Organic Carbon (\%)} = \frac{10(\text{B} - \text{S}) \times 0.003 \times 100}{\text{B} \times \text{Wt of the sample taken (g)}}$$

B- Titre value of the blank in mL

S- Titre value of the sample in mL

3.5.7.4 Available N in soil : The alkaline potassium permangante method of Subbiah and Asija (1956) was followed for the estimation of available N content in soil. 20 g of soil was taken in 800 ml dry Kjeldahl flask and 20 mL of distilled water was added.

Then 100 mL of each of 0.32 % $KMnO_4$ and 2.5 % NaOH solutions were added. The froth formation during boiling was prevented by adding liquid paraffin (1 mL) and bumping by adding a few glass beads. The contents were distilled in Kjeldahl assembly at a steady rate and liberated ammonia was collected in a conical flask (250 mL) containing 20 mL of boric acid solution (with mixed indicator). The pink colour of the Boric acid solution turns to green with the absorption of ammonia. 150 mL of distillate collected in about 30 minutes was titrated with 0.02 N H_2SO_4 till the original shade (Pinkish) was obtained. Blank was performed without soil. Available nitrogen in soil was expressed in Kg ha^{-1}

$$\text{Available Nitrogen} = \frac{(A - B) \times N \times 0.014 \times 100}{\text{Weight of the soil}}$$

Where,

A (mL) = Volume of standard acid required for soil
B (mL) = Volume of standard acid required for blank
N = Normality of sulphuric acid.

3.5.7.5 Available P in soil : Available P in soil was determined by Olsen's method described by Olsen *et al.* (1954). 2.5 g air dried soil was placed into a 250 mL conical flask and add a pinch of Darco charcoal and 50 ml of Sodium bicarbonate extract (0.5 M) was added. Then the contents were shaken for 30 minutes and filtered through Whatman No1 filter paper, 5 mL of clear and colorless filtrate was transfered into a 25 mL volumetric flask, 2-3 drops of p-nitrophenol was added, it turns to yellow color. Then the filtrate was titrated with 5N H_2SO_4 drop by drop till yellow color disappears, to this 5 mL of Olsen's extract solution was added then diluted up to 20 mL with distilled water. Then 4 mL of ascorbic acid was added and volume was made up to 25 mL and shaken well. After 5 minutes and the intensity of the blue color developed was measured using 660 nm, blank was run without soil, and standard curve of P was plotted. Available P was expressed in Kg ha^{-1}.

$$\text{Available Phosphorus} = R \times \frac{\text{Total Volume of Extract} \times 25 \times 2.24}{\text{Weight of the soil} \times \text{Volume of Aliquot}} \times 2.29$$

Available P in Soil (Kg ha^{-1}) = R× 513

Where R= Concentration of P (ppm) in aliquot obtained from standard curve

3.5.7.6 Available K in soil : Available potassium content from soil was extracted by using 1N neutral normal ammonium acetate as described by Jackson (1973). The concentration of potassium in the extractant was determined by flame photometer and expressed in Kg ha^{-1}.

$$\text{Available Potassium} = \text{R} \times \frac{\text{Total Volume of Extract} \times 2.24}{\text{Weight of the soil}} \times 1.2$$

Available K in Soil (Kg ha^{-1}) = R× 13.44

Where R= Concentration of K (ppm) in extract

3.5.7.7 Dehydrogenase activity : Method was described by Casida *et al.* (1964). The enzyme activity in 1 gm soil was determined by adding 0.05 g of CaCO3, 2.5 ml of distilled water and by using 1 mL of 3 % TTC where it is reduced to light pink TPF on the incubation for 24 h. Later it was dissolved in 10 ml of methanol and finally made up to 25 mL. The intensity of the red color was measured on a spectrophotometer at 485 nm. Expressed as µg of TPF g^{-1} soil d^{-1}.

3.5.7.8 Phosphatase activity : The procedure followed was of Eivazi and Tabatabai (1977) for alkaline phosphatases. The principle in the estimation of Phosphatase enzyme activity is that the soil extract from 1g of soil was allowed to react with para nitro phenol, which was estimated colorimetrically.

Phosphatase activity was estimated by taking 1g of soil sample and mixed it with 0.2 ml of toluene 4 mL of MUB (Modified Universal Buffer) and 1mL of disodium para nitrophenol solution. This solution was kept in an incubator at 37 °C for 1 h. Later 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were mixed properly and the intensity of the yellow color of filtrate was read on spectrophotometer at 420 nm. Expressed as µg pNP g^{-1} soil h^{-1}

3.6 INFLUENCE OF DEVELOPED ENDOPHYTIC MICROBIAL CONSORTIA ON MAIZE AGAINST WATER STRESS UNDER FIELD CONDITIONS

A field experiment was conducted to investigate the effect developed endophytic microbial consortia on soil health, growth and yield of maize under moisture stress conditions.

3.6.1 Location of the experiment

The field experiment was carried out at Regional Agricultural Research Station, Anakapalle (Plate 3.4 and 3.5).

Table 3.4. Chemical and microbial properties of initial field Soil

pH	7.8
EC (dS m^{-1})	0.18
OC (%)	0.223
Available N in Soil (kg ha^{-1})	83.46
Available P in Soil (kg ha^{-1})	40.21
Available K in Soil (kg ha^{-1})	172.83
Alkaline phosphatase activity (µg pNP g^{-1}soil h^{-1})	38.76
Dehydrogenase activity (µg of TPF g^{-1} of soil day^{-1})	23.21
Bacterial population (Log CFU g^{-1} of soil)	8.24
Fungal population (Log CFU g^{-1} of soil)	4.82
Actinomycetes population (Log CFU g^{-1} of soil)	3.66

3.6.2 Physical and chemical properties of the soil

The soil of the experimental site was sandy loam with good drainage. Composite soil samples were collected randomly from the experimental field prior to sowing and analysed for the physical, chemical and microbiological properties by adopting standard procedures at Biofertilizer unit and Department of Soil Science, RARS, Anakapalle.

3.6.3 Soil Microbiological Properties of the Experimental site

Viable population of bacteria, fungi, actinomycetes was analyzed by the standard serial dilution plate count method (Vlassak *et al.*, 1992). The microbial colonies appearing after the stipulated time period of incubation were counted as Colony forming units (CFU) g^{-1} dry weight of the soil sample. The microbial populations were expressed as number of colony forming units per gram of soil

Table 3.5. Details of the treatments used in the field experiment

Crop	Maize
Variety	PIONEER 3396
No. of treatments	10
No. of replications	3
Plot size	4m X 4.8m
Spacing	60cm X 20cm
Experimental design	Randomized Block Design
Date of sowing	27-12-2020
Date of harvesting	17-04-2021
Treatments	T_1:3 Irrigations + 50 % RDF + MC T_2:3 Irrigations + 75 % RDF + MC T_3:3 Irrigations + 100 % RDF + MC T_4:5 Irrigations + 50 % RDF + MC T_5:5 Irrigations + 75 % RDF + MC T_6:5 Irrigations + 100 % RDF + MC T_7:7 Irrigations + 50 % RDF + MC T_8:7 Irrigations + 75 % RDF + MC T_9:7 Irrigations + 100 % RDF + MC T_{10}: Full Irrigations + 100 % RDF (Control) (RDF: Recommended dose of fertilizer; MC: Microbial consortium)

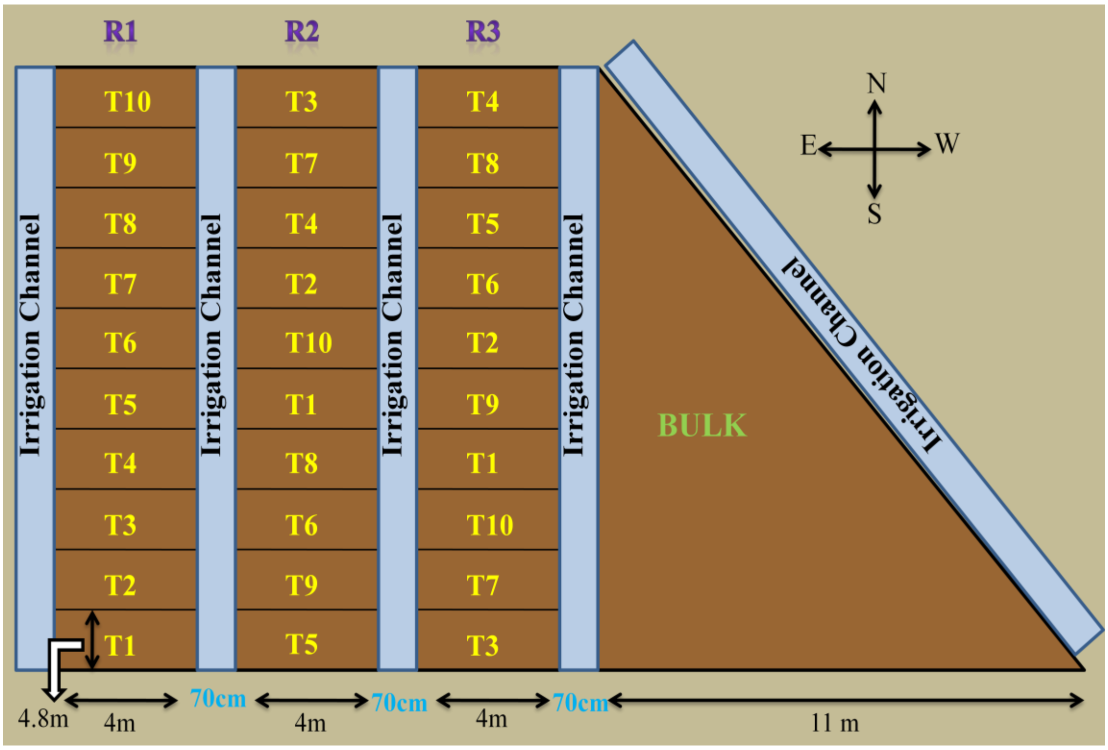


Fig. 3.1. Layout of field experiment

3.6.5 Cultural Practices

3.6.5.1 Land preparation : The experimental field was ploughed with a tractor drawn cultivator and properly leveled with leveling plank to bring the soil to a fine tilth, the experimental field was provided with irrigation and drainage channels and the field was divided into the individual plot as per the layout plan (Fig. 3.1).

3.6.5.2 Fertilizer and microbial inoculums : The fertilizers were applied as per the treatment combinations. The crop was supplied with recommended dose of fertilizers. Nitrogen @240 Kg ha^{-1}, phosphorus @ 80 Kg ha^{-1} and potassium @ 80 Kg ha^{-1} in the form of urea, Single Super Phosphate and muriate of potash, respectively. Entire dose of phosphorus was applied as basal at the time of sowing. Potassium was applied in two split applications *ie.,* half split as basal and 2nd split at flowering stage. Nitrogen was applied through urea in three split applications.

Soil application of the developed microbial consortia on maize crop under *in vitro* against water stress mitigation and plant growth promoting was done 2 days after sowing using lignite as carrier . Mixed the broth cultures aseptically with sterilized lignite and incubated for 24 hours. After incubation purity was checked by serial dilution and plating method for cell viability of recommended count (10^8 to 10^9 CFU mL^{-1}). Followed by foliar application of microbial consortium twice at 15 days interval was done

3.6.5.3 Seed source and sowing : Obtained maize seed material (PIONEER 3396) from local market and bacterized with the developed consortium after surface sterilizing the seeds. Treated seeds were sown in lines by dibbling one seed per hill with spacing with a spacing of 60×20 cm on 26th December 2020. In control plots non bacterized seeds were sown.

3.6.5.4 Gap filling and thinning : After ten days of sowing, gap filling and thinning operations were carried out in order to maintain optimum plant population in all the treatment plots.

3.6.5.5 Weed management : Hand hoeing was taken up twice at 20 and 50 DAS for the control of weeds during the critical period of crop weed competition and earthing up of the soil was done around the plant after weeding.

3.6.5.6 Water management : The first two irrigations were given equally to all the treatments at 15 days interval. Moisture stress was imposed from 30 DAS in controlled treatments at interval of 10-15 days managing critical growth stages and normal irrigations were maintained in control.

3.6.5.7 Plant protection : Fall army worm and stem borer infestation was observed 25 DAS and to control damage caused by the pest sparying of biocontrol agent *Metarrhizium anisopliae* (Ma-35) at the rate of 5 g/L within the whorls of each plant was done twice at 15 days interval.

3.6.6 Pre-harvest observations on crop growth

Five plants were tagged in each net plot area for recording observations that did not involve destructive sampling. All the observations were recorded on these plants at vegetative, flowering and harvesting stages. Randomly selected five plants from net plot were uprooted at each time for recording dry matter production and for endophytic microbial count.

3.6.6.1 Plant height (cm) : Plant height was recorded at different intervals *viz.*, vegetative, flowering and harvesting stage by measuring from the ground level to the tip of the top most leaf upto tasseling stage and after tasseling to the tip of the tassel and expressed in cm.

3.6.6.2 Biomass yield (g) : Five plants representing the population were randomly harvested at each observation for recording dry matter production. The plants were removed along with root system. The roots were separated from each selected plant, above ground parts were cleaned, transferred to properly labelled brown paper bags and partially dried in the sun for 2 days and then dried in hot air oven at a temperature of 60 °C to a constant weight.

3.6.6.3 Microbial population enumerated at different growth stages : Microbial population was enumerated at three different stages and estimated soil health by analysis of microbial count in rhizospheric zone and endosphere.

Plate. 3.4. General view of the field experiment showing influence of endophytic microbial consortia on maize under moisture stress

a) Land preparation b) Sowing of maize

c) Foliar application of Microbial consortium d) Application of *Metarhizium anisopliae* in whorls

e) Dehusking and shelling of maize f) Stover yield

Plate 3.5. Various field operations showing the study of endophytic microbial consortia on maize under moisture stress

3.6.7 Plant Biochemical Analysis

3.6.7.1 Chlorophyll Stability Index (CSI) : The total chlorophyll content was estimated by the method given by Arnon (1949). Expressed as mg g^{-1} fresh weight. Chlorophyll stability index was estimated by using the method given by Murthy and Majumdar (1962).

3.6.7.2 Relative Water Content (RWC) (see section 3.10.3)

3.6.7.3 H_2O_2 estimation : Hydrogen peroxide estimation in the leaf samples method was described by Patterson *et al*. (1984). Expressed as µmol of H_2O_2.

3.6.7.4 Proline estimation : The proline content in the leaf samples were determined by the method given by Bates *et al.* (1973). Expressed as mg g^{-1} dry weight.

3.6.7.5 Peroxidase estimation : Peroxidase was estimated as per the method is given by Harde *et al*. (2019) g^{-1} min^{-1}.

3.6.7.6 Nitrogen concentration : Nitrogen content (%) was estimated by the micro kjeldhal method (Piper, 1960).

3.6.7.7 Phosphorus concentration : The phosphorus content was determined by vanadomolybdo phosphoric acid yellow color method (Piper, 1960).

3.6.7.8 Potassium concentration : The potassium content in the triacid digest was determined by using ELICO Flame photometer (Piper, 1960).

3.6.7.9 Nutrient (N, P, K) uptake : The nutrient uptake was calculated using the following formula and expressed in Kg ha^{-1}

$$\text{Nutrient uptake}\left(\frac{\text{Kg}}{\text{hactare}}\right) = \frac{\text{Nutrient content (\%)} \times \text{Dry matter production (Kg/ha)}}{100}$$

3.6.8 Soil Chemical Analysis

3.6.8.1 pH in the soil samples : pH of the slurry samples was determined in 1:2.5 substrate water suspension by using digital pH meter (Systronics µ pH system 361) (Jackson, 1973).

3.6.8.2 Electrical conductivity (EC) in the soil samples : The electrical conductivity of slurry samples was determined in saturation extract by using Elico CM 180 conductivity meter (Jackson, 1973).

3.6.8.3 Organic carbon percentage in the samples : Organic carbon content of the soils was determined by Walkley and Black (1934) rapid titration method and results were expressed in percentage.

3.6.8.4 Available nitrogen in soil : The available nitrogen was estimated by the alkaline potassium permanganate method as described by Subbiah and Asija (1956) using KELPLUS and the results were expressed as Kg ha^{-1}.

3.6.8.5 Available phosphorus in soil : Available phosphorus in the soils expressed as Kg ha^{-1} was estimated as described by Olsen *et al.* (1954) and phosphorus in the extract was determined using spectrophotometer at 680 nm wavelength.

3.6.8.6 Available potassium in soil : Available potassium in the soils was extracted by employing neutral normal ammonium acetate and determined by aspirating the extract into the ELICO Flame photometer (Jackson, 1973) and results were expressed were in Kg ha^{-1}.

3.6.8.7 Dehydrogenase activity : Dehydrogenase enzyme activity was estimated using method described by Casida *et al.*, (1964). Expressed as μg of TPF g^{-1} soil day^{-1}.

3.6.8.8 Phosphatase activity : The procedure followed for alkaline phosphatase enzyme estimation was given by Eivazi and Tabatabai (1977). Expressed as μg pNP g^{-1} soil h^{-1}.

3.6.9 Harvesting

Harvesting was done at physiological maturity, judged visually when about 95 per cent grains were turned into golden colour. Initially the border rows were harvested. Later the net plot ear heads were harvested and stover bundled. The post harvest observations were recorded from the harvest samples. The cobs from net plot area were shelled separately and after sun drying, the net plot grain yields and straw yield were recorded treatment wise and reported in kg ha^{-1}

3.6.10 Yield Attributes

3.6.10.1 Test weight (100 seed weight) (g) : Five samples each of 100 grains were collected randomly from the net plot produce treatment wise and weighed, averaged and expressed in grams.

3.6.10.2 Grain yield (Kg ha^{-1}) : The kernels from the air-dried maize cobs of each net plot were separated, cleaned and dried to obtain at least 13 per cent moisture. Weight of grains of each plot was recorded separately and expressed as grain yield in Kg ha^{-1}.

3.6.10.3 Stover yield (Kg ha^{-1}) : The stover obtained from each net plot was weighed after it was completely sun dried and expressed as stover yield in Kg ha^{-1}.

3.6.10.4 Protein (%) estimation : The protein content in maize grains is estimated by Micro Kjeldal method (Humphries 1956). Approximately 0.1 g of raw material was hydrolyzed with 2.5 mL concentrated sulfuric acid (H_2SO_4) containing 1 g of two copper catalyst mixture in a heat block (Kjeltec system 2020 digestor) at 420 °C for 2 h. The tubes are cooled to room temperature after digestion. The digest solution was ensured to be totally clear and the tubes were attached to KELPLUS automatic nitrogen distillation unit. In 150 mL conical flask took 20 mL of 4 % boric acid containing mixed indicator and placed at receiver tube. After the completion of distillation for 9 minutes conical flask from the receiver tube was removed and the distillate was titrated against 0.02N H_2SO_4 till the pinkish shade appeared. The blank was run simultaneously without sample. The nitrogen content in sample was estimated by using the formula

$$\%\,\mathrm{N} = \frac{\mathrm{TV} \times 0.00028 \times 100}{\mathrm{W}}$$

Where TV= Titration value (Sample titration value-Blank titration value)
W= Weight of sample

The protein content in maize can be estimated estimated from nitrogen value (Galicia et al., 2009) Protein (%) = % Nitrogen value × 6.25 (Conversion factor for maize).

3.6.10.5 Starch (%) estimation : The starch content in maize grains was estimated by Anthrone method (Clegg, 1956). Starch is hydrolysed into simple sugars by dilute acids and the quantity of simple sugars is measured calorimetrically. The sample is treated with 80 % alcohol to remove sugars and then starch is extracted with 52 % perchloric acid. In hot acidic medium starch is hydrolysed to glucose and dehydrated to hydroxymethyl furfural. This component forms green color ed product with anthrone.

Around 0.1 g of sample was homogenized with 80 % ethanol to remove sugars. Centrifugation was done and the residue was washed repeatedly with hot 80 % ethanol till the washings do not give color with anthrone reagent. Then the residue was dried in water bath.to the residue 5 mL of distilled water and 6.5 mL of perchloric acid was added. Then centrifugation was done at 0 ºC for 20 min supernantant was saved and 0.2 mL of supernatant was made upto 1mL volume by distilled water simultaneously working standard were prepared by taking glucose at the rate of 0.2, 0.4, 0.6, 0.8 and made the volume to 1mL by distilled water. To the each tube 4mL of anthrone reagent was added and heated for 8 minutes in a boiling water bath. The intensity of green to dark green color was read at 630nm. Starch content was measured by using following formula (Clegg, 1956).

Starch content = Glucose content (obtained from standard curve) × 0.9 (Conversion factor)

3.6.11 Economics

a) Cost of cultivation: The cost of inputs (seeds and fertilizers) required per hectare and operational costs (man hours and electricity)

Seeds (20 Kg ha^{-1})	5500/-
Fertilizers (240:80:80 NPK/ha)	8300/-
Human labor (350/- per day)	29,750/- per ha
Electricity	3000/-
Miscellaneous	1000/-
Total	**47550/-**
*100 % RDF -8300/-, 75 % RDF-6225/-, 50 % RDF- 4150/- *Taken per irrigation cost- 500/-	

b) Gross returns: Gross returns was calculated by considering the prevailing market price of maize grains (Rs. 20/- Kg^{-1})

c) Net returns: Net returns (Rs ha^{-1}) was calculated by deducting the cost of cultivation from gross returns per hectare basis.

Net returns (Rs ha^{-1}) = Gross returns (Rs ha^{-1}) – Cost of cultivation (Rs ha^{-1})

d) Rate of returns (B:C ratio): The rate of returns per rupee spent was calculated by working out the ratio between net returns and cost of cultivation to get benefit cost ratio.

3.7 STATISTICAL ANALYSIS

The data obtained in different experiments was statistically analyzed using Completely Randomized Design (CRD) as per the procedures given by Snedecor and Cochran (1967). Data on different characters were subjected to analysis of variance as given by Gomez and Gomez (1984) for Randomized Block Design (RBD). Statistical significance was tested by F–value at 0.05 level of probability and critical difference was worked out where ever the effects were significant.

Chapter – IV

Results and Discussion

Chapter IV

RESULTS AND DISCUSSION

The results of the research work entitled **“DEVELOPMENT OF ENDOPHYTIC MICROBIAL CONSORTIUM TO MITIGATE STRESS CONDITIONS IN MAIZE CROP (*Zea mays* L.)”** were presented in this chapter. The experiments were conducted at Advanced Post Graduate Center, Lam, Guntur and Regional Agricultural Research Station, Anakapalle during 2019-2021. Maize plant samples were collected from major maize growing areas of Andhra Pradesh. Isolated endophytic bacteria from various plant parts of maize were characterized by performing morphological and biochemical traits. All the isolates were screened for their osmotolerance and plant growth promoting traits. The effective isolates were identified molecularly by 16S rRNA gene sequencing and re-examined for endophytic colonization. The confirmed endophytic bacteria were developed into consortium and their influence on stress mitigation and plant growth promotion in maize was studied both in pot and field conditions. The results obtained are explained and discussed in this chapter.

4.1 ISOLATION AND SCREENING OF ENDOPHYTIC BACTERIA FROM MAIZE PLANT SAMPLES

4.1.1 Plant sample collection

The major maize crop growing areas of Andhra Pradesh were selected and plant samples were collected from different regions of AP Srikakulam, Vijayanagaram, Visakhapatnam, East Godavari, Guntur, Kurnool and Ananthapuram *viz.,* during the Kharif and Rabi seasons of 2019. During each collection sterile techniques were used, labeled immediately and placed in thermocol box with cooling pads to maintain dry and cooling conditions to avoid excessive drying and moisture accumulation. At the time of sample collection information like growth stage of crop, previously harvested crop, type of soil and the latitude and longitude were recorded (Table 4.1). Point map was constructed for locating area of sample collected using QGIS software (Fig. 4.1).

4.1.2 Isolation and morphological characterization of endophytic bacteria

Endophytic bacteria were isolated by surface sterilizing maize plant tissues including roots, stem, leaf and kernels. A total of 106 culturable endophytic bacterial morphotypes were isolated and pure cultures were obtained by quadrant streak method. Morphological and physiological characterization of isolates was done based on colony shape, size, elevation, surface, margin, color, pigmentation, motility, grams

Table 4.1: Details of sampling site across Andhra Pradesh

S. No	District	Mandal	Crop growth stage	Previous crop	Soil type	Latitude and Longitude
1.	Visakhapatnam	Anakapalli	Grain filling	Maize	Red soil	17.6896◦N 83.0024◦E
2.	Nandyal	Allagadda	Flowering	sesame	Red soil	15.158173◦N 78.53815◦E
3.	Guntur	Tenali	Grain filling	Rice	Black soil	16.2379◦N 80.6444◦E
		Repalle	Grain filling	maize	Red loam	16.0174◦N 80.8295
		Venigandla	Vegetative	Cotton	Black cotton	16.2129◦N 80.2847◦E
		Nidumukkala	Grain filling	Maize	Black soil	16.1148◦N 80.2085◦E
4.	Vizianagaram	Bobbili	Flowering	Brinjal	Sandy	18.3014◦N 23.1649◦E
		Saluru	Grain filling	Maize	Sandy	18.3014◦N 23.1649◦E
		Gantyada	Grain filling	Rice	Sandy loam	18.0014◦N 23.2686◦E
5.	Srikakulam	Chilakapalem	Grain filling	Maize	sandy	18.2735 ◦N 83.8071◦E
		Ponduru	Grain filling	Rice	Sandy loam	18.3030 ◦N 83.7063◦E
		Subadrapuram	Flowering	Rice	Red soil	18.2271 ◦N 83.7116 ◦E
		Pydibhimavaram	Grain filling	Maize	Clay	18.1460 ◦N 83.6281 ◦E
6.	East Godavari	ARS, Peddapuram	Grain filling	Maize	Red soils	17.0712 ◦N 82.1479 ◦E
		Jaggampeta	Flowering	Maize	Loamy soil	17.1417 ◦N 82.0798 ◦E
		Rangampeta	Grain filling	Rice	Clay loam	17.0850 ◦N 81.9788 ◦E
7.	Anantapur	Garladinne	Grain filling	Paddy	Black soil	14.8256◦N 77.5935◦E
		Lolur	Flowering	Cotton	Red soil	14.8007 ◦N 77.5982 ◦E
		Vadiampeta	Grain filling	Bhendi	Red soil	14.7599 ◦N 77.6165 ◦E

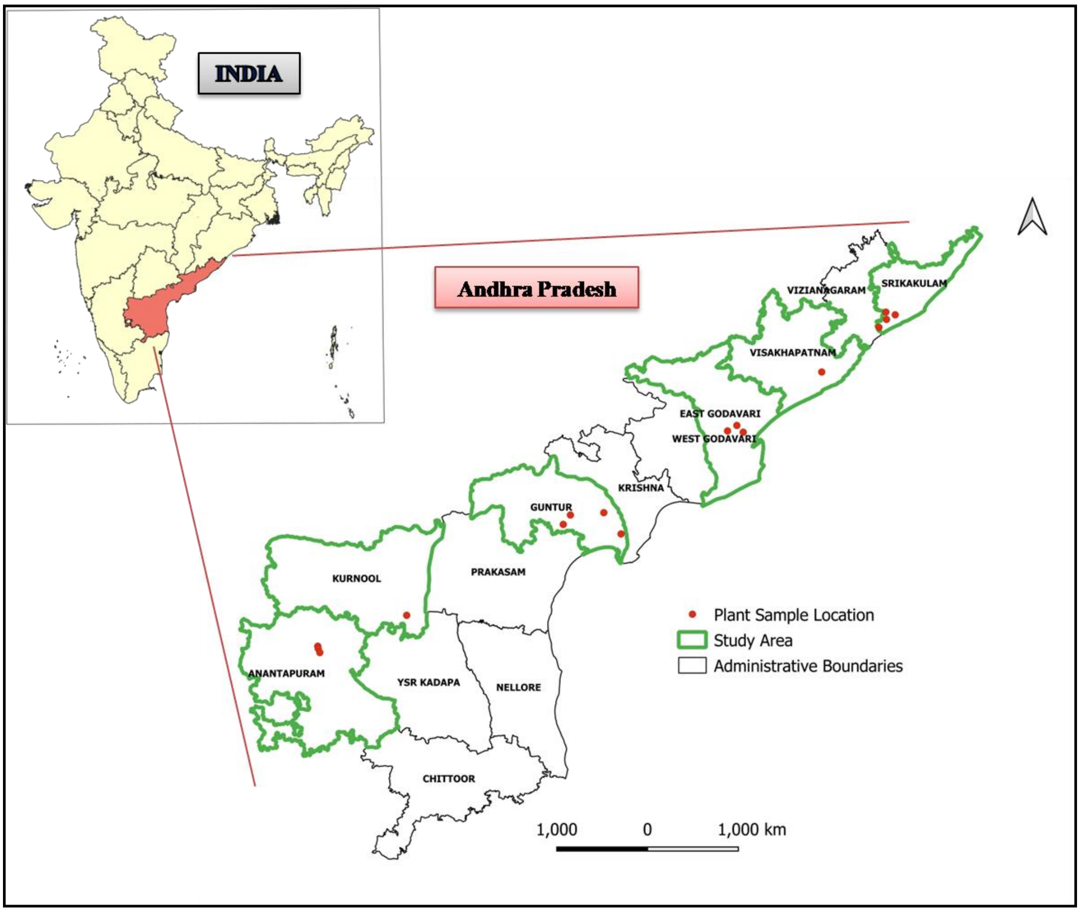


Fig. 4.1. Location map of maize plant samples collection across Andhra Pradesh

reaction and cell shape (Table 4.2). The isolates were designated with codes representing the place of isolation (1st letter indicating mandal), part of tissue (2nd letter indicating R-Root, S-Stem, L-Leaf, C-Kernal) and endophytic colonization.

Out of all the bacterial isolates 32 were isolated from root, 30 from the kernel, 24 from leaf and 20 from stem. Among 106 obtained isolates 80 % were found to be Gram negative (–ve) and 20 % of isolates were gram positive (+ve). Around 81 % of isolates (86) were found to have rod shaped cells with varied size ranging from small rods to long rods and 19 % of the isolates found to have cocci shaped cells. About 73.6 % of isolates (78) were found to have motility function while 26.4 % (28) of bacterial isolates were found to be non motile. Colonies of varied sizes like small, medium and large were observed among the obtained endophytic isolates with round to irregular colony shape and varied pigmentation showing white, cream, buff, pink, yellow and orange colored colonies.

Table 4.2. Morphological and physiological characters of endophytic bacteria isolated from maize

S. No.	Plant Tissue	Isolate Code	Gram Reaction	Cell Shape	Motility	Colony Size	Colony Shape	Edge	Elevation/ Surface	Pigmentation
1	Root	AR3E1	-Ve	Long Rods	Non motile	Medium	Round	Entire	Flat	White
2	Root	AR3E2	-Ve	Rods	Motile	Small	Round	Entire	Raised	Pink
3	Root	AR3E3	-Ve	Rods	Motile	Medium	Round	Entire	Flat	White
4	Root	AR3E4	-Ve	Rods	Motile	Medium	Round	Entire	Flat	Yellow
5	Root	ALR2E1	-Ve	Rods	Motile	Medium	Irregular	Irregular	Flat	White
6	Root	ALR2E2	-Ve	Rods	Motile	Medium	Round	Entire	Flat	Buff
7	Root	ALR2E3	-Ve	Rods	Motile	Medium	Round	Entire	Raised	Cream
8	Root	ALR2E4	+Ve	Rods	Motile	Large	Irregular	Irregular	Raised	White
9	Root	ALR2E5	-Ve	Rods	Motile	Small	Round	Entire	Convex	Orange
10	Root	ALR2E6	+Ve	Cocci	Motile	Medium	Irregular	Irregular	Flat	Cream
11	Root	ALR1E2	-Ve	Rods	Motile	Medium	Round	Entire	Flat	Buff
12	Root	ALR1E3	-Ve	Rods	Motile	Medium	Round	Entire	Raised	Cream
13	Leaf	ALL1E1	-Ve	Cocci	Non motile	Medium	Round	Entire	Raised	Yellow
14	Leaf	ALL1E2	+Ve	Cocci	Non motile	Medium	Round	Entire	Raised	White
15	Kernel	GCE1	+Ve	Cocci	Non motile	Small	Round	Entire	Flat	Cream
16	Kernel	GCE2	-Ve	Short Rods	Motile	Small	Round	Entire	Convex	Buff
17	Kernel	GCE3	-Ve	Rods	Motile	Small	Round	Entire	Convex	Orange
18	Kernel	GCE4	-Ve	Long Rods	Motile	Large	Irregular	Irregular	Flat	White
19	Kernel	GCE5	-Ve	Long Rods	Motile	Small	Round	Entire	Convex	Cream
20	Kernel	GCE6	-Ve	Short Rods	Motile	Large	Irregular	Irregular	Rhizoid	White
21	Root	GR3E1	-Ve	Rods	Motile	Medium	Round	Entire	Flat	Buff
22	Root	GR2E1	-Ve	Rods	Motile	Medium	Round	Entire	Raised	Cream
23	Stem	BS2E1	-Ve	Rods	Non motile	Small	Round	Entire	Convex	Orange
24	Stem	VS1E1	-Ve	Cocci	Non motile	Medim	Irregular	Irregular	Raised	White
25	Stem	VS1E2	-Ve	Cocci	Non motile	Medium	Round	Entire	Rhizoid	Yellow
26	Stem	VS1E3	-Ve	Rods	Motile	Medium	Round	Entire	Rhizoid	White
27	Stem	VS1E4	-Ve	Rods	Motile	Small	Round	Entire	Flat	Cream

S.No	Plant Tissue	Isolate Code	Gram Reaction	Cell Shape	Motility	Colony Size	Colony Shape	Edge	Elevation/ Surface	Pigmentation
28	Leaf	VL1E1	-Ve	Rods	Motile	Large	Irregular	Irregular	Convex	Orange
29	Root	VR1E1	+Ve	Rods	Non motile	Small	Round	Etire	Flat	Orange
30	Root	VR1E2	-Ve	Cocci	Motile	Small	Round	Etire	Covex	Yellow
31	Leaf	VL1E2	-Ve	Rods	Motile	Medium	Round	Entire	Flat	White
32	Stem	RS1E1	-Ve	Rods	Non motile	Medium	Round	Entire	Flat	Yellow
33	Stem	RS1E2	-Ve	Rods	Non motile	Medium	Irregular	Irregular	Flat	White
34	Root	RR1E1	-Ve	Cocci	Motile	Medium	Round	Entire	Raised	Yellow
35	Root	RR1E2	+Ve	Cocci	Motile	Medium	Round	Entire	Raised	White
36	Root	RR1E3	+Ve	Cocci	Motile	Small	Round	Entire	Flat	Cream
37	Root	TR3E1	-Ve	Short Rods	Motile	Small	Round	Entire	Convex	Buff
38	Leaf	NL3E1	-Ve	Cocci	Motile	Large	Round	Entire	Raised	White
39	Leaf	NL3E2	-Ve	Rods	Non motile	Large	Oval	Entire	Flat	Yellow
40	Leaf	NL3E3	-Ve	Rods	Motile	Medium	Round	Entire	Raised	Yellow
41	Leaf	NL3E4	-Ve	Long Rods	Motile	Large	Round	Entire	Raised	White
42	Leaf	NL3E5	-Ve	Short Rods	Motile	Medium	Round	Entire	Raised	Cream
43	Kernel	NC3E1	+Ve	Cocci	Motile	Large	Round	Irregular	Raised	White
44	Kernel	NC3E2	-Ve	Rods	Motile	Medium	Round	Entire	Raised	Yellow
45	Kernel	NC3E3	+Ve	Cocci	Motile	Medium	Round	Entire	Flat	Cream
46	Stem	NS3E1	-Ve	Short Rods	Motile	Large	Round	Entire	Convex	White
47	Stem	NS3E2	-Ve	Rods	Non motile	Medium	Round	Entire	Raised	Cream
48	Root	NR3E1	-Ve	Long Rods	Non motile	Large	Round	Entire	Convex	White
49	Root	NR3E2	-Ve	Long Rods	Non motile	Medium	Round	Entire	Raised	Cream
50	Kernel	PdC3E1	-Ve	Short Rods	Motile	Medium	Round	Entire	Flat	White
51	Kernel	PdC3E2	-Ve	Rods	Motile	Large	Round	Entire	Convex	White
52	Kernel	PdC3E3	-Ve	Rods	Motile	Medium	Round	Entire	Flat	White
53	Kernel	PdC3E4	-Ve	Long Rods	Motile	Large	Round	Entire	Rhizoidal	White
54	Kernel	PdS3E1	+Ve	Rods	Motile	Large	Irregular	Irregular	Raised	White
55	Stem	PdS3E2	+Ve	Rods	Motile	Large	Round	Entire	Rhizoidal	White
56	Stem	PdS3E3	-Ve	Long Rods	Motile	Medium	Round	Entire	Raised	White

S.No	Plant Tissue	Isolate Code	Gram Reaction	Cell Shape	Motility	Colony Size	Colony Shape	Edge	Elevation/ Surface	Pigmentation
57	Leaf	PdL3E1	-Ve	Short Rods	Non motile	Medium	Round	Entire	Raised	Cream
58	Leaf	RgL3E1	-Ve	Rods	Non motile	Medium	Round	Entire	Flat	Cream
59	Leaf	RgL3E2	-Ve	Rods	Non motile	Medium	Round	Entire	Rhizoid	Cream
60	Leaf	RgL3E3	+Ve	Rods	Motile	Small	Round	Entire	Raised	Orange
61	Leaf	RgL3E4	-Ve	Rods	Non motile	Large	Round	Entire	Convex	White
62	Kernel	RgC3E1	-Ve	Long Rods	Motile	Medium	Round	Irregular	Rhizoidal	Cream
63	Kernel	RgC3E2	+Ve	Rods	Motile	Large	Round	Entire	Flat	White
64	Kernel	JC3E1	+Ve	Rods	Motile	Medium	Round	Entire	Flat	White
65	Kernel	JC3E2	-Ve	Rods	Motile	Medium	Round	Entire	Flat	Cream
66	Kernel	PC3E1	-Ve	Cocci	Motile	Large	Round	Entire	Raised	White
67	Kernel	PC3E2	-Ve	Rods	Motile	Large	Oval	Entire	Flat	Yellow
68	Kernel	PC3E3	-Ve	Rods	Motile	Medium	Round	Entire	Raised	Yellow
69	Kernel	PC3E4	-Ve	Short Rods	Motile	Large	Round	Entire	Convex	White
70	Root	PR3E1	-Ve	Rods	Motile	Medium	Round	Entire	Raised	Cream
71	Root	PR3E2	-Ve	Long Rods	Motile	Large	Round	Entire	Convex	White
72	Leaf	PL3E1	+Ve	Rods	Motile	Large	Round	Entire	Flat	White
73	Leaf	PL3E2	+Ve	Rods	Motile	Medium	Irregular	Irregular	Flat	White
74	Leaf	PL3E3	-Ve	Long Rods	Motile	Medium	Round	Entire	Flat	White
75	Leaf	PL3E4	-Ve	Rods	Non motile	Small	Round	Entire	Raised	Pink
76	Root	CR3E1	-Ve	Rods	Non motile	Medium	Round	Entire	Flat	White
77	Root	CR3E2	-Ve	Rods	Motile	Medium	Round	Entire	Flat	Yellow
78	Kernel	CC3E1	-Ve	Rods	Non motile	Medium	Irregular	Irregular	Flat	White
79	Kernel	CC3E2	-Ve	Rods	Motile	Medium	Round	Entire	Flat	Cream
80	Kernel	CC3E3	+Ve	Cocci	Motile	Small	Round	Irregular	Raised	Yellow
81	Kernel	CC3E4	-Ve	Short Rods	Motile	Large	Irregular	Irregular	Rhizoid	White
82	Stem	CS3E1	-Ve	Rods	Motile	Medium	Round	Entire	Flat	Buff
83	Stem	CS3E2	-Ve	Rods	Motile	Medium	Round	Entire	Raised	Cream
84	Stem	PyS3E1	-Ve	Rods	Non motile	Small	Round	Entire	Convex	Orange
85	Stem	LS3E2	-Ve	Cocci	Non motile	Medium	Irregular	Irregular	Raised	White

S.No	Plant Tissue	Isolate Code	Gram Reaction	Cell Shape	Motility	Colony Size	Colony Shape	Edge	Elevation/ Surface	Pigmentation
86	Root	PyR3E1	-Ve	Rods	Motile	Large	Round	Entire	Convex	White
87	Kernel	PyC3E1	-Ve	Rods	Motile	Medium	Round	Entire	Flat	White
88	Kernel	SuC3E1	-Ve	Long Rods	Motile	Large	Round	Entire	Rhizoidal	White
89	Kernel	SuC3E2	+Ve	Rods	Motile	Large	Irregular	Irregular	Raised	White
90	Kernel	SuC3E3	+Ve	Rods	Motile	Large	Round	Entire	Rhizoidal	White
91	Root	SuR3E1	-Ve	Long Rods	Motile	Medium	Round	Entire	Raised	White
92	Root	SuR3E2	-Ve	Rods	Motile	Medium	Round	Entire	Flat	Buff
93	Leaf	SuL3E1	-Ve	Rods	Motile	Medium	Round	Entire	Raised	Cream
94	Leaf	SuL3E2	+Ve	Rods	Motile	Large	Irregular	Irregular	Raised	White
95	Root	VaR3E1	+Ve	Rods	Motile	Large	Irregular	Undulate	Wrinkled	White
96	Root	VaR3E2	-Ve	Rods	Non motile	Small	Round	Entire	Convex	Orange
97	Root	VaR3E3	-Ve	Cocci	Motile	Medium	Irregular	Irregular	Raised	White
98	Leaf	VaL3E1	-Ve	Small Rods	Motile	Medium	Round	Entire	Flat	Buff
99	Stem	VaS3E1	-Ve	Cocci-Oval	Non motile	Medium	Round	Entire	Flat	White
100	Leaf	KL3E1	-Ve	Cocci	Motile	Medium	Round	Entire	Raised	Yellow
101	Leaf	KL3E2	-Ve	Rods	Non motile	Large	Round	Entire	Convex	White
102	Stem	KS3E1	-Ve	Rods	Motile	Small	Round	Entire	Flat	Yellow
103	Stem	KS3E2	-Ve	Cocci	Non motile	Medium	Round	Entire	Flat	White
104	Stem	LS3E1	-Ve	Rods	Motile	Small	Round	Entire	Flat	Green
105	Leaf	LL3E1	-Ve	Rods	Motile	Small	Round	Entire	Raised	Pink
106	Stem	LS3E3	-Ve	Rods	Non motile	Large	Round	Entire	Convex	White

In the studies of Zinniel *et al.* (2002) preliminary characterization of endophytic bacteria showed that approximately equal percentages of gram-positive (41 %) and gram-negative (42 %) bacteria were recovered from the agronomic crop plants, whereas gram-negative bacteria (50 %) were isolated more frequently than gram-positive bacteria (21 %) from prairie plant. And more of gram-negative bacteria were found to be motile than gram-positive bacteria.

4.1.3 Determining bacterial growth under water potential of –0.65 MPa

Osmotolarance of bacterial isolates was measured by growing in nutrient broth supplemented with Polyethylene Glycol (PEG) 6000 to get varied water potential. Since PEG is a flexible, water-soluble polymer, it can be used to create very high osmotic pressures. It also is unlikely to have specific interactions with biological chemicals. These properties make PEG one of the most useful molecules for applying osmotic pressure in biochemistry experiments, in particular when using the osmotic stress technique. Per cent decrease in the bacterial growth in treatments supplemented with 10% PEG compared to control (No PEG) was measured for all the bacterial isolates (Table 4.3).

Few bacterial isolates ALR1E2, NL3E5, NC3E2, PdC3E3, JC3E2, KL3E1, KS3E2, LS3E1 were found to have increased growth at 10% PEG than the controlled condition. Mexal *et al.* (1975) demonstrated that beyond 5 % PEG, O_2 concentration was inversely proportional to PEG concentration. Endophytic bacteria may be categorized as microaerophic, facultative aerobic or anaerobic based on their plant tissue colonization ability where O_2 concentration is low. Considering low available O_2 levels as favourable condition for the growth of endophytic bacteria, supplementing growth media with 10 % PEG favoured the growth conditions of endophytic bacteria and increased growth rate has been observed in the present study. Isolates (36) with less per cent of decrease in their growth at 10 % PEG and improved growth were selected for further screening.

4.1.4 Nitrogen fixation

Among all the isolated (106) bacterial endophytes 43 isolates *i.e.,* 40 % of total isolates were found to have capability of biological nitrogen fixation (BNF). The isolates were inoculated on N-Free agar plates supplemented with BTB indicator, and those which turned color of inoculated plates from green to blue (Plate 4.1c) were considered as positive for nitrogen fixation. The atmospheric N_2 is converted to ammonia by nitrogen fixers which results in increased pH *invitro* and the color change phenomenon of BTB indicator from green to blue with increased pH above neutral represents nitrogen fixing ability of isolates. The N_2 fixing ability of endophytic bacteria could be more efficient than the rhizhospheric as there will be less concentration of O_2 inside the plant and competition for nutrients will be less. The identified nitrogen fixing bacteria were isolated from all the tissues of plant while most of N_2 fixing isolates were found to be root colonizers (12) followed by leaf and kernel tissues from which 11 N_2 fixers in each plant part were obtained and least number of N_2 fixers (9) were isolated from stem. This may indicate that roots are primary colonizers for evading endophytes from rhizosphere where abundancy diazotrophic bacteria is higher and the phyllosphere has been shown to provide appropriate conditions for colonization of diazotrophic bacteria that are able to fix atmospheric nitrogen (Abadi *et al.,* 2020). Many endophytic prokaryotes were reported to have N fixing ability due to less competition for nutrients and less O_2 concentration inside the plant (Döbereiner *et al.* 1995., Kuan *et al.* 2016., Yadav and Yadav, 2017).

4.1.5 Phosphorus solubilization

Out of all the endophytic bacteria 64 isolates have formed solubilization zone on Pikovskayas agar plates (Plate 4.1a) supplemented with 0.5% tri calcium phosphate indicating that 60 % of isolates have varied range of phosphate solubilizing ability. According to Silva Filho and Vidor (2000), solubilization indices below 2 are considered low, between 2 and 3 medium and above 3 high. In present study, 43 isolates were found to be have no solubilization ability while 27 isolates have shown low solubization indices (SI <2) and 14 isolates have shown medium SI (2-3), whereas 27 endophytic isolates were found have high phosphorus SI (>3). Maximum solubilization was shown by the strain KL3E1 (6.0), KL3E2 (6.0) followed by LS3E3 (5.8) (Table 4.3)

The phosphorus solubilizing bacteria can be used as bioinoculants for the crops growing in phosphorus limited areas as Oteino *et al.* (2015) demonstrated that the inoculation of endophytes into the rhizosphere increased growth in plants suffering from limited phosphate supply. The endophytic bacteria belonging to genera *Bacillus*, *Klebsiella, Microbacterium, Pantoea, Paenibacillus*, and *Pseudomonas*, have been reported to solubilize phoshorous (Kour *et al.*, 2020 a, b). *Pseudomonas fluorescens* and *Bacillus megaterium* strains were found to record higher phosphate solubilization efficiency on pikovskayas plates (El-Komy, 2005).

4.1.6 Potassium solubilization

Among all the isolated bacteria 19% were found to have potassium solubilization capacity. The potassium solubilization indices of maize endophytic bacteria were observed in 20 isolates in the range of 1.0 to 1.6 and the highest SI was shown by the strains LS3E3 (1.60) followed by JC3E2 (1.59) (Table 4.3). Number of K solubilizers is found to be less as compared to P solubilizers and N_2 fixers in present investigation which indicates that the isolates may use different mechanisms or different organic acids for solubilizing phosphorus and potassium.

Similar results were reported by Marag and suman (2018) that maize endophytic bacteria were with less K solubilizing ability as compared to P and Zn solubilization. The clear zone formation on selective medium was taken as criteria for screening P and K solubilizing bacteria (Plate 4.1b) in the present study, Azizah *et al.* (2020) has suggested that the larger clear zone is not always because of larger colony instead the exessive release of polysaccharides and enzyme activity.

4.1.7 IAA production

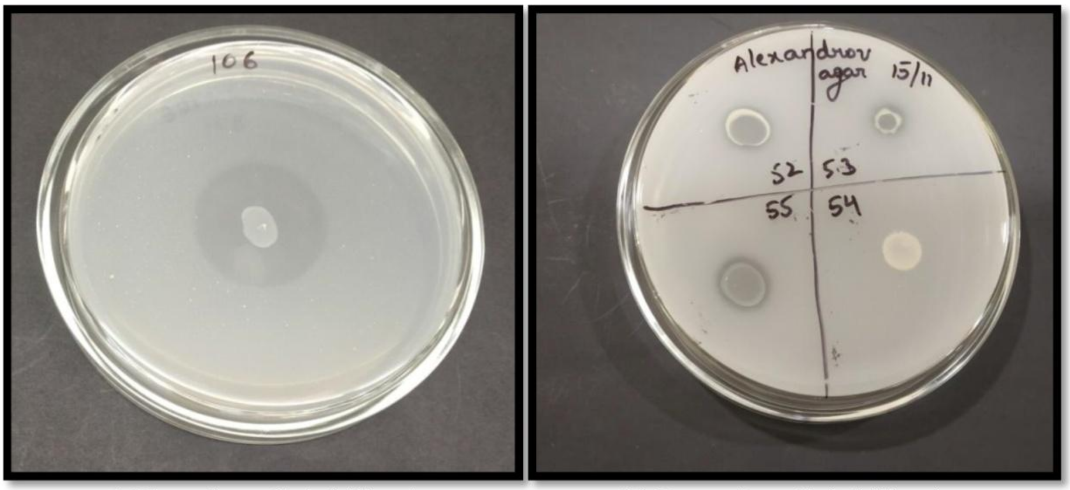
Out of 106 bacterial isolates 38 % were found positive for IAA production. Based on the color intensity developed after adding the Salkowski reagent (Plate 4.1e) and incubation the IAA production is categorized into low (no color), Medium (pale pink) and High (dark pink) production ability. Of the screened 106 isolates 64 strains were found to be low or no producers of IAA (Table 4.3). Medium level of IAA production was shown by 30 strains while 12 bacterial isolates (VS1E1, RS1E2, TR3E1, PdC3E2, PdS3E2, RgL3E4, RgC3E2, JC3E2, PyC3E1, LS3E2, LS3E3 and LL3E1) were found to be having high production of IAA.

In this study, IAA production ability was assessed for all bacterial endophytes using tryptophan as supplement and was found highly variable IAA producers. IAA synthesizing bacteria of different genera are well known to make use of tryptophan as a main precursor for IAA biosynthesis pathways (Spaepen *et al.*, 2007). On the basis of red color intensity *Pantoea* sp. CCBAU15488 was reported as IAA producer and found to nodulate soybean (Li *et al.*, 2008). The investigation of Etesami *et al.* (2014a) has also reported that *Pseudomonas putida* as an IAA-producing endophytic bacterium isolated from berseem clover plants nodules. The endophytic bacterial genera *Bacillus*, *Microbacterium, Psychrobacillus* and *Lysinibacillus* have been isolated from maize roots and reported to have ability to produce IAA (Yu *et al.*, 2016).

4.1.8 Siderophore Production

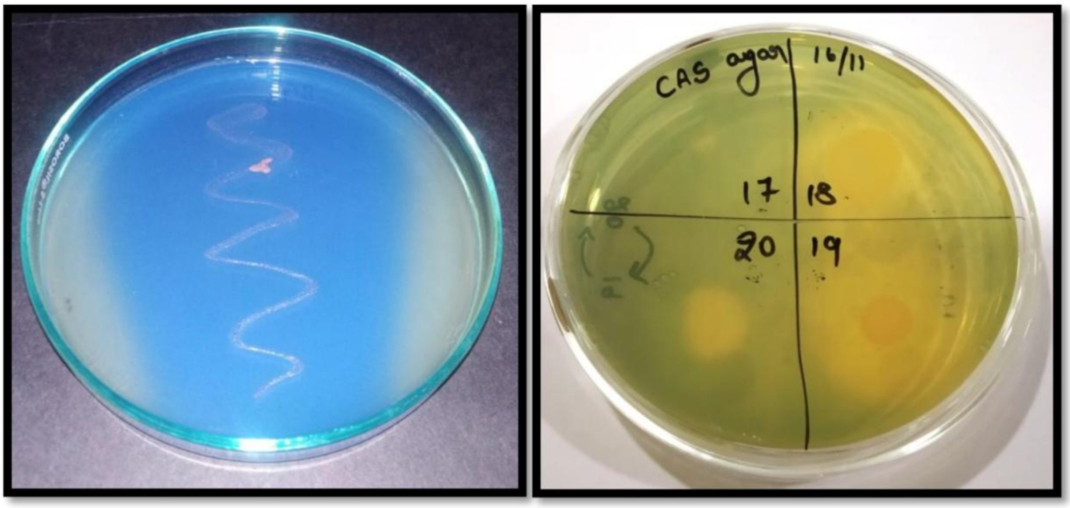
Iron is required in large quantity for plants, as it is involved in various decisive biological processes, such as chlorophyll biosynthesis, respiration and biological nitrogen fixation. Siderophore are low molecular weight iron-chelating compounds released by many microorgansims and make them successful competitors in colonization of roots, stem and leaf than pathogenic microorganism. The formation of orange halo zone around the colonies on CAS agar indicates siderophore production (Plate 4.1d). Bacteria producing siderophore form colonies with an orange halo because iron is removed from the original blue CAS–Fe (III) complex during siderophore production. Out of all 26 isolates were found to be positive for siderophore production in plate assay and selected for quantitative evaluation. The isolates which formed large orange halo zone were considered as efficient siderophores in the primary screening and the efficient isolates identified are NL3E3, NC3E2, PdC3E2, PdC3E1, RgL3E4, JC3E2, VaR3E1, KL3E1, KS3E1, LS3E1 and LS3E3 (Table 4.3).

Siderophore are Fe chelating biological compounds which bacteria synthesize, chelate Fe and make it available to the plant system. In present study the most of the siderophore producing bacteria were isolated from stem and leaf and their colonization may alter with the plant growth stage as per the report of Loaces *et al.* (2011), during plant growth the community of siderophore producing endophytic bacteria *Burkholderia, Enterobacter, Pseudomonas* and *Sphingomonas* were atlered and in roots at tillering stage of *Oryza sativa*, *Pantoea* were highest and at subsequent stages highest in leaves.



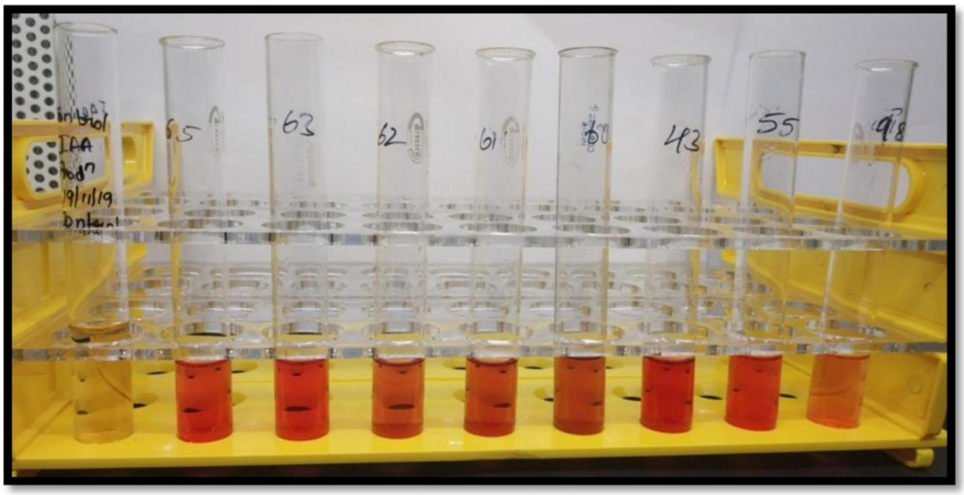
a) Phosphate Solubilization

b) Potassium Solubilization



c) Nitrogen Fixation

d) Siderophore Production



e) IAA Production

Plate. 4.1. Plant growth promoting traits of endophytic bacterial isolates

Table 4.3. Evaluation of endophytic bacteria against water stress (osmotolerance) and plant growth promoting traits

S.No	Plant Tissue	Isolate Code	Bacterial Growth (OD)		% decrease in OD	N_2 Fixation ability	P Solubilization Index	K Solubilization Index	IAA Production	Siderophore Production
			-0.05 MPa (Control)	-0.65 MPa (10% PEG)						
1	Root	AR3E1	1.182	0.576	51.269	+	2.92	–	+	–
2	Root	AR3E2	0.856	0.624	27.103	+	2.92	–	+	–
3	Root	AR3E3	1.165	0.847	27.296	–	–	–	–	–
4	Root	AR3E4	0.447	0.357	20.134	+	–	–	–	–
5	Root	ALR2E1	0.088	0.067	23.864	–	–	–	–	–
6	Root	ALR2E2	1.020	0.748	26.667	–	1.38	–	+	–
7	Root	ALR2E3	0.864	0.627	27.431	+	–	–	–	–
8	Root	ALR2E4	0.142	0.052	63.380	–	–	–	–	+
9	Root	ALR2E5	0.908	0.711	21.696	+	1.58	–	+	–
10	Root	ALR2E6	1.037	0.624	39.826	–	1.50	–	–	–
11	Root	ALR1E2	0.576	0.847	-47.049	+	2.33	–	+	–
12	Root	ALR1E3	1.100	0.357	67.545	–	–	–	–	–
13	Leaf	ALL1E1	0.281	0.067	76.157	+	–	–	–	–
14	Leaf	ALL1E2	1.530	0.748	51.111	–	3.38	–	–	–
15	Kernel	GCE1	1.097	0.627	42.844	–	2.58	–	–	–
16	Kernel	GCE2	1.388	0.407	70.677	+	2.33	–	+	+
17	Kernel	GCE3	1.346	0.589	56.241	–	2.85	–	-	–
18	Kernel	GCE4	0.615	0.245	60.163	+	1.37	–	-	–
19	Kernel	GCE5	0.673	0.256	61.961	+	1.47	–	-	–
20	Kernel	GCE6	0.841	0.412	51.011	–	1.26	–	-	–
21	Root	GR3E1	0.158	0.045	71.519	–	1.61	–	+	–
22	Root	GR2E1	0.159	0.098	38.365	–	–	–	+	–
23	Stem	BS2E1	1.468	1.092	25.613	–	1.26	–	–	+
24	Stem	VS1E1	1.463	0.826	43.541	–	3.69	1.29	++	+
25	Stem	VS1E2	1.067	0.546	48.828	+	–	–	–	–

S.No	Plant Tissue	Isolate Code	Bacterial Growth (OD)		% decrease in OD	N_2 Fixation	PSI	KSI	IAA	Siderophore
			-0.05 MPa (Control)	-0.65 MPa (10% PEG)						
26	Stem	VS1E3	0.124	0.045	63.710	–	–	–	+	–
27	Stem	VS1E4	0.404	0.245	39.356	–	–	–	–	–
28	Leaf	VL1E1	1.607	0.845	47.418	+	–	–	–	–
29	Root	VR1E1	0.964	0.847	12.137	+	1.35	1.25	+	+
30	Root	VR1E2	1.107	0.578	47.787	+	–	–	–	–
31	Leaf	VL1E2	1.024	0.751	26.660	-	–	–	+	–
32	Stem	RS1E1	0.124	0.075	39.516	+	–	–	–	–
33	Stem	RS1E2	0.330	0.124	62.424	+	–	–	++	–
34	Root	RR1E1	0.422	0.301	28.673	–	–	–	–	–
35	Root	RR1E2	1.660	1.010	39.157	–	1.40	–	–	–
36	Root	RR1E3	0.318	0.104	67.296	–	1.20	–	–	–
37	Root	TR3E1	0.328	0.144	56.098	–	1.43	–	++	–
38	Leaf	NL3E1	0.854	0.247	71.077	–	–	–	–	–
39	Leaf	NL3E2	1.388	0.407	70.677	+	3.93	1.18	+	+
40	Leaf	NL3E3	0.865	0.675	21.965	+	5.45	–	+	++
41	Leaf	NL3E4	1.532	0.921	39.883	+	5.16	–	+	+
42	Leaf	NL3E5	0.572	0.971	-69.755	+	3.92	–	–	–
43	Kernel	NC3E1	1.360	0.978	28.088	–	2.27	–	+	–
44	Kernel	NC3E2	1.308	1.444	-10.398	+	5.63	–	+	++
45	Kernel	NC3E3	1.466	1.040	29.059	–	2.67	–	–	–
46	Stem	NS3E1	1.409	0.854	39.390	+	2.80	–	–	–
47	Stem	NS3E2	1.298	0.741	42.912	–	–	–	–	–
48	Root	NR3E1	1.503	1.439	4.258	+	1.73	–	+	+
49	Root	NR3E3	1.503	1.133	24.617	–	2.11	–	–	–
50	Kernel	PdC3E1	1.177	0.478	59.388	+	1.35	–	–	–
51	Kernel	PdC3E2	1.182	0.576	51.269	–	1.87	–	++	–

S. No.	Plant Tissue	Isolate Code	Bacterial Growth (OD)		% decrease in OD	N_2 Fixation	PSI	KSI	IAA	Siderophore
			-0.05 MPa (Control)	-0.65 MPa (10% PEG)						
52	Kernel	PdC3E3	1.390	1.442	-3.741	+	2.67	1.22	+	+
53	Kernel	PdC3E4	1.503	1.133	24.617	–	1.43	1.42	-	-
54	Kernel	PdC3E1	1.392	1.031	25.934	+	4.41	-	+	++
55	Stem	PdS3E2	1.709	1.440	15.740	+	3.79	1.21	++	+
56	Stem	PdS3E3	1.440	0.645	55.208	–	–	–	–	–
57	Leaf	PdL3E1	0.918	0.241	73.747	–	–	–	–	–
58	Leaf	RgL3E1	0.842	0.241	71.378	–	–	–	–	–
59	Leaf	RgL3E2	0.412	0.102	75.243	–	–	–	–	–
60	Leaf	RgL3E3	0.691	0.418	39.508	–	–	–	–	–
61	Leaf	RgL3E4	1.503	1.133	24.617	++	4.92	1.35	++	++
62	Kernel	RgC3E1	1.040	0.784	24.615	–	1.35	–	–	–
63	Kernel	RgC3E2	1.177	1.087	7.647	+	4.43	–	++	+
64	Kernel	JC3E1	0.908	0.711	21.696	+	1.32	1.22	-	+
65	Kernel	JC3E2	1.276	1.288	-0.940	+	4.77	1.59	++	++
66	Kernel	PC3E1	0.588	0.431	26.701	–	–	–	–	–
67	Kernel	PC3E2	0.514	0.124	75.875	–	1.25	–	–	–
68	Kernel	PC3E3	0.902	0.614	31.929	+	-	–	–	–
69	Kernel	PC3E4	1.345	0.841	37.472	–	2.25	–	–	–
70	Root	PR3E1	0.245	0.107	56.327	–	–	–	+	–
71	Root	PR3E2	0.107	0.087	18.692	–	–	–	–	–
72	Leaf	PL3E1	0.856	0.324	62.150	+	5.33	–	–	–
73	Leaf	PL3E2	1.331	1.092	17.956	+	2.27	1.15	–	+
74	Leaf	PL3E3	0.400	0.210	47.500	+	–	–	–	–
75	Leaf	PL3E4	0.106	0.024	77.358	–	–	–	–	–
76	Root	CR3E1	0.839	0.421	49.821	+	–	–	–	–
77	Root	CR3E2	0.911	0.325	64.325	–	–	–	+	–

S. No.	Plant Tissue	Isolate Code	Bacterial Growth (OD)		% decrease in OD	N_2 Fixation	PSI	KSI	IAA	Siderophore
			-0.05 MPa (Control)	-0.65 MPa (10% PEG)						
78	Kernel	CC3E1	0.205	0.103	49.756	+	–	–	–	–
79	Kernel	CC3E2	0.675	0.241	64.296	+	5.40	–	–	–
80	Kernel	CC3E3	1.554	0.734	52.767	+	5.47	1.18	–	+
81	Kernel	CC3E4	0.814	0.321	60.565	+	1.40	–	+	–
82	Stem	CS3E1	0.550	0.214	61.091	+	–	–	–	–
83	Stem	CS3E2	0.851	0.614	27.850	–	–	–	–	–
84	Stem	PyS3E1	0.973	0.612	37.102	+	–	–	+	–
85	Stem	LS3E2	0.709	0.457	35.543	+	2.43	–	++	–
86	Root	PyR3E1	0.529	0.246	53.497	+	1.08	–	+	–
87	Kernel	PyC3E1	0.740	0.138	81.351	–	1.15	–	++	–
88	Kernel	SuC3E1	0.469	0.174	62.900	–	–	–	–	–
89	Kernel	SuC3E2	0.561	0.241	57.041	+	–	–	–	–
90	Kernel	SuC3E3	0.783	0.324	58.621	–	–	–	–	–
91	Root	SuR3E1	0.763	0.229	69.987	–	1.30	1.23	–	–
92	Root	SuR3E2	0.613	0.354	42.251	–	–	1.50	–	–
93	Leaf	SuL3E1	0.896	0.574	35.938	–	–	–	–	–
94	Leaf	SuL3E2	1.015	0.641	36.847	–	1.72	–	–	–
95	Root	VaR3E1	0.284	0.234	17.606	++	3.33	–	+	++
96	Root	VaR3E2	0.245	0.089	63.673	–	–	–	–	–
97	Root	VaR3E3	0.908	0.711	21.696	–	1.93	–	–	–
98	Leaf	VaL3E1	0.620	0.324	47.742	–	3.62	1.22	+	+
99	Stem	VaS3E1	1.859	1.254	32.544	+	1.53	1.14	–	–
100	Leaf	KL3E1	1.103	1.635	-48.232	+	6.00	1.1	–	++
101	Leaf	KL3E2	1.554	0.734	52.767	–	6.00	1.25	+	–
102	Stem	KS3E1	1.177	1.087	7.647	++	3.92	1.25	+	++

S. No.	**Plant Tissue**	**Isolate Code**	**Optical Density (OD)**		**% decrease in OD**	**N_2 Fixation**	**PSI**	**KSI**	**IAA**	**Siderophore**
			-0.05 MPa (Control)	**-0.65 MPa (10% PEG)**						
103	Stem	KS3E2	1.345	1.528	-13.606	+	1.47	1.29	–	–
104	Stem	LS3E1	1.133	1.575	-39.011	++	4.08	–	+	++
105	Leaf	LL3E1	1.499	1.205	19.613	++	4.21	–	++	+
106	Stem	LS3E3	1.388	0.845	39.121	++	5.8	1.6	++	++

*OD- Optical Density, N- Nitrogen, P- Phosphorus, K- Potassium, IAA- Indole Acetic Acid , – No, + Medium , ++ High

4.2 BIOCHEMICAL CHARACTERIZATION AND QUANTITATIVE EVALUATION OF SCREENED ENDOPHYTIC BACTERIA AGAINST OSMOTOLERANCE AND PLANT GROWTH PROMOTING TRAITS

All the 106 endophytic bacterial isolates were screened based on their growth in low water potential of –0.65 MPa for osmotolerance and evaluated plant growth promoting traits qualitatively. Identified 36 efficient water stress (drought) mitigating and plant growth promoting endophytic bacteria for further screening quantitatively and biochemically characterized.

4.2.1 Biochemical characterization of bacterial endophytes

According to the Bergey's manual of determinative bacteriology, the physiology and biochemical characteristics of the selected isolates was determined (Plate 4.2). All the isolates are positive for catalase production except 3 isolates (PdC3E2, PdC3E3 and VaL3E1). Isolate VaL3E1 is negative for citrate utilization while remaining strains have shown positive result. 20 isolates were found to be H_2S producers, 8 isolates are positive for indole production, 4 isolates are positive for MR test and 25 isolates are positive for VP test. Isolates NL3E5, PdC3E3 and PdS3E1 are found to be negative for gelatin liqification while remaining 33 isolates have shown positive result (Table 4.4).

Ammonia production is an important trait that indirectly affect the plant growth. In the present study 27 isolates were found to be positive for ammonia production by changing color after addition of Nessler's reagent, indicating that these isolates may accumulate nitrogen in plants and promotes root and shoot elongation which indirectly influencing seed vigor index. Comparable results were reported by Marques *et al.* (2010) that ammonia producing bacteria accumulate and provide nitrogen to host plant , elongation of plant root and shoot, consequently increasing plant biomass. Similarly Fouda *et al.* (2021) showed the potency of bacterial endophytes for ammonia production ranging between low to high based on color change of inoculated growth media after adding Nesseler's reagent.

Sharma *et al.* (2014) characterized bacterial isolates with Zn solubilisation efficiency for their morphological and biochemical studies, isolates 1J and 19D were gram negative produced round shaped, raised colonies having shining surface, smooth margin and light yellow to off white in color. Isolate 19D produced yellow-green pigment when streaked on King's B medium. Biochemical characterization revealed 19D positive for oxidase, citrate and nitrate reduction. Isolate 1J was positive for, oxidase, citrate utilization and nitrate reduction.

The carbohydrate utilization of endophytic bacterial isolates was evaluated for dextrose, sucrose, maltose and lactose (Table 4.5). All the tested 36 bacterial isolates were found to have ability to utilize dextrose as carbon source while 33 isolates were observed to be sucrose utilizers except 3 isolates (AR3E2, AlR2E5 and PdC3E4). Except the strain PdC3E4 remaining all bacterial isolates were found positive for maltose utilization. Lactose was observed to be utilized by only 16 isolates while remaining 20 bacterial isolates were found negative for lactose utilization as carbon source. Dextrose was found to be mostly used carbon source while lactose was leastly used (Plate 4.3). Our results were supported by similar observations reported by Shahab and Ahmed (2008) by testing 10 rhizospheric bacteria for their carbon sources utilization including Glucose, Fructose, Sucrose and Lactose. Out of all glucose was found to be most favorable carbon source for P solubilization while lactose is the least favorable carbon source.

4.2.2 Evaluation of endophytic bacterial growth at varied water potential (Osmotolerance)

Osmotolaerance of bacterial isolates was measured by growing in nutrient broth supplemented with PEG 6000 at concentration of 10%, 20%, 30% and 40% to get varied water potentials of –0.65, –1.57, –2.17 and –2.70 MPa respectively. Bacterial growth was measured by taking optical density at 600nm using spectrophotometer. Per cent decrease in the bacterial growth in treatments compared to control (No PEG) having water potential -0.05 MPa was measured. The table 4.6 showing the optical density of bacterial growth at varied water potential and the per cent decrease in their growth with respect to control.

Out of all 21 isolates were found to show less than 25% decrease in their growth at –0.65MPa (10%PEG), while 8 isolates have shown 25-50% decrease and 7 isolates with 50-75% decrease in their growth with respect to control. At –1.57MPa (20%PEG) of water potential 3 isolates (VaR3E1, LS3E1 & VS1E1) have shown less than 25% decrease in their growth while decreased growth in the range of 25-50% and

50-75% were shown by 15 and 16 isolates respectively and more than 75% decrease in growth was expressed by 2 isolates. The bacterial isolate VaR3E1 is observed to grow at –2.17MPa of water potential with less than 25% decrease in the growth where as 4 isolates have shown 25-50% decrease in growth, 10 isolates with 50-75% and 21 isolates with more than 75% decrease in the growth at –2.17MPa. All the isolates were found to show more than 25% decrease in growth at –2.70MPa water potential, bacterial isolate NL3E3 have shown 48% decrease in growth, 10 isolates with 50-75% and 25 isolates with more than 75% decrease in the growth were observed (Table 4.7).

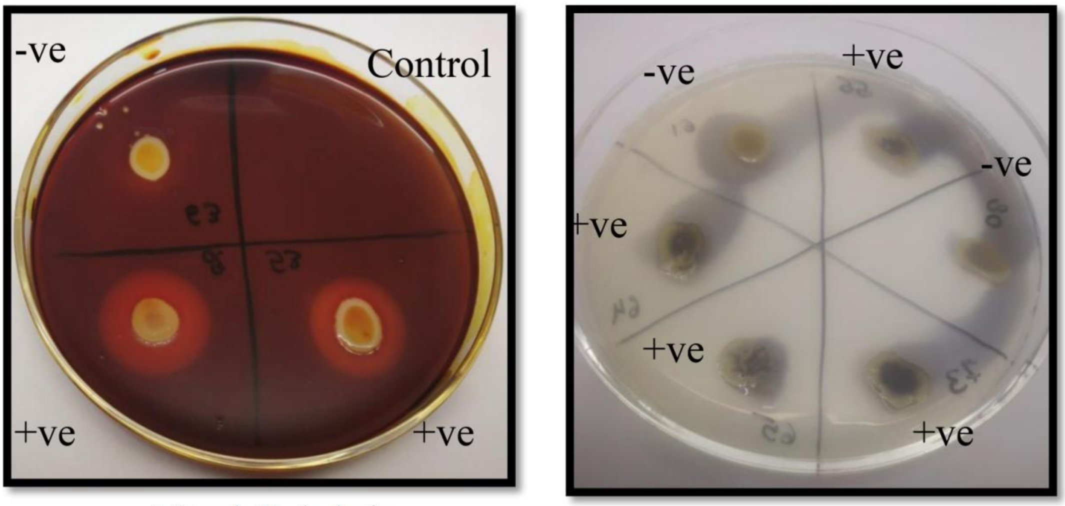
Bacterial isolates with less per cent decrease in their growth even at higher water potential were considered as osmotolerant or water stress resilient. In present investigation the endophytic bacterial strains VaR3E1 (23.94%), NL3E3 (45.45%), VS1E1 (46.62%), PL3E2 (47.18%) and LL3E1 (54.07%) were found to be efficient osmotolerants with less per cent decrease in the growth at higher water potential of –2.17MPa (30% PEG 6000).

Many maize seed endophytic bacteria exhibited tolerance to salinity (10%) and osmotic stress at 40% PEG6000 (Bodhankar *et al.,* 2017). The previously identified endophytic bacteria with osmotolerance potential that protects plant from drought stress are *Bacillus* sp.(Grover *et al.* 2014., Kushwaha *et al.* 2020 and Vardharajula *et al.* 2011) *Enterobacter cloacae* (Sandhya *et al.*, 2017) *Klebsiella* sp., *Pantoea alhagi* (Lei *et al.* 2017 and Chen *et al.* 2017) *Pseudomonas* sp and *Bacillus cereus* (Dubey *et al.,* 2021).

Table 4.7. Per cent decrease in bacterial growth at varied water potential

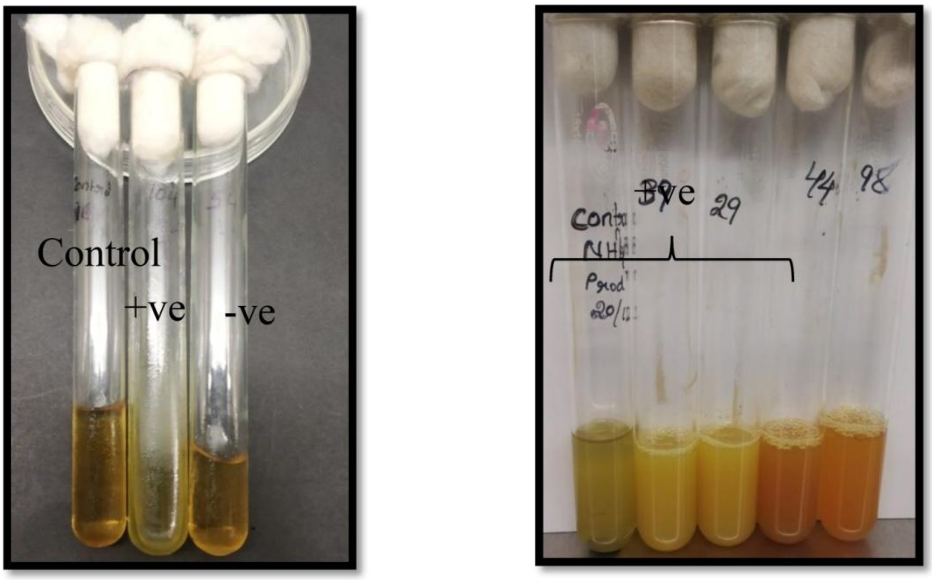
Water Potential	**Per cent decrease in bacterial growth**			
	<25%	25-50%	50-75%	>75%
–0.65MPa	21	8	7	0
–1.57MPa	3	15	16	2
–2.17MPa	1	4	10	21
–2.70MPa	0	1	10	25

Values in each column represents number of bacterial isolates



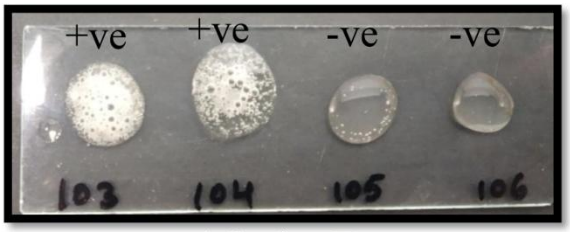
a) Starch Hydrolysis

b) Oxidase Test



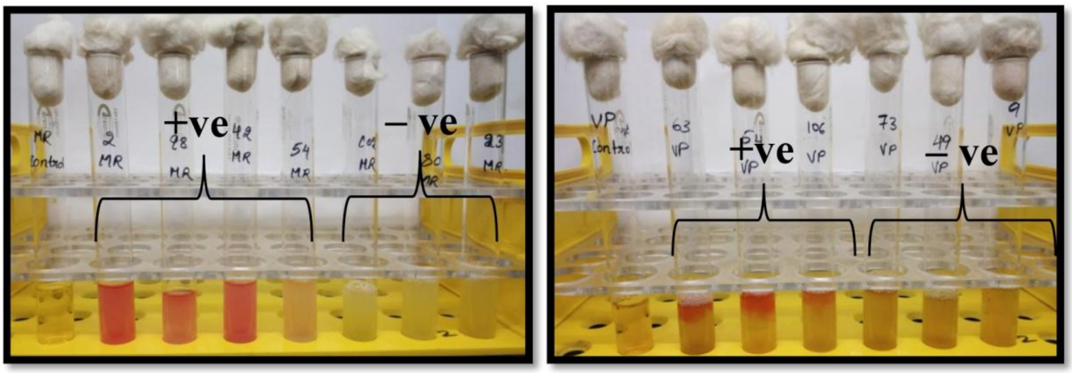
c) Gelatin Liquification

d) Ammonia Production

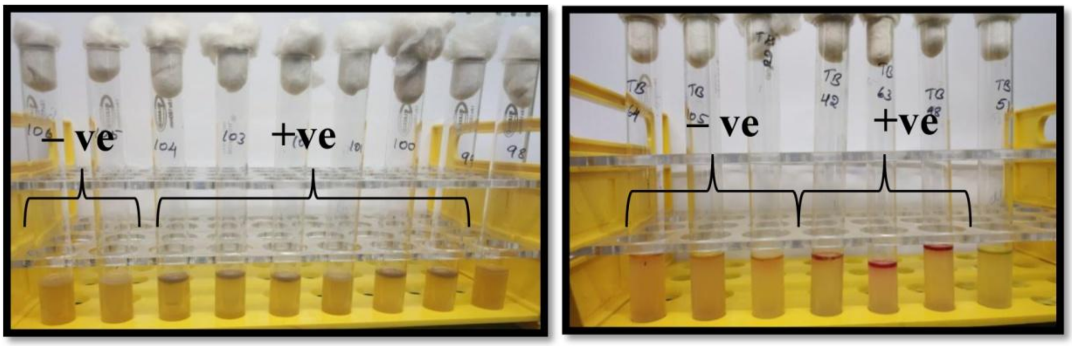


e) Catalase Test

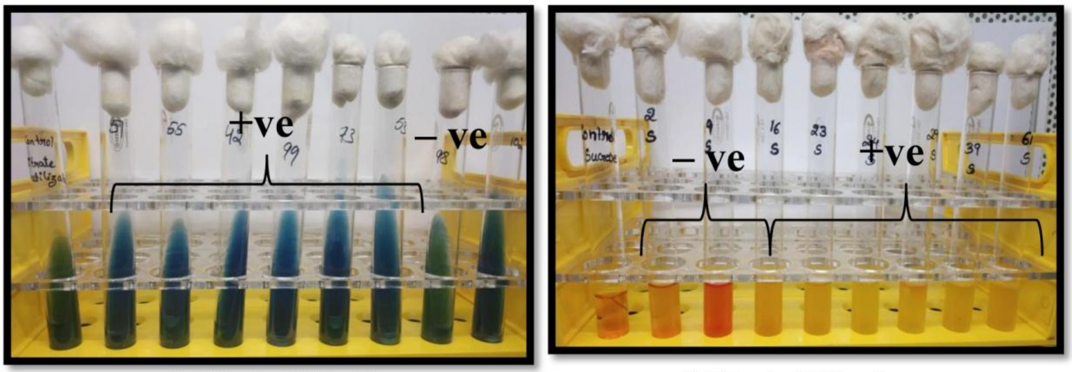
Plate 4.2. Biochemical characterization of endophytic bacterial isolates



a) Methyl Red Test b) Voges Proskauer Test



c) H_2S Production d) Indole Production



e) Citrate Utilization f) Citrate Utilization

Plate 4.3. Biochemical characterization of endophytic bacterial isolates

Table 4.4. Biochemical characterization of bacterial endophytes

S. No	Isolate Code	Catalase	Oxidase	Starch Hydrolysis	Citrate Utilization	H_2S Test	Indole Production	MR	VP	Gel Liquification	Ammonia Production
1	AR3E2	+	–	–	+	–	+	+	+	+	+
2	AlR2E5	+	+	–	+	+	–	–	–	+	–
3	GC3E2	+	–	+	+	+	–	–	+	+	+
4	BS2E1	+	+	+	+	+	–	–	+	+	–
5	VS1E1	+	+	+	+	+	–	–	–	+	+
6	VR1E1	+	+	–	+	+	–	–	+	+	+
7	NL3E2	+	+	–	+	+	–	–	+	+	+
8	NL3E3	+	–	–	+	+	–	–	+	+	+
9	NL3E4	+	+	+	+	+	–	–	–	+	–
10	NL3E5	+	–	+	+	–	+	+	–	–	+
11	NC3E1	+	+	–	+	+	–	–	+	+	–
12	NC3E2	+	+	–	+	+	–	–	+	+	+
13	NR3E1	+	+	+	+	+	–	–	+	+	+
14	NR3E3	+	+	–	+	+	–	–	–	+	+
15	PdC3E2	–	–	+	+	–	–	–	–	+	+
16	PdC3E3	–	+	–	+	–	–	–	–	–	–
17	PdC3E4	+	–	–	+	–	–	–	+	+	+
18	PdS3E1	+	–	–	+	–	–	+	+	–	+
19	PdS3E2	+	+	+	+	–	–	–	+	+	+
20	RgL3E4	+	+	+	+	+	–	–	+	+	+
21	RgC3E2	+	–	+	+	–	+	–	+	+	–
22	JC3E1	+	–	–	+	–	+	–	–	+	+
23	JC3E2	+	–	+	+	–	+	–	+	+	+
24	PL3E2	+	+	+	+	+	–	–	–	+	+
25	CC3E3	+	–	–	+	+	–	–	–	+	+
26	VaR3E1	+	–	+	+	+	–	–	+	+	+
27	VaL3E1	–	–	+	–	–	+	+	–	+	+
28	VaS3E1	+	+	+	+	+	–	–	+	+	–
29	KL3E1	+	+	–	+	+	–	–	+	+	+
30	KL3E2	+	+	–	+	–	–	–	+	+	–
31	KS3E1	+	+	–	+	+	–	–	+	+	+
32	KS3E2	+	+	+	+	+	–	–	+	+	–
33	LS3E1	+	–	+	+	+	–	–	+	+	+
34	LS3E2	+	–	+	+	–	+	–	+	+	+
35	LS3E3	+	–	+	+	–	–	–	+	+	+
36	LL3E1	+	+	–	+	–	+	–	+	+	+

Table 4.5. Evaluation of bacterial endophytes for their ability to utilize various carbon sources

S.No	Isolate no	Dextrose	Sucrose	Maltose	Lactose
1	AR3E2	+	–	+	–
2	AlR2E5	+	–	+	–
3	GC3E2	+	+	+	–
4	BS2E1	+	+	+	+
5	VS1E1	+	+	+	–
6	VR1E1	+	+	+	–
7	NL3E2	+	+	+	–
8	NL3E3	+	+	+	–
9	NL3E4	+	+	+	–
10	NL3E5	+	+	+	–
11	NC3E1	+	+	+	+
12	NC3E2	+	+	+	+
13	NR3E1	+	+	+	+
14	NR3E3	+	+	+	–
15	PdC3E2	+	+	+	+
16	PdC3E3	+	+	+	+
17	PdC3E4	+	–	–	–
18	PdS3E1	+	+	+	+
19	PdS3E2	+	+	+	+
20	RgL3E4	+	+	+	+
21	RgC3E2	+	+	+	+
22	JC3E1	+	+	+	+
23	JC3E2	+	+	+	+
24	PL3E2	+	+	+	–
25	CC3E3	+	+	+	+
26	VaR3E1	+	+	+	–
27	VaL3E1	+	+	+	–
28	VaS3E1	+	+	+	–
29	KL3E1	+	+	+	–
30	KL3E2	+	+	+	+
31	KS3E1	+	+	+	–
32	KS3E2	+	+	+	–
33	LS3E1	+	+	+	+
34	LS3E2	+	+	+	–
35	LS3E3	+	+	+	+
36	LL3E1	+	+	+	–

Table 4.6. Evaluation of endophytic bacterial growth at varied water potential

Isolate no.		OD at varied water potential					Per cent decrease in OD			
	Isolate code	–0.05 MPa (Control)	–0.65 MPa	–1.57 MPa	–2.17 MPa	–2.70 MPa	–0.65 MPa	–1.57 MPa	–2.17 MPa	–2.70 MPa
1	AR3E2	1.182	0.576	0.392	0.201	0.128	51.269	66.84	82.99	89.17
2	AlR2E5	0.908	0.711	0.671	0.417	0.309	21.696	26.10	54.07	65.97
3	GC3E2	1.388	0.407	0.381	0.207	0.086	70.677	72.55	85.09	93.80
4	BS2E1	1.468	1.092	0.864	0.52	0.371	25.613	41.14	64.58	74.73
5	VS1E1	1.463	0.826	1.123	0.781	0.595	43.541	23.24	46.62	59.33
6	VR1E1	0.964	0.937	0.594	0.227	0.084	2.801	38.38	76.45	91.29
7	NL3E2	1.388	0.407	0.381	0.207	0.086	70.677	72.55	85.09	93.80
8	NL3E3	0.865	0.675	0.564	0.472	0.442	21.965	34.80	45.43	48.90
9	NL3E4	1.532	0.921	0.473	0.195	0.2	39.883	69.13	87.27	86.95
10	NL3E5	0.572	0.971	0.337	0.246	0.011	-69.755	41.08	56.99	98.08
11	NC3E1	1.36	0.978	0.256	0.265	0.224	28.088	81.18	80.51	83.53
12	NC3E2	1.308	1.444	0.49	0.301	0.187	-10.398	62.54	76.99	85.70
13	NR3E1	1.503	1.439	0.583	0.299	0.239	4.258	61.21	80.11	84.10
14	NR3E3	1.503	1.133	0.708	0.123	0.239	24.617	52.89	91.82	84.10
15	PdC3E2	1.182	0.576	0.392	0.201	0.128	51.269	66.84	82.99	89.17
16	PdC3E3	1.39	1.442	0.526	0.197	0.100	-3.741	62.16	85.83	92.81
17	PdC3E4	1.503	1.133	0.708	0.123	0.239	24.617	52.89	91.82	84.10
18	PdS3E1	1.392	1.031	0.602	0.346	0.206	25.934	56.75	75.14	85.20
19	PdS3E2	1.709	1.440	0.728	0.537	0.432	15.740	57.40	68.58	74.72
20	RgL3E4	1.503	1.133	0.708	0.123	0.239	24.617	52.89	91.82	84.10
21	RgC3E2	1.177	1.087	0.383	0.215	0.251	7.647	67.46	81.73	78.67
22	JC3E1	0.908	0.711	0.671	0.417	0.309	21.696	26.10	54.07	65.97
23	JC3E2	1.276	1.288	0.738	0.34	0.559	-0.940	42.16	53.35	56.19
24	PL3E2	1.331	1.092	0.852	0.703	0.446	17.956	35.99	47.18	66.49
25	CC3E3	1.554	0.734	0.876	0.259	0.164	52.767	43.63	83.33	89.45
26	VaR3E1	0.284	0.234	0.220	0.216	0.123	17.606	24.12	23.94	56.69
27	VaL3E1	0.62	0.324	0.336	0.311	0.118	47.742	45.81	49.84	80.97
28	VaS3E1	1.859	1.254	0.381	0.242	0.1	32.544	79.51	86.98	94.62
29	KL3E1	1.103	1.635	0.633	0.373	0.228	-48.232	42.61	66.18	79.33
30	KL3E2	1.554	0.734	0.876	0.259	0.164	52.767	43.63	83.33	89.45
31	KS3E1	1.177	1.087	0.383	0.215	0.251	7.647	67.46	81.73	78.67
32	KS3E2	1.345	1.528	0.336	0.195	0.144	-13.606	75.02	85.50	89.29
33	LS3E1	1.133	1.575	0.852	0.439	0.216	-39.011	24.80	61.25	80.94
34	LS3E2	1.499	1.205	0.612	0.351	0.025	19.613	59.17	76.58	98.33
35	LS3E3	1.388	0.845	0.689	0.207	0.086	39.121	50.36	85.09	93.80
36	LL3E1	0.908	0.711	0.671	0.417	0.309	21.696	26.10	54.07	65.97

4.2.3 Quantitative evaluation of plant growth promoting traits of endophytic bacteria

4.2.3.1 Phosphate solubilization : All the identified efficient bacterial isolates (36) showed distinct phosphate-solubilizing abilities in quantitative study varying in the range of 68 to 573 µg/mL. Maximum release of soluble P_2O_5 was shown by the isolates KL3E1 (573µg/mL), KL3E2 (568µg/mL), LS3E3 (512µg/mL), NC3E2 (468µg/mL) and CC3E3 (461µg/mL) while the isolate VR1E1 was found to release 68 µg/mL of soluble P_2O_5 recording lowest among all solubilizing isolates (Table 4.8)

The phosphorus available in soil is cannot to be utilized by plants as most of inorganic phosphorus is bound to minerals like calcium, iron and aluminium forming tricalcium phosphate $(Ca_3PO_4)_2$, aluminium phosphate (Al_3PO_4), iron phosphate (Fe_3PO_4), etc., or immobilized in phytate hence although concentrations of phosphorus is high in soil it is always limited for plant growth. Thus to improve crop production the fixed mineral phosphates in soil and applied phosphates need to be solubilized by phosphate solubilizing bacteria. Amid different ways of mineral phosphate solubilization, the production of organic acids is the main mechanism (Khan *et al.* 2009). In present study most of the significant phosphate solubilizers belong to enterobacteriacea family based on biochemical characterization and the results are in similar with investigations of de Abreu *et. al.* (2017) who demonstrated genus *Bacillus* and different species of Enterobacteriaceae as efficient phosphate solubilizers.

Many studies have reported endophytic bacteria as potential phosphate solubilizers, Panigrahi and Rath (2019) isolated *Kosakonia cowanii* MK834804 from medicinal plant *Aegle marmelos* and found to produce many organic acids into the medium and reported as potential phosphate solubilizing endophytic bacteria. Noori *et al.* (2018) have isolated non rhizobial endophytic bacteria *K.cowanii* and *K. pneumonia* from the nodules of alfalfa and found to be efficient phosphate solubilizers and IAA producers. The bacterial strains *Enterobacter, Microbacterium, Pantoea, Pseudomonas* isolated from corn sap were found to be potential phosphate solubilizers (Ali *et al.*, 2018). From wheat endophytic bacterial genera *Pantoea*, *Pseudomonas, Enterobacter*, *Burkholderia*, *Azotobacter* and *Citrobacte r*were isolated and reported to have phosphate solubilizing property (Verma e*t al.*, 2015).

Four endophytic isolates P18, P31, P35, and P42 isolated from roots of potato plant were found to have solubilization capacity of rock phosphate in the range of 240-350 μg/mL (Dawwam *et al.*, 2013). In studies of Zhu and She (2018) *Pantoea* sp. Strain BG25 an endophytic bacteria isolated from *Ammodendron bifolium* exhibited the highest phosphate solubilization ability at the level of 699.16 μg/mL, and the lowest solubilization ability of 68.05 μg/mL was detected in *Klebsiella* sp. strain AG48.

4.2.3.2 Potassium solubilization : In present study 50 % of the maize endophytic bacterial strains were found to have capability of potassium solubilization. The isolates were found to have potassium solubilization in the range of 9 to 39 mg/L quantitatively. Eighteen isolates were shown potassium solubilization capacity of which maximum was shown by PdS3E1 (39 mg/L) and JC3E2 (38 mg/L) while minimum K solubilization was observed in the isolate KL3E1 (9 mg/L) (Table 4.8)

The major quantity of potassium in soil is present in various forms like feldspar, muscovite, biotite, orthoclase, illite and vermiculite which are not available for plant uptake. The bioavailability of K to plants may be increased by microbes through various mechanisms such as mineralization, decomposition and release of nutrients (Rajawat *et al.*, 2020). In the present investigation bacterial strains PdS3E1 and JC3E2 isolated from maize stem and kernel were found to have maximum potassium solubilization capacity however potassium solubilizing ability and number of K solubilizers are found to be less (50%) as compared to phosphate solubilizers. Similar results were reported by Marag and Suman (2018) that maize endophytic bacteria were with less K solubilizing ability as compared to P and Zn solubilization.

Results obtained in present study are in correlation with Parmar *et al.* (2016) isolated potassium solubilizers using mica from the soil samples of maize rhizosphere, on Aleksandrov medium and the highest solubilization (46.52 µg mL^{-1}) was observed in isolate KSB-1 *Bacillus licheniformis* followed by KSB-3 (42.37 µg mL^{-1}) which was identified as *Bacillus subtilis*.

Yaish *et al.* (2015) demonstrated the potential of endophytic bacteria from date palm (*Phoenix dactylifera* L.) seedling roots for K and Zn solubilization and for their ability to help plants grow under saline conditions, of which *Bacillus* *endophyticus* strain 2DT, *Acinetobacter pittii* , *Achromobacter* sp. were able to solubilize K .

Similarly, in various studies different K-solubilizing endophytic bacteria have been isolated from different host plants and the efficient K solubilizing bacterial genera reported are *Bacillus, Klebsiella, Enterobacter, Microbacterium, Alcaligenes, Achromobacter* and *Acinetobacter* (Kour *et al.* 2020c., Verma *et al.* 2019 and Verma *et al.* 2014).

4.2.3.3 IAA production : IAA is an important auxin required for plants to control important physiological processes including cell enlargement, division and tissue differentiation. IAA production among bacterial isolates in the present study varied significantly. Minimum production of IAA was shown by the isolate PL3E2 producing 12 µg/mL while maximum production of IAA was shown by the isolates LL3E1 (165µg/mL), RgL3E4 (163µg/mL), PdS3E2 (149µg/mL), PdC3E2 and JC3E2 producing 134 µg/mL of IAA (Table 4.8). Among all the identified efficient isolates more than 75% were recorded as significant IAA producers in current work and bacteria colonizing leaf and stem were found to be elite producers of IAA.

Similar results were obtained by Beneduzi *et al.* (2013) as they found 71% of endophytic bacteria isolated from sugarcane stem and root were positive for IAA production in the range of 0.1-264 µg mL^{-1} in 3 days. Previous findings with IAA producing bacteria includes, *Methylobacterium* colonizing leaf can produce IAA, which in turn increases plants IAA concentrations and promotes plant growth (Ivanova *et al.* 2001., Madhaiyan *et al.* 2006 and Zhang *et al.* 2021). The genera *Klebsiella, Pantoea, Kocuria* showed the highest abilities to produce IAA, ranging from 17.09 to 58.66 µg mL^{-1} (Zhu and She, 2018). The endophytic bacterial genera *Bacillus, Microbacterium, Psychrobacillus* and *Lysinibacillus* have been isolated from maize roots and reported to have ability to produce IAA (Yu *et al.*, 2016).

In the current study bacterial isolates have shown wide range of IAA production from12 µg/mL to 165µg/mL. The level of IAA biosynthesis in bacteria have been shown to affected by various genetic factors like the position of auxin biosynthesis genes with numerous copies which may alter IAA production (Patten and Glick, 1996) and many pathways present in a single bacterial species which could be induced differently (Manulis *et al.,* 1998). Hence, the wide range of IAA production

(12-165 μg/mL) in present study was observed because different isolates are having different genes or pathways for the IAA production.

4.2.3.4 Siderophore production : The production of siderophores by endophytic bacteria helps the plants to acquire bioavailable iron by process of sequesteration and inhibits the growth of pathogens by competing for nutrients and thus limiting the iron availability to them. In the current investigation the multitrait plant growth promoting isolates were evaluated quantitatively for siderophore production and 21 bacterial isolates were found to have ability to produce more than 50% siderophore forming units. Efficient siderophore producing isolates were observed as VaR3E1, JC3E2 and KS3E1 with 93%, 92% and 91% of siderophore forming units respectively (Table 4.8).

In the study of Singh *et al.* (2020) quantitatively siderophore production was found highest in *Burkholderia arboris* CSRS12 (92.29%) followed by *Acinetobacter baumannii* (TA1PS with 65.54% unit of siderophore production. Ten endophytic bacteria out of 14 isolated from roots and nodules of *Pisum sativum* and *Cicer arietinum* plants were quantitatively analyzed for siderophore production and found to have above 65% siderophore production capacity in of CAS-liquid assay (Maheshwari *et al.,* 2019).

The endophytic bacterial genera reported as iron sequesters in previous studies are *Pantoea, Klebsiella, Enterobacter, Pseudomonas* isolated from corn sap (Ali *et al.,* 2018), *Bacillus licheniformis K. pneumonia* and *Pseudomonas* sp. isolated from *P.nigrum* (Jasim *et al.,* 2013), *Pseudomonas* isolated from banana (Catherine *et al.,* 2012).

4.2.3.5 Exopolysaccharides : The exopolysaccharide production was recorded in the range of 6 to 65 mg/mL by 29 isolates. The isolates LS3E3, LS3E1, VaL3E1 and KS3E2 have produced 65, 61, 61 and 60 mg/mL of EPS respectively (Table 4.8).

EPS acts as a microenvironment that has water retention capability thus protecting bacterial cells from exposure to water stress (Naseem *et al.,* 2018) and under unfavourable environmental conditions the plants may be protected by EPS produced by bacterial strains (Silambarasan *et al.,* 2019 b). PGP *Pseudomonas* strains were found to produce copious amount of exopolysaccharide under stress (Sandya *et al.*, 2010).

Vardharajula *et al.* (2011) reported similar results with three *Bacillus* sp. They were studied for the ability to tolerate osmatic stress and produce EPS under different water potentials. EPS production in all the three *Bacillus* sp. strains increased with increasing water stress. Among the isolates, strain HYD-17 showed the highest production of EPS. Maize endophytic bacteria *Burkholderia phytofirmans* (PsJN) and *Enterobacter* sp. (FD17) were reported to produce exopolysaccharides (Akhtar *et al.*, 2015)

4.2.3.6 ACC deaminase Activity : The presence of ACC deaminase activity in bacteria was found on the basis of ability of bacteria to utilize ACC as a source of nitrogen. On the basis of preliminary qualitative plate assay 2 isolates (RgC3E2, JC3E1) have shown high growth, 16 isolates have shown moderate growth, 14 isolates have shown low growth and 4 strains were found to have no growth (Table 4.8, Plate 4.4b).

In the conditions of drought, salinity and pathogenicity, essential plant growth metabolite ethylene level increases, resulting in negative effects of epinasty and premature senescence thus reducing crop yields (Bharti and Barnawal, 2018). Plant growth promoting bacteria with ACC deaminase activity regulates ethylene levels by metabolizing ACC which is an immediate precursor for ethylene biosynthesis (Dar *et al.*, 2018). According to few studies for endophytic bacteria ACC deaminase activity plays pivotal role in its ability to stimulate plant growth (Hardoim *et al.*, 2008).

We found more than 50% of the isolates were positive for ACC deaminase activity while the isolates RgC3E2 and JC3E1 were elite producers based on the qualitative evaluation. The previous studies reported many genera which showed positive result for ACC deaminase activity including *Bacillus megaterium* and *Pantoea agglomerans* (Silambarasan *et al.*, 2019a), *Pseudomonas stutzeri* (Han *et al.*, 2015), *Klebsiella oxytoca*and, *Klebsiella variicola* (Zheng *et al.*, 2014) salt tolerant endophytic bacteria *B.aryabhattai* (Siddikee *et al.*, 2010) and *B.megaterium* (Chinnaswamy *et al.*, 2018). Xu *et al.* (2014), reported that *Bacillus subtilis* (HYT-12-1) having PGP traits along with ACC deaminase activity (112.02 nmol α-ketobutyrate mg^{-1} protein h^{-1}).

Table 4.8. Evaluation of plant growth promoting traits of endophytic bacterial isolates of maize

S. No	Isolate code	Plant tissue	N_2 fixation	Soluble P_2O_5 (µg/mL)	Soluble K_2O (mg/L)	IAA (µg/mL)	Percent Siderophore Production (%)	EPS (mg/mL)	ACC Deaminase	HCN
1	AR3E2	Root	+	137	–	58	–	19	++	–
2	AlR2E5	Root	+	73	–	54	–	22	++	–
3	GC3E2	Kernel	+	116	–	67	48	–	++	–
4	BS2E1	Stem	–	76	–	17	54	–	++	–
5	VS1E1	Stem	–	207	12	21	11	24	++	–
6	VR1E1	Root	+	68	14	30	65	12	+	–
7	NL3E2	Leaf	+	218	32	68	52	26	+	–
8	NL3E3	Leaf	+	354	–	74	76	36	+	–
9	NL3E4	Leaf	+	326	–	94	–	37	–	–
10	NL3E5	Leaf	+	147	–	15	–	–	++	–
11	NC3E1	Kernel	–	94	–	89	9	6	+	–
12	NC3E2	Kernel	+	468	–	54	87	32	+	–
13	NR3E1	Root	+	140	–	68	64	53	++	–
14	NR3E3	Root	–	134	–	24	–	55	++	–
15	PdC3E2	Kernel	–	114	–	134	–	58	++	–
16	PdC3E3	Kernel	+	147	32	81	59	–	–	–
17	PdC3E4	Kernel	–	126	20	38	–	22	++	–
18	PdS3E1	Stem	+	281	39	87	86	41	–	–
19	PdS3E2	Stem	+	289	–	149	67	–	+	–
20	RgL3E4	Leaf	++	321	18	163	91	48	++	–
21	RgC3E2	Kernel	+	268	–	145	21	60	+++	–
22	JC3E1	Kernel	+	104	36	26	64	56	+++	–
23	JC3E2	Kernel	+	286	38	134	92	42	++	–
24	PL3E2	Leaf	+	138	21	12	38	42	+	–
25	CC3E3	Kernel	+	461	16	17	58	14	+	–
26	VaR3E1	Root	++	230	24	75	93	57	++	–
27	VaL3E1	Leaf	–	248	16	64	74	61	+	–
28	VaS3E1	Stem	+	146	26	23	–	51	++	–
29	KL3E1	Leaf	+	573	9	24	85	–	+	–
30	KL3E2	Leaf	–	568	27	67	–	30	–	–
31	KS3E1	Stem	++	214	21	87	91	43	+	–
32	KS3E2	Stem	+	112	–	21	–	60	+	–
33	LS3E1	Stem	++	243	–	86	82	61	++	++
34	LS3E2	Stem	+	149	–	117	65	30	+	–
35	LS3E3	Stem	++	512	34	128	87	65	++	–
36	LL3E1	Leaf	++	347	–	165	75	–	+	–

IAA-Indole Acetic Acid, EPS- Exopolysaccharides, –No, + Low, ++ Medium, +++ High

4.2.3.7 HCN production : HCN production is considered as indirect mechanisms of growth promotion as it provides antifungal activity. In the present study endophytic bacterial strain LS3E1 is the only isolate which has shown positive result for HCN production (Plate 4.4a).

Few bacteria inhibit the disease development indirectly by synthesizing HCN, there by strengthening the host's disease resistance mechanism (Whipps *et al.,* 2001). Previous studies reported HCN production by *Pseudomonas* spp., out of 16 endophytic bacteria isolated from roots of *Cassia tora*, three bacterial strains namely *Pseudomonas* sp. CT5,*P. putida*, CT4 and *A. tumefaciens* CT2 produced HCN (Kumar *et al.,* 2014). Similarly, out of seven bacterial strains isolated from medicinal plant *Mussaenda roxburghii*, two isolates namely *Pseudomonas* sp. and *Acinetobacter* sp. produced HCN (Pandey *et al.,* 2015). These reports indicate that endophytic bacteria have the ability to produce HCN and helping in inhibiting plant disease development. Maize endophytic bacteria *Pseudomonas aeruginosa*was estimated to produce ammonia, siderophore, HCN, IAA, cytokinins and gibberellins (Sandhya *et al.,* 2017).

4.2.4 Evaluation of efficient stress mitigating and plant growth promoting endophytic bacteria for special traits

Based on the osmotolerance and plant growth promoting abilities 16 efficient bacterial isolates were selected, characterized molecularly (Sec 4.3) and evaluated for special traits including seed germination, seed vigor, *in-vitro* antagonistic activity against pathogenic fungi and intrinsic antibiotic sensitivity.

4.2.4.1 Seed vigor :

Many of the isolates from current investigation have improved seedling length, % germination and seed vigor index compared to control (Plate 4.5a). Maximum root length was obtained in seedlings treated with the bacterial isolates *Pantoea dispersa* (KS3E1) measuring 38.33 cm and *Priestia megaterium* (PdS3E1) measuring 38.27 cm of root length which are significantly higher than control (no inoculum) having root length of 30.47 cm. significantly higher shoot length was obtained in seedlings treated with *Pantoea dispersa* (KS3E1) measuring 19.83 cm followed by *Methylorubrum populi* (LL3E1) with 19.40 cm of shoot length. Maximum germination per cent was shown by *Bacillus licheniformis* (VaR3E1) recording 95.33% followed by *Priestia megaterium* (PdS3E1) and *Klebsiella pneumonia* (RgL3E4) recording 94.67% which are significantly higher than the control (88%). The seed vigor index is significantly higher for *Pantoea dispersa* (5390.8) followed by *Priestia megaterium* (5334.9) and *Bacillius licheniformis* (5265.2). The per cent germination and seed vigor index were recorded less in seedlings treated with *Pseudomonas aeruginosa* (Table 4.9).

Table 4.9. Effect of maize seed bacterization by different endophytes on germination and seed vigor

Isolate code	Isolate Name	Root length (cm)	Shoot length (cm)	% Germination	Seed Vigor Index
AR3E2	*Gordonia hongkongensis*	30.17^{ef}	16.33^{ef}	90.00^{abcd}	4185^{fgh}
VR1E1	*Microbacterium hydrothermale*	29.93^{f}	19.30^{abc}	88.67^{bcd}	4365^{defg}
NL3E3	*Kosakonia radicincitans*	32.30^{d}	17.17^{de}	86.00^{d}	4256^{efgh}
NC3E2	*Kosakonia cowanii*	31.30^{def}	18.37^{abcd}	88.00^{cd}	4370^{defg}
PdS3E1	*Priestia megaterium*	38.27^{a}	18.07^{cd}	94.67^{ab}	5335^{ab}
PdS3E2	*Priestia aryabhattai*	30.23^{ef}	18.07^{cd}	92.67^{abc}	4473^{def}
RgL3E4	*Klebsiella pneumoniae*	27.50^{g}	14.23^{gh}	94.67^{ab}	3949^{gh}
JC3E2	*Priestia megaterium*	31.97^{de}	17.93^{cd}	94.00^{abc}	4688^{cde}
PL3E2	*Priestia aryabhattai*	27.77^{g}	14.97^{fg}	90.00^{abcd}	3846^{h}
CC3E3	*Cellulosimicrobium funkei*	34.17^{c}	12.83^{h}	93.33^{abc}	4389^{defg}
VaR3E1	*Bacillus licheniformis*	36.07^{b}	19.17^{abc}	95.33^{a}	5265^{ab}
KL3E1	*Kosakonia cowanii*	34.47^{bc}	18.17^{bcd}	94.00^{abc}	4947^{bc}
KS3E1	*Pantoea dispersa*	38.33^{a}	19.83^{a}	92.67^{abc}	5391^{a}
LS3E1	*Pseudomonas aeruginosa*	32.23^{d}	19.21^{abc}	76.67^{e}	3992^{gh}
LS3E3	*Klebsiella pneumoniae*	31.63^{def}	19.23^{abc}	94.00^{abc}	4784^{cd}
LL3E1	*Methylorubrum populi*	34.63^{bc}	19.40^{abc}	93.33^{abc}	5044^{abc}
CONTROL	No inoculum	30.47^{def}	17.30^{de}	88.00^{cd}	4204^{fgh}
CD		1.74	1.43	5.60	393
CV		3.23	4.88	3.69	5

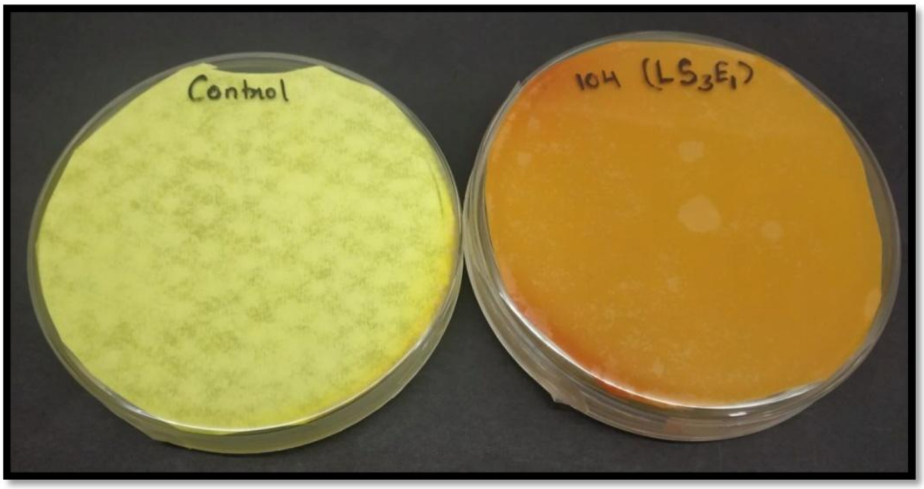
Values in each column are means of three replications compared using Duncans Multiple Range Test(DMRT). Same letters in the column are not significantly different between treatments at $P < 0.05$

Ullah *et al.,* (2017) has reported that improved root length and plant density was observed when inoculated with endophytic bacteria via various mechanisms including the production of plant hormones, ammonia, making bioavailability of nutrients and antagonistic action to phytopathogens. Inoculation of endophytic bacteria has reported increase germination rate by 20-40% in maize seeds and highest of 40% increase was given by *Enterobacter* sp. FD 17 (Naveed *et al.,* 2014a).

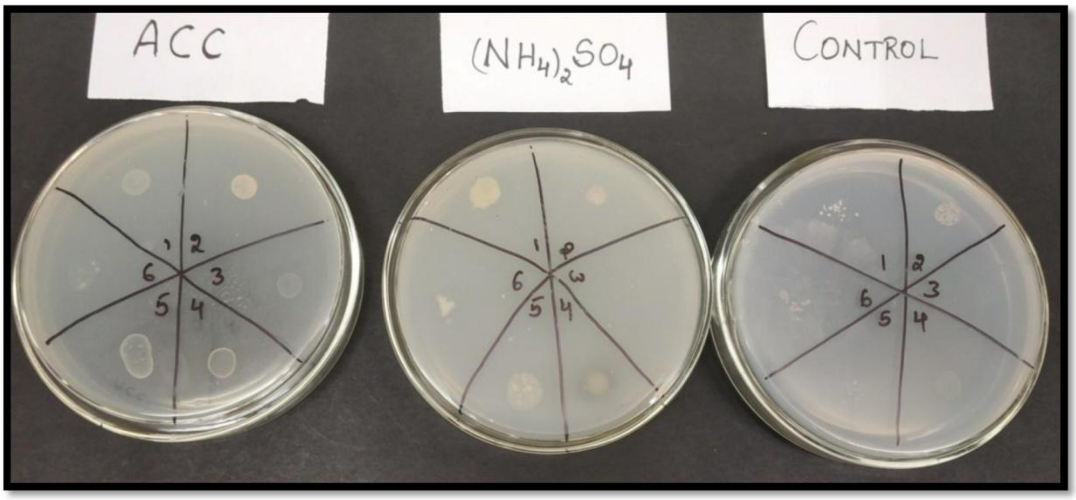
Seedling vigor, germination percentage and plant biomass were reported to get enhanced when the seeds were treated with *B. amyloliquefaciens* EPP90 (Kushwaha *et al.,* 2020). Inoculation of endophytic bacterium *Herbaspirillum* spp at the rate of 10 to 20% (v/v) increased seed vigor rate and total seed germination rate 80-95% and 90-100%, respectively (Briatia *et al.,* 2016). The increased seedling length and germination per cent may be due to the production of IAA by endophytic bacteria. Though many isolates from our study improved seedling length and % germination but further investigation is needed for tracking influence of isolates under given water potential against seedling growth.

4.2.4.2 Antagonistic activity under i*n-vitro* conditions :

Efficient 16 endophytic bacterial isolates were further tested for their antagonistic activity towards pathogenic fungi of maize i.e., *Rhizoctonia solani, Fusarium oxysporium* and *Exserohilum turcicum*. Highest percent of mycelium inhibition of *Rhizoctonia solani* was done by the bacterial isolates *Kosakonia radicincitans* (NL3E3) with 96% and *Pseudomonas aeruginosa* (LS3E1) with 96% followed by *Klebsiella pneumonia* (RgL3E4) inhibiting 95% of fungal mycelium. The isolate with less antagonistic activity against *Rhizoctonia solani* was found to be *Gordonia hongkongensis* (AR3E2) with 8% of mycelium inhibition. Highest percent of mycelium inhibition of *Fusarium oxysporium* was done by the bacterial isolates *Pseudomonas aeruginosa* (LS3E1) with 92% followed by *Bacillus licheniformis* (VaR3E1) with 89% while least percent of mycelia inhibition against *Fusorium oxysporium* was shown by *Microbacterium hydrothermale* (VR1E1) with 6%. The maximum atagonistic activity against pathogenic fungi *Exserohilum turcicum* was shown by the bacterial isolates *Bacillus licheniformis* (VaR3E1) with 84% followed by *Klebsiella pneumonia* (LS3E3) with 62% of mycelium inhibition while lowest per cent of mycelial inhibition was shown by *Cellulosimicrobium funkei* (CC3E3) with 6% against *Exerohilum turcicum* (Table 4.10).



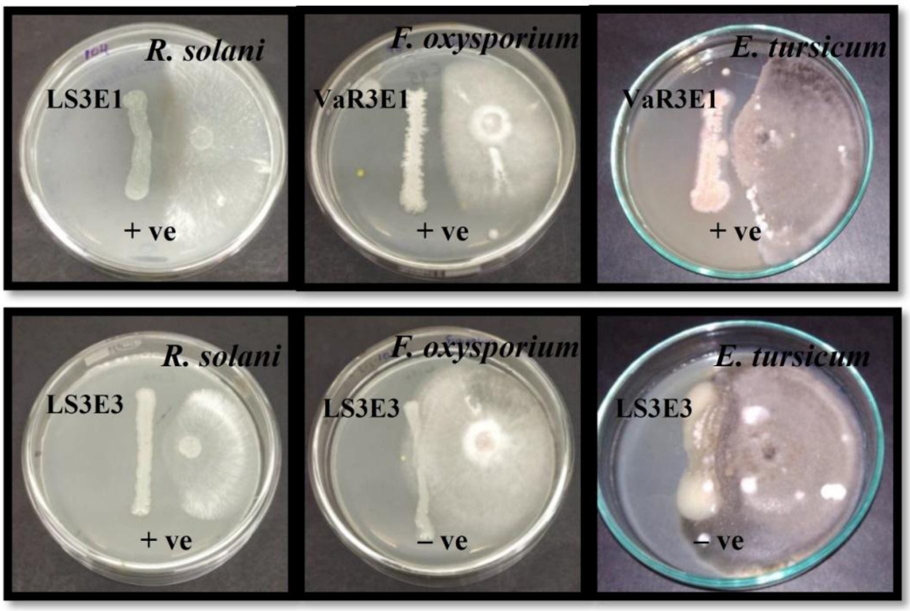
a) HCN Production



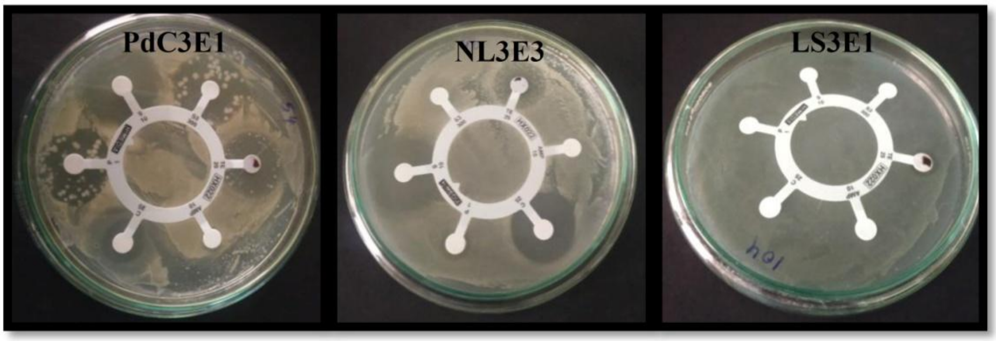
b) ACC deaminase Activity

Plate. 4.4. Plant growth promoting traits of endophytic bacterial isolates

a) Seedlings of endophytic bacteria treated maize seeds
(Isolate numbers – S.No of isolates in table 4.3)



b) Antagonistic Activity



c) Antibiotic Sensitivity

Plate. 4.5. Evaluation of endophytic bacteria for special traits

Bacterial isolates *Bacillus licheniformis* and *Pseudomonas aeruginosa* have shown antagonism effect for the three tested pathogenic fungi (Plate 4.5b). Isolates *Kosakonia radicincitans* and *Kosakonia cowanii* were antagonistic to *Rhizoctonia solani* and *Fusarium oxysporium* while the strains of *Klebsiella pneumoniae* were antagonistic to *Rhizoctonia solani* and *Exserohilum turcicum*. The bacterial isolates *Priestia megaterium* (82% and 74%), *Priestia aryabhattai* (76% and 72%) and *Methylorubrum populi* (71%) were found antagonistic to only *Rhizoctonia solani*.

In present study *Bacillus licheniformis* and *Pseudomonas aeruginosa* were found to have antagonistic activity towards all the three tested pathogenic fungi with maximum per cent of mycelium inhibition in the range of 84-95%. The results are similar with the findings of Esikova *et al.,* (2021) as the genera *Bacillus* and *Pseudomonas* were characterized by high activity against fungal phytopathogens. In current invetigation the efficient antagonistic bacteria were isolated from root and stem of maize and the results were comparable with Mugiastuti *et al.* (2020) that the endophytic *Bacillus* sp, isolated from maize root (BK.A1; BK.A3; PP.A5) and stem (PPD.B2) can suppress the growth of *R.solani* by more than 50% and considered to be prominant.

Similarly *Bacillus* strains isolated from pearl millet niche showed antagonistic activity by inhibiting mycelium growth of *Sclerotium rolfsii* (45 – 78%) and *Rhizoctonia solani* (47 – 80 %) (Kushwaha *et al.,* 2020) and strong antagonistic activity was exhibited by maize endophytic *Bacillus* spp. against the pathogen *Fusarium moniliforme* (Gond *et al.,* 2015).

The efficient antagonistic bacteria may induce the systemic resistance against pathogenic fungi in host plants and can be applied as biopesticides. The results were in positive correlation with the findings of Pandey *et al.* (2012) that *Pseudomonas aeruginosa* PW09 isolated from wheat found to trigger an induced systemic resistance in cucumber plants infected with fungal pathogen *Sclerotium rolfsii*. Similarly, 4 endophytic bacteria isolated from maize plant niche characterized successfully and found have the potential to be developed as biopesticides to control maize disease, especially *R. solani* and *Pantoea* sp. (Prihatiningsih and Soesanto, 2020).

Soybean root endophytes *Pseudomonas otitidis*, *Pseudomonas* sp and *Bacillus cereus* exhibited antagonistic activity against *F. oxysporum*, inhibiting fungal growth by 97%, 98% and 98.5% respectively (Dubey *et al.,* 2021). Several maize endophytic bacteria were reported previously for having antagonistic properties against pathogenic fungi (Naveed *et al.,* 2014a; Bodhankar *et al.,* 2017; Ali *et al.,* 2018; Rana *et al.,* 2021).

Table 4.10. Antagonistic activity of endopytic bacterial isolates against fungal pathogens

S.No	Isolate code	Isolate Name	% inhibition of mycelial growth		
			Rhizoctonia solani	***Fusarium oxysporium***	***Exserohilum turcicum***
1	AR3E2	*Gordonia hongkongensis*	8	12	10
2	VR1E1	*Microbacterium hydrothermale*	10	6	7
3	NL3E3	*Kosakonia radicincitans*	96	68	8
4	NC3E2	*Kosakonia cowanii*	75	72	16
5	PdS3E1	*Priestia megaterium*	82	7	12
6	PdS3E2	*Priestia aryabhattai*	76	7	10
7	RgL3E4	*Klebsiella pneumoniae*	95	15	54
8	JC3E2	*Priestia megaterium*	74	12	10
9	PL3E2	*Priestia aryabhattai*	72	10	12
10	CC3E3	*Cellulosimicrobium funkei*	10	8	6
11	VaR3E1	*Bacillus licheniformis*	94	89	84
12	KL3E1	*Kosakonia cowanii*	92	74	15
13	KS3E1	*Pantoea dispersa*	12	10	8
14	LS3E1	*Pseudomonas aeruginosa*	96	92	56
15	LS3E3	*Klebsiella pneumoniae*	94	17	62
16	LL3E1	*Methylorubrum populi*	71	12	23
17	Control	No inoculum	0	0	0

4.2.4.3 Intrinsic antibiotic sensitivity : Out of all (16) studied effective endophytic bacteria many were found resistant to two antibiotics Ampicillin and Penicillin G (Table 4.11, Plate 4.5c). The isolates *Kosakonia radicincitus* and *Kosakonia cowanii* were found resistant to Ampicillin, Penicillin G and Sulphatriad recording more number of antibiotics than the remaining isolates. Thus, *Kosakonia* strains of maize endophytes studied in present research *K. radicincitans* and *K.cowanii* were assumed as the potential isolates to compete against other microbes as the antibiotic resistant strains have the ability to compete with other strains.

Invitro screening of maize endophytic bacteria *Enterobacter cloacae* showed resistance against 15 different antibiotics (Maqbool *et al.,* 2021). Endophytic bacteria FTR isolated from maize niche was resistant to eight antibiotics out of 19 tested and reported as the most potential endophyte to compete against other microbes (Sandhya *et al.,* 2017). Broad spectrum resistant strains have been reported from the palm tree endophytes *Enterobacter cloacae* subsp. *Cloacae* and *Acinetobacter pitti* against many antibiotics (Yaish *et al.,* 2015).

Table 4.11. Assay of intrinsic antibiotic sensitivity of endophytic bacterial isolates

S. No	Isolate code	Ampicillin (10 mcg)	Chloramphenicol (25 mcg)	Penicillin-G (0.6 mcg)	Streptomycin (10 mcg)	Sulphatriad (300 mcg)	Tetracyclin (25 mcg)
1	AR3E2	S	S	R	S	S	S
2	VR1E1	R	S	R	S	S	S
3	NL3E3	R	S	R	S	R	S
4	NC3E2	R	S	R	S	R	S
5	PdS3E1	R	S	S	S	S	S
6	PdS3E2	R	S	S	S	S	S
7	RgL3E4	R	S	R	S	S	S
8	JC3E2	R	R	R	S	S	S
9	PL3E2	R	S	S	S	S	S
10	CC3E3	R	S	R	S	S	S
11	VaR3E1	R	S	R	S	S	S
12	KL3E1	R	S	R	S	R	S
13	KS3E1	R	S	R	S	S	S
14	LS3E1	S	S	S	R	S	R
15	LS3E3	R	S	R	S	S	S
16	LL3E1	S	S	S	R	S	R

R- Resistant (no zone formation) S- Sensitive (zone formed)

4.3 MOLECULAR CHARACTERIZATION OF EFFICIENT STRESS MITIGATING AND PLANT GROWTH PROMOTING ENDOPHYTIC BACTERIAL ISOLATES BY USING 16S rRNA GENE SEQUENCING

4.3.1 Molecular characterization of efficient endophytic bacteria by 16S rRNA gene sequencing

Based on qualitative and quantitative screening of maize endophytic bacteria against osmotolerance and plant growth promotion and biochemical characterization following 16 effective isolates were identified AR3E2, VR1E1, NL3E3, NC3E2, PdS3E1, PdS3E2, RgL3E4, JC3E2, PL3E2, CC3E3, VaR3E1, KL3E1, KS3E1, LS3E1, LS3E3 and LL3E1. Gram's reaction, cell shape and morphological characterstics of efficient bacterial isolates are given table 4.12 and fig.4.2. Most of the isolates are found to be having rod shape cells, colony size ranging from small to large, with round colony shape and entire edge with varied pigmentation and elevation. Further characterization of selected effective isolates at molecular level by 16S rRNA gene sequencing has been carried out. Bacterial DNA was isolated from all selected isolates following published protocols and the 16S rRNA gene was subjected to sequencing. The obtained gene sequences (Appendix-III) were compared with available sequences in the GenBank databases using the NCBI and BLASTn. The nearest match from GenBank data showing ≥99-100 % similarity with the sequences of our isolates was reported (Table 4.13).

Six isolates *Priestia megaterium* strain GAD181_5, *Priestia aryabhattai* strain GAD181_6, *Priestia megaterium strain* GAD181_8, *Priestia aryabhattai* strain GAD181_9, *Bacillus licheniformis* strain GAD181_11, *Pantoea dispersa* strain GAD181_13, *Pseudomonas aeruginosa* strain RZS9, *Methylorubrum populi* GAD181_16 are grouped under phylum Firmicutes. Seven isolates *Kosakonia radicincitans* strain GAD1813, *Kosakonia cowanii* strain GAD1814, *Klebsiella pneumoniae* strain GAD181_7, *Kosakonia cowanii* strain GAD181_12, *Klebsiella pneumoniae* strain DSM 30104, *Pantoea dispersa* strain GAD181_13 and *Pseudomonas aeruginosa* are classified under phylum Proteobacteria. Three endophytic bacterial isolates namely *Gordonia hongkongensis* strain GAD1811, *Microbacterium hydrothermale* strain GAD1812 and *Cellulosimicrobium funkei* strain GAD181_10 are identified as phylum Actinobacteria.

In the present study *Bacillus* and its closely related genus *Priestia* were identified as most dominant group of isolates in maize. Based on the molecular and strong phylogenetic evidence, Gupta *et al.* (2020) has proposed to recognize 17 *Bacillus* species clades, *Priestia gen. nov* is one of those as novel genera based on strong evidence of molecular and phylogenetic research studies. Out of 16 identified bacterial isolates in maize crop in present investigation 5 isolates were belong the genera *Bacillus*. Isolate VaR3E1 was identified as *Bacillus licheniformis*, isolates PdS3E1 and JC3E2 were identified as *Priestia megaterium* (previously *B.megaterium*), isolates PdS3E2 and PL3E2 were identified as *Priestia aryabhattai* (previously *B.aryabhattai*). Similar findings were reported by Bodhankar *et al.* (2017) that the dominant genera of maize seed endophytic bacteria are *Bacillus* affiliated with phylum Firmicutes. In the phyllosphere of maize the most dominant genus identified was *Bacillus* and the species identified were *B. licheniformis*, *B. paralicheniformis*, *B. megaterium*, *B. subtilis*, *B. pumilus*, *B. niabensis* and *B. amyloliquefaciens* (Abadi *et al.*, 2020). Recently, *Priestia aryabhattai* was reported to vary significantly from previously described B*acillus* genus based on characterization of cellular morphology, development cycle and 16S rRNA gene sequence (Esikova *et al.,* 2021).

Table 4.12. Physiological and morphological characters of screened endophytic bacteria

S. No.	Isolate code	Gram's reaction	Cell shape	Colony size	Colony shape	Edge	pigmentation	Elevation
1	AR3E2	-ve	Rods	Small	Round	Entire	Pink	Raised
2	VR1E1	+ve	Rods	Small	Round	Etire	Orange	Flat
3	NL3E3	-ve	Rods	Medium	Round	Entire	Yellow	Raised
4	NC3E2	-ve	Rods	Medium	Round	Entire	Yellow	Raised
5	PdS3E1	+ve	Rods	Large	Irregular	Entire	White	Rhizoidal
6	PdS3E2	+ve	Rods	Large	Irregular	Entire	Cream	Rhizoidal
7	RgL3E4	-ve	Rods	Large	Round	Entire	White	Convex
8	JC3E2	-ve	Rods	Medium	Round	Entire	Cream	Flat
9	PL3E2	+ve	Rods	Medium	Irregular	Irregular	White	Flat
10	CC3E3	+ve	Rods	Small	Round	Irregular	Yellow	Raised
11	VaR3E1	+ve	Rods	Large	Irregular	Undulate	White	Wrinkled
12	KL3E1	-ve	Cocci	Medium	Round	Entire	Yellow	Raised
13	KS3E1	-ve	Rods	Small	Round	Entire	Yellow	Flat
14	LS3E1	-ve	Rods	Small	Round	Entire	Green	Flat
15	LS3E3	-ve	Rods	Large	Round	Entire	White	Convex
16	LL3E1	-ve	Rods	Small	Round	Entire	Pink	Raised

In the current study, pink pigmented bacterial strain *Methylorubrum populi* was isolated from the leaf samples of maize. Green and Ardley (2018) have re-classified *Methylobacterium* species as *Methylorubrum populi comb. nov.* the 16S *rRNA* gene of endophytic bacteria transmitted through rice seed were sequenced, analysed and identified as *Methylorubrum* sp., *Methylobacterium* sp., *Micrococcus* sp. and *Pseudomonas* sp. (Krishnamoorthy *et al.,* 2020)

The isolates identified as *Enterobacteriaceae* family in the current study are NL3E3 (*K. radicincitans*), NC3E2 and KL3E1 (*K. cowanii*), RgL3E4 and LS3E3 (*Klebsiella pneumoniae*). Brady *et al.* (2013) has proposed new genus *Kosakonia* for few species of *Enterobacter* as it is one of the largest genera within the family of *Enterobacteriaceae.* The new genus designated species include *K. radicincitans*, *K. cowanii*, *K. arachidis and K. oryzae.*

In present investigation *Pseudomonas aeruginosa* (LS3E1) was identified as one of the endophytic bacteria isolated from maize stem with many plant growth promoting attributes. Similar pattern of isolation and identification was reported by Rai *et al.* (2007) who identified *Pseudomonas aeruginosa* and *P florescence* as the prevailing group of cultivable endophytes colonizing bacteria in maize stem.

The molecularly identified endophytic bacteria in present study were in correlation with many previous studies, the genera *Bacillus, Pantoea, Klebsiella, Burkholderia* were isolated from maize roots (Ikeda *et al.*, 2013), *Bacillus licheniformis*, *Bacillus aryabhattai*, *Pantoea dispersa, Pantoea cypripedii*, *Pantoea* sp., *Klebsiella* sp., *Klebsiella variicola* and *Agrobacterium larrymoorei* have been isolated from different tissues of composite PC-4 maize plants at different growth stages and identified molecularly by 16S rRNA gene sequence analysis (Marag *et al.*, 2018), *Cellulosimicrobium*, *Stenotrophomonas*, *Enterobacter,* and *Bacillus* were isolated from maize (Ikeda *et al*., 2020)

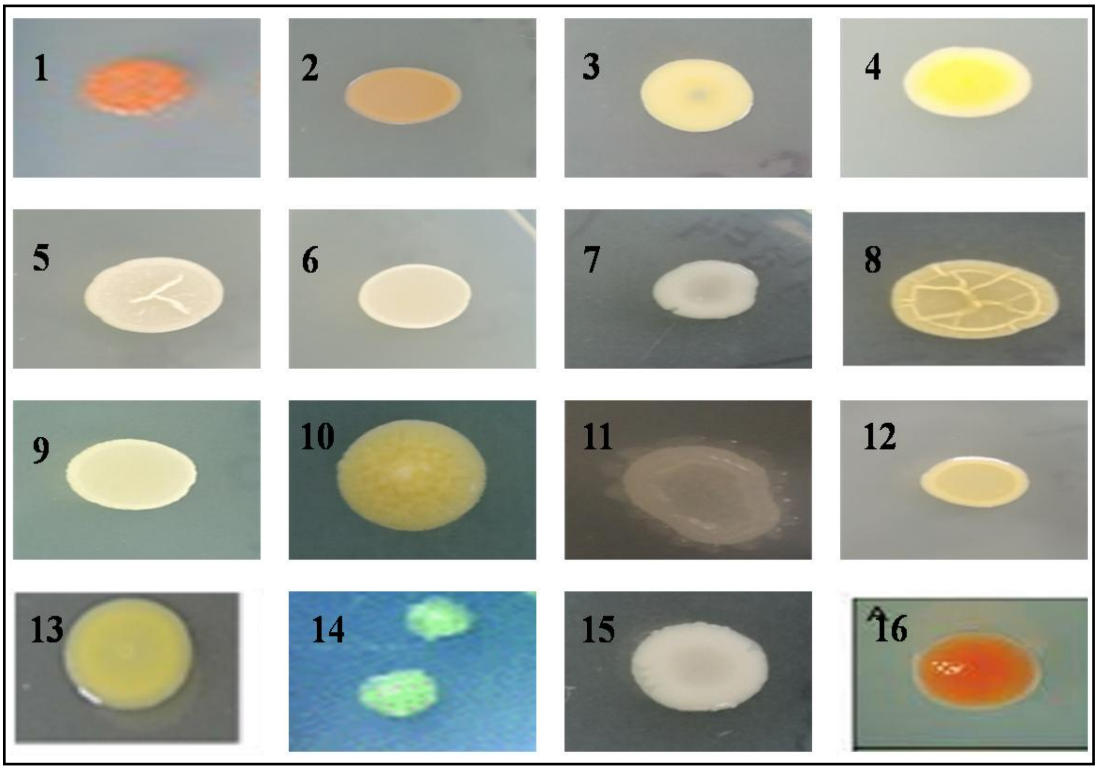


Fig. 4.2. Colony morphology of endophytic bacterial isolates (1-16 isolates according to S. No in Table 4.13)

Table 4.13. 16S rRNA gene sequence based identification of effective stress mitigating and plant growth promoting endophytic bacteria

S. No	**Isolate code**	**Identified bacteria (homologous with sequence run in NCBI database)**	**Similarity**	**NCBI Accession number**
1	AR3E2	*Gordonia hongkongensis strain GAD1811*	99.93%	MW822550
2	VR1E1	*Microbacterium hydrothermale strain GAD1812*	100.00%	MW822553
3	NL3E3	*Kosakonia radicincitans strain GAD1813*	99.93%	MW822555
4	NC3E2	*Kosakonia cowanii strain GAD1814*	99.80%	MW822597
5	PdS3E1	*Priestia megaterium strain GAD181_5*	100.00%	MW880711
6	PdS3E2	*Priestia aryabhattai strain GAD181_6*	100.00%	MW880712
7	RgL3E4	*Klebsiella pneumoniae strain GAD181_7*	99.66%	MW880713
8	JC3E2	*Priestia megaterium strain GAD181_8*	99.93%	MW880714
9	PL3E2	*Priestia aryabhattai strain GAD181_9*	100.00%	MW880715
10	CC3E3	*Cellulosimicrobium funkei strain GAD181_10*	99.79%	MW880716
11	VaR3E1	*Bacillus licheniformis strain GAD181_11*	100.00%	MW880717
12	KL3E1	*Kosakonia cowanii strain GAD181_12*	99.86%	MW880718
13	KS3E1	*Pantoea dispersa strain GAD181_13*	99.59%	MW880719
14	LS3E1	*Pseudomonas aeruginosa*	99.93%	----
15	LS3E3	*Klebsiella pneumoniae* strain DSM 30104	99.60%	MW812251
16	LL3E1	*Methylorubrum populi* GAD18_16	99.86%	MZ484406

4.3.2 Phylogenetic tree analysis

Further classification of bacterial isolates can be observed in phylogenetic tree. Using the UPGMA method evolutionary history was inferred after multiple alignment of data by CLUSTAL X program. The evolutionary history of analyzed taxa was represented by taking 1000 replicates to infer bootstrap consensus tree. The values shown next to the branches are the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test. By using the maximum likelihood composite method the evolutionary distances were computed and the base substitutions per site are 0.050 units (Fig. 4.3). Evolutionary analyses were conducted in MEGA X (Kumar *et al.,* 2018). The final sequences were submitted to GenBank (NCBI) and obtained accession numbers (Table 4.13).

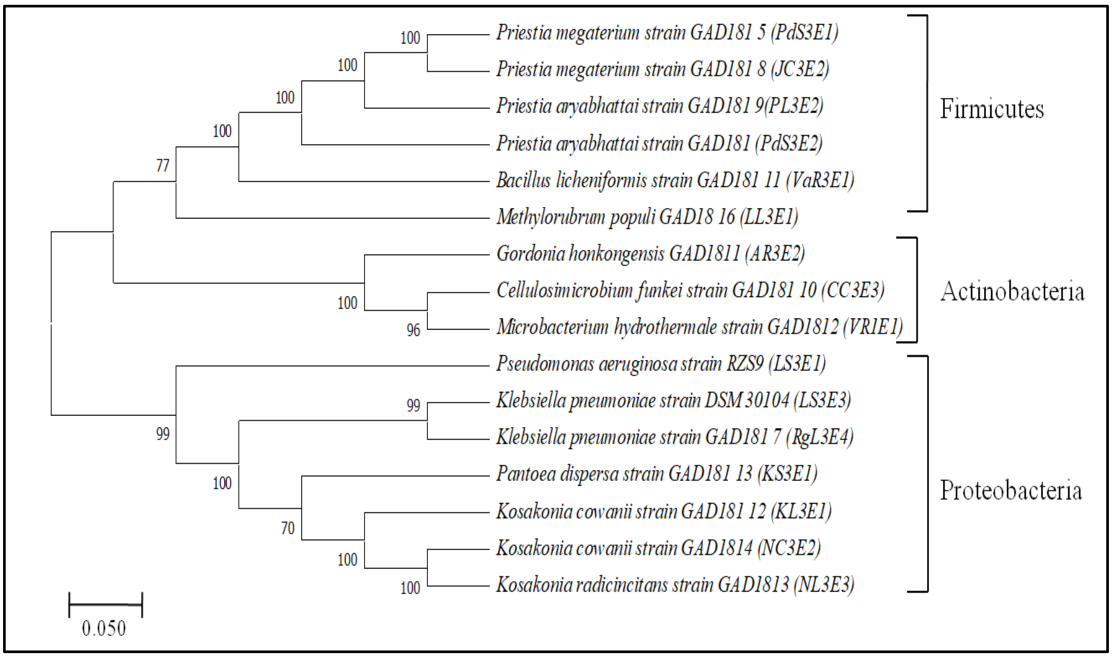


Fig. 4.3. **Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the relationship among strains endophytic bacteria of maize**

In firmicutes of current study *Bacillus* and *Priestia* strains possessed identical 16S rRNA gene sequences sharing 100% sequence similarities while *M. populi* sharing 77% of the sequence similarities with other fimicutes. The two strains of *klebsiella* sharing 99% similarities in 16S rRNA gene sequences while three *Kosakonia* strains possessed 100% sequence similarities. *P.dispersa* shared 70% sequence similarity with the all *Kosakoni*a strains. *Pseudomonas* share 99% of 16S rRNA sequence similarity with the other proteobacteria investigated in current study.

In actinobacteria *C. funkei* and *M. hydrothermale* possessed 96% sequence similarities while *G. hongkongensis* possessed 100% sequence similarity with other two actinobacteria (Fig. 4.3). Diversed endophytic bacterial species have been isolated from a single plant. The diversity in isolated endophytes was influenced by different factors that include host specificity, plant age, type of tissue and geographical distribution (Kobayashi and Palumbo, 2000).

4.4 COMPATIABILITY AND ENDOPHYTIC COLONIZATION ABILITY OF EFFECTIVE BACTERIAL ISOLATES

The effective osmotolerant and plant growth promoting isolates were further screened based on molecular characterization, antagonistic activity and seed vigor index for development of microbial consortium and selected 7 isolates namely *Kosakonia radicincitans* (NL3E3), *Priestia megaterium* (PdS3E1), *Priestia aryabhattai* (PL3E2) *Bacillus licheniformis* (VaR3E1), *Pseudomonas aeruginosa* (LS3E1), *Klebsiella pneumonia* (LS3E3) and *Methylorubrum populi* (LL3E1) for compatiability and endophytic colonization studies. All isolates were identified to show efficiency in multiple plant growth promoting traits and osmotolerance. Special characteristic abilities of each selected isolate were discussed in detail.

Firstly, *Kosakonia radicincitans* (NL3E3) has recorded 48.90% of decreased growth at higher water potential of -2.70 MPa (40% PEG 600) which is much lesser than other isolates and inhibited the mycelial growth of *R.solani* and *F. oxysporium* at the rate of 96% and 68% respectively there by having antagonistic property and osmotolerance at best. The isolate *Priestia megaterium* (PdS3E1) was selected based on its ability to solubilize potassium at maximum level of 39 mg/L, recorded 86% siderophore unit production, shown significantly higher germination percentage (94.67%) and seed vigor index (5334.9). *Priestia aryabhattai* (PL3E2) was found to excel in exopolysaccharide production (42 mg/mL) and improved germination percentage (90%). The isolate *Bacillus licheniformis* (VaR3E1) has shown lesser decrease in the growth (56.69%) even at higher water potential of -2.70 MPa (40% PEG 600), maximum siderophore units (93%) were produced, efficient in EPS production (57mg/mL), found to have ACC deaminase activity, nitrogen fixing activity, shown significantly higher germination percentage (95.33%) and found antagonistic all the three tested pathogenic fungi *R. solani*, *F. oxysporium* and *E. turcicum* at 94%, 89% and 84% of mycelium inhibition respectively.

The isolate *Pseudomonas aeruginosa* (LS3E1), has shown 82% of siderophore unit production, produced EPS at maximum of 61 mg/mL, found to have nitrogen fixing ability, ACC deaminase activity and HCN production and found antagonistic all the three tested pathogenic fungi *R. solani*, *F. oxysporium* and *E. turcicum* at 96%, 92% and 56% of mycelium inhibition respectively. The isolate *Klebsiella pneumonia* (LS3E3) was found excelled in solubilization of phosphorus at the rate of 512 µg/mL of soluble P_2O_5, 128 µg/mL of IAA production, 87% production of siderophore units, highest production of EPS recording 65 mg/mL, found to have nitrogen fixing ability, ACC deaminase activity and antagonistic to *R. solani* and *E. turcicum* at 94% and 62% of mycelium inhibition respectively. The isolate *Methylorubrum populi* (LL3E1) was found to grow at higher water potential (-2.17 MPa) with lesser per cent decrease in growth (54.07%), shown maximum IAA production (165 µg/mL) ability and higher germination per cent (93.33%).

4.4.1 Compatiability of endophytic bacteria

The selected efficient endophytic bacteria were tested for their compatibility on nutrient agar medium by cross streaking method (Plate 4.6). *Kosakonia radicincitans* (NL3E3) was found compatiable with the isolates *Priestia megaterium*, *Priestia aryabhattai* and *Pseudomonas aeruginosa* while the growth was inhibited by the isolates *Bacillus licheniformis*, *Klebsiella pneumonia* and *Methylorubrum populi*. The endophytic bacterial isolate *Priestia megaterium* (NL3E3) was found compatiable with all the tested isolates except *Pseudomonas aeruginosa* as an inhibition zone was observed around *P. aeruginosa* when streaked on a mat of *P. megaterium* on solid nutrient agar medium. An inhibition zone was observed around *P. aeruginosa* and *M. populi* when streaked on *Priestia aryabhattai* (PL3E3) bacterial lawn. *Bacillus licheniformis* (VaR3E1) was found compatiable with all the tested isolates except for *K. radicintans*. The endophytic bacterial isolate *Pseudomonas aeruginosa* (LS3E1) was found compatiable with only *B. licheniformis* and *K. pneumonia* while inhibiting the growth of all other tested isolates. *Klebsiella pneumonia* (LS3E3) was found compatiable with *P. megaterium, B. licheniformis* and *M. populi.* The pink pigmented methylotrophic bacteria *Methylorubrum populi* (LL3E1) was found compatiable with *P. megaterium, B. licheniformis* and *K. pneumonia* (Table 4.14)

Our results were in accordance with Nagpal *et al.* (2019) who studied the plate assay method for testing compatiability between *Mesorhizobium* strains and endophytic bacteria isolated from chickpea. They found no mutual inhibition between *Mesorhizobium* LGR4 and all endophytes (NE6 NE8, RB-1 andRE8). But, *Mesorhizobium* LGR-5 was observed to be incompatible with nodule endophyte NE6 as it formed inhibition zone and endophytic bacterial isolates RB-1 and NE6 showed mutual inhibition with *Mesorhizobium* isolate LGR-12.

Similarly, Deepa and Mathew (2017) carried compatibility studies of different five bacterial isolates selected from *invitro* screening for consortium development and were subjected to mutual compatibility test by cross streak method. No clearance zone was observed at the juncture of TRB-1 x VSB-1, TRB-1 x EkRB-1 and VSB-1 x EkRB-1 combination, which indicated the compatibility among the isolates.

Our results are in similar with Subramanian *et al.* (2015) who reported that endophytes *Methylobacterium oryzae* CBMB20 and *Bacillus megaterium* LNL6 did not produce any zone of clearance on the plates preseeded with *Bradyrhizobium japonicum* MN110. Mansotra *et al.* (2015a) also reported positive interaction between *Mesorhizobium* sp. and *Piriformospora indica* with different *Pseudomonas* sp. in plate culture method.

Choure and Dubey (2012) developed plant growth promoting microbial consortium based on interaction studies. *In vitro* dual culture studies on the interaction of one strain to another, revealed no mutual growth inhibition among *Streptomyces fredii* KCC5, *Pseudomonas fluorescens* and *Azotobacter chroococcum* AZK2.

4.4.2 Endophytic colonization of effective bacterial isolates

4.4.2.1 Inoculation of maize plants with bacterial endophytes :

The effective bacterial cultures were inoculated to maize individually and in combinations as consortium based on their compatiability with each other. Four methods of inoculation including seed bacterization, soil drenching, stem injection and foliar application were followed to ensure the infection of applied bacterial culture into plants because any one method may not be similarly effective for all the bacterial strains.

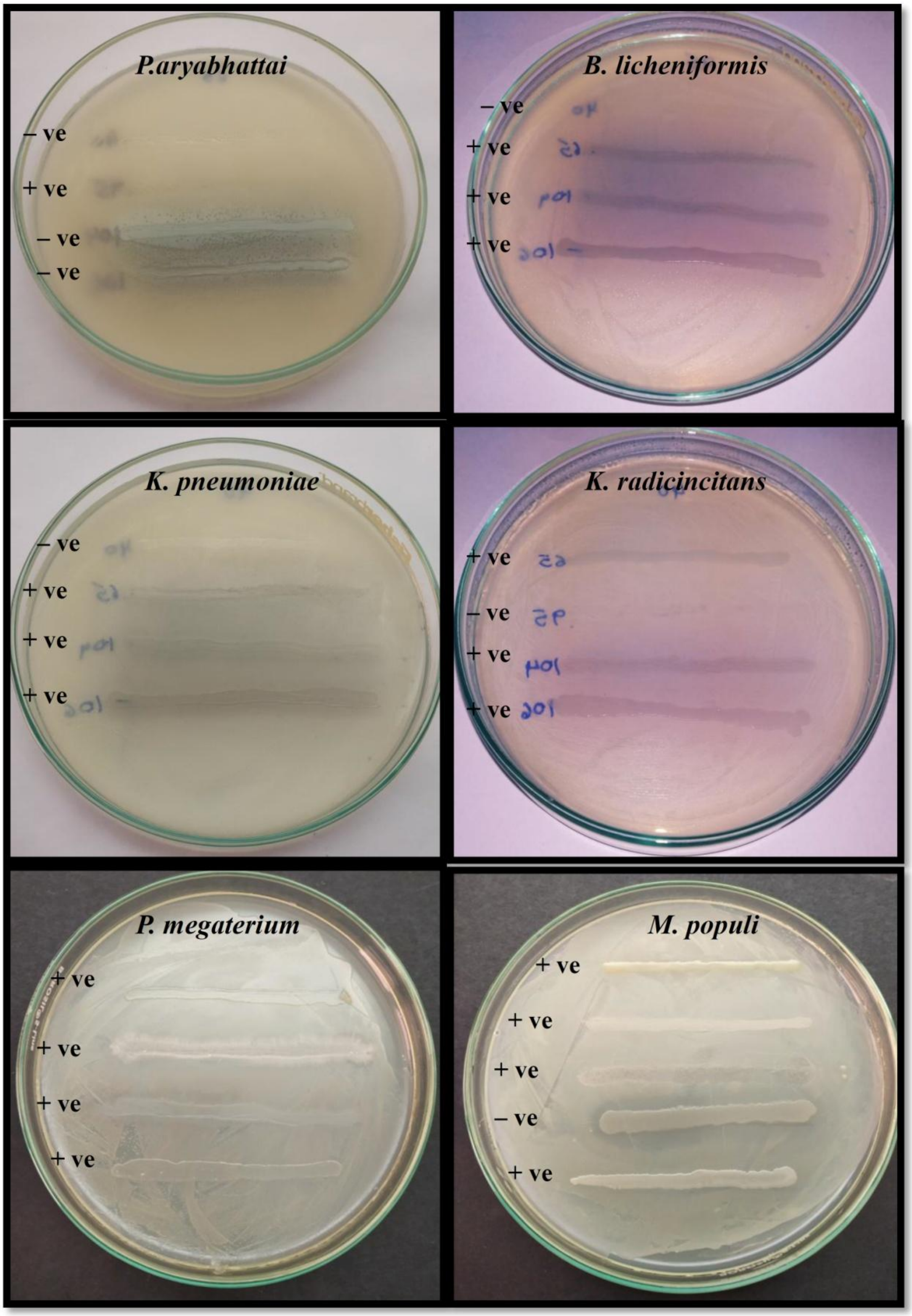


Plate 4.6. Compatiability among the effective bacterial isolates

Table 4.14. Evaluation of compatiability among the effective endophytic bacterial isolates

	NL3E3	PdS3E1	PL3E2	VaR3E1	LS3E1	LS3E3	LL3E1
***Kosakonia radicincitans* (NL3E3)**	+	+	+	-	+	–	–
***Priestia megaterium* (NL3E3)**	+	+	+	++	–	++	++
***Priestia aryabhattai* (PL3E2)**	+	+	+	+	–	+	–
***Bacillus licheniformis* (VaR3E1)**	–	+	+	+	+	++	++
***Pseudomonas aeruginosa* (LS3E1)**	+	–	–	+	+	+	–
***Klebsiella pneumoniae* (LS3E3)**	–	++	+	+ +	+	+	+
***Methylorubrum populi* (LL3E1)**	–	+	–	++	–	++	+

– Inhibition zone formed, + Low bacterial growth, + More bacterial growth

Bressan and Borges (2004) demonstrated that nonpathogenic endophytes can be recovered from maize plants following inoculation by different delivery methods. However, it depends on methods and endophytic bacterial isolate. Isolatespecific attributes appear to be important in determining whether an isolate will colonize plants internally. The results suggest a method will have to be tested specifically for the endophytic bacterial isolate to be delivered. Therefore, practical delivery of different isolates to maize plants may require different methods and the relationship of the biological control properties to the ability of the endophytic bacterial isolate to colonize plant tissues has yet to be examined

4.4.2.1 Re-isolation of endophytic bacteria from inoculated plants :

Pot experiment was conducted to verify that the same bacteria inoculated to surface sterilized maize seeds sown in sterile soil could be re-isolated from these seedlings (to fulfill Koch’s postulate), and examined their endophytic competence (infection and persistence characteristics) by determining which of the endophytic bacteria had the ability to colonize and persist at high levels in plant hosts by experimentally inoculating plants (Plate 4.7).

Seven bacterial isolates were inoculated in maize plants as individual isolates and in consortium. Based on morphological characterization of re isolated bacterial colonies by comparing with inoculated isolates, microbial load in each tissue (root, stem and leaf) were taken at 40-45 days after sowing. As Zinniel *et al.* (2002) has suggested that the colony morphologies of the endophytes recovered after experimental inoculation were indistinguishable from the colony morphologies of the inoculated organisms.

The isolates were found to colonize plants at the levels ranging from 4.30 to 5.26 Log_{10} CFU g^{-1}. Maximum colonization of inoculated isolates was observed in roots followed by stem and least in leaves. Among the recovered isolates the population density of *Bacillus licheniformis* was found to be highest recording maximum of 5.26 Log_{10} CFU g^{-1} in roots of maize while the lowest population density was shown by *Kosakonia radicincitus* both in individual inoculation as well as in consortium. From our study it was also interpreted that the inoculated bacterial isolate could have movement in plant tissues during growth stages as particular tissue specific isolate was observed to re isolated also from other tissues of plant. As in case of *Priestia megaterium* and *Priestia aryabhattai* which are leaf colonizers at first but they were re isolated from all the three tissues. *Bacillus licheniformis* which was initially root colonizer could only re isolated from roots and stem but not the leaf. *Pseudomonas aeruginosa* was also re isolated from only roots and stem indicating that non colonizer of leaf. *Klebsiella pneumonia* which was initially stem colonizer was re isolated from all the three tissues while *Methylorubrum populi*, a leaf colonizer was isolated from only roots and leaves but not stem. (Table 4.15).

Results obtained in present investigation are in positive correlation with many previous studies. In a greenhouse study, 28 of 86 prairie plant endophytes were found to colonize their original hosts at 42 days postinoculation at levels of 3.5 to 7.7 $\log_{10}$ CFU/g (fresh weight). Numerous long-range greenhouse studies and field experiments were done with 9 endophytic bacteria from corn and 8 sorghum bacterial endophytes have shown significant colonization density and the average colonization levels were 6.1±0.1 $\log_{10}$ CFU/g (fresh weight). Prairie endophytic bacteria LB030, PD039, and SG041 were found to colonize the original hosts in a consistent trend in the range of 3.8 and 5.7 $\log_{10}$ CFU/g (fresh weight) (Zinniel *et al.*, 2002)

Table 4.15. Re-isolation of endophytic bacteria from inoculated plants

Treatments		**Log$_{10}$ CFU/g (fresh weight)**		
		Root	**Stem**	**Leaf**
T1 (*Kosakonia radicincitans* NL3E3)		4.60	4.30	–
T2 (*Priestia megaterium* NL3E3)		5.20	5.11	4.60
T3 (*Priestia aryabhattai* PL3E2)		4.90	4.60	4.90
T4 (*Bacillus licheniformis* VaR3E1)		**5.26**	4.85	–
T5 (*Pseudomonas aeruginosa* LS3E1		4.30	4.60	–
T6 (*Klebsiella pneumoniae* LS3E3)		5.08	5.00	4.78
T7 (*Methylorubrum populi* LL3E1)		4.78	–	5.08
T8 (No inoculum- indegenious bacteria)		4.48	–	4.60
MC1 (T1+T3+T6)	**NL3E3**	4.90	–	–
	PL3E2	–	–	4.48
	LS3E3	4.90	5.00	–
MC2 (T1+T5+T6)	**NL3E3**	4.60	4.30	4.60
	LS3E1	4.78	4.48	–
	LS3E3	5.08	4.90	**4.30**
MC3 (T1+T2+T4+T6)	**NL3E3**	–	–	4.48
	NL3E3	5.11	4.90	–
	VaR3E1	5.23	4.78	–
	LS3E3	4.90	5.04	4.60
MC4 (T2+T4+T6+T7)	**NL3E3**	5.04	4.60	4.00
	VaR3E1	5.20	5.00	–
	LS3E3	5.08	4.90	4.60
	LL3E1	4.60	–	4.85

The indigenous endophytic microbial population may vary between 2.0 and 6.0 log$_{10}$ CFU per g for sweet corn, cotton, alfalfa, sugar beet, potato and squash. Similar results were reported for endophytic bacteria of tomato and pomato inoculated by root and seed drenching, having population titres ranging between 3.0 and 5.0 log10 CFU/g of plant tissue (Kobayashi and Palumbo, 2000)

Eighty-three endophytic bacteria were isolated from roots, stems, and leaves of *A. bertolonii* and classified by restriction analysis of 16S rDNA (ARDRA) and partial 16S rDNA sequencing in 23 different taxonomic groups. To evaluate the plant colonization ability of the isolated endophytes, 23 isolates were selected among the different ARDRA types, and infection of sterile grown plant seedlings was performed. All the tested bacterial isolates were successfully recovered within one or both plant portions shoots (stem plus leaflets) and roots. A higher number of ARDRA types were found in roots than in stems and leaves. A small fraction only of ARDRA types was exclusively found in stems or leaves (Barzanti *et al.*, 2007)

4.4.2.2 Qualitative comparision of recovered isolates with inoculated isolates :

The highly similar colony morphologies of re-isolated endophytic bacteria when compared with inoculated bacterial colonies were selected for further confirmation of their identity. About 15 re isolated strains RI$_{1}$T1R, RI$_{7}$T2S, RI$_{4}$T3R, RI$_{5}$T12R, RI$_{6}$T10S, RI$_{8}$T7L, RI$_{10}$T12L, RI$_{9}$T5S, RI$_{11}$T9S, RI$_{2}$T9L, RI$_{3}$T11R, RI$_{12}$T4R, RI$_{13}$T10L, RI$_{14}$T9S and RI$_{15}$T12R were further examined for antibiotic sensitivity and carbon source utilization.

a) Profiling of antibiotic sensitivity

Based on colony morphology RI$_{1}$T1R was identified as *Pseudomonas* and their antibiotic sensititivity has also shown similar pattern as of inoculated *P. aeruginosa.* The re-isolated strains RI$_{7}$T2S and RI$_{3}$T11R were identified as *Priestia megaterium* and their antibiotic sensitivity assay has shown that all the tested antibiotics were in similar pattern with inoculated *P. megaterium* except for Streptomycin (10 mcg) against RI$_{7}$T2S which has shown resistant. RI$_{4}$T3R and RI$_{14}$T9S were identified as *P. aryabhattai* morphologically but they differed in sensitivity against Streptomycin (10 mcg) and Tetracyclin (25 mcg) respectively when compared with the inoculated isolate. The isolates RI$_{5}$T12R, RI$_{12}$T4R and RI$_{15}$T12R were phenotypically identified.

as *Bacillus licheniformis* and the trend of antibiotic sensitivity of all the three isolates were found similar with the inoculated isolate *B.licheniformis*. The isolates RI$_{6}$T10S, RI$_{2}$T9L and RI$_{11}$T9S were identified as *Klebsiella pneumoniae* former two isolate was found similar with the inoculated isolate in antibiotic assay while the later isolate was varied with the sensitivity against Ampicillin (10 mcg). The re-isolated strains RI$_{8}$T7L and RI$_{10}$T12L were phenotypically identical with *Methylorubrum populi* and the results of antibiotic sensitivity assay has also obtained in similar with the inoculated isolate (Table 4.16).

For gram-negative bacteria, resistance to ampicillin was most common (75%), followed by resistance to kanamycin (70%), resistance to streptomycin (36%), and resistance to tetracycline (27%). Gram-positive strains demonstrated a similar pattern of antibiotic resistance, although they were more likely to be resistant to kanamycin (51%) than to ampicillin (39%) (Zinniel *et al.*, 2002).

b) Profiling of carbon source utilization

All the re-isolated bacterial strains have shown similar pattern of carbon source utilization as of their inoculated bacterial isolates except for four including $RI_{11}T9S$**,** $RI_{12}T4R$, $RI_{13}T10L$ and $RI_{14}T9S$ which are expected as the isolates *Klebsiella pneumonia, Bacillus licheniformis, Kosakonia radicincitus* and *Priestia aryabhattai* respectively (Table 4.17).

Zinniel *et al.* (2002) demonstrated that carbon source utilization tests of re-isolated prairie plant endophytes identified LB030 as *M. testaceum,* PD039 as *Clavibacter michiganensis* subsp. *insidiosus* (equivalent to DSM 20157 and ATCC 10253), and SG041 as *Curtobacterium citreum* (equivalent to DSM 20528, IFO 12677, and ATCC 15828).

4.4.2.3 Molecular comparision of recovered isolates with inoculated isolates : The recovered isolates which were confirmed to be inoculated ones based on morphology, antibiotic sensitive assay and carbon source utilization assay (RI_1T1R, RI_7T2S, RI_4T3R, RI_5T12R, RI_6T10S, RI_8T7L, $RI_{10}T12L$, RI_2T9L and RI_3T11R) were further examined for their similarity with 16S rRNA gene sequences as of inoculated bacterial isolates (Appendix-III).

The 16S rRNA gene of re-isolated strain RI_5T12R was 99.82% homologous with the 16S rRNA gene of the *Bacillus licheniformis.* The recovered isolate RI_6T10S has exhibited 98.92 % of similarity of 16S rRNA gene with *Klebsiella pneumonia* gene. The 16S rRNA gene of re-isolated strain $RI_{10}T12L$ was found 99.40% of homologous with 16S rRNA gene of *Methylorubrum populi*. The 16S rRNA gene of the recovered isolate RI_3T11R has shown 97.06% and 97.07% of similarity with the 16S rRNA gene of the strains *Priestia megaterium* and *Priestia aryabhattai* (Table 4.18). In phylogentic tree analysis RI_3T11R was found to have similarity with the cluster of *P. megaterium* and *P.aryabhattai,* re-isolates RI_4T3R and RI_5T12R were found to have similarity with *B. licheniformis* with 95% and 99% respectively. The recovered strains RI_8T7L, $RI_{10}T12L$ and RI_2T9L were found to have 100% similarity with the inoculated isolate *M. populi*. RI_1T1R have shown 88% similarity index with *P.aeruginosa* (Fig. 4.4). By analyzing the 16S rRNA gene sequence by PCR amplification the isolates B. *licheniformis, K. pneumonia, M. populi, P. megaterium* and *P. aryabhattai* were confirmed as endophytic colonizers as they have fulfilled Koch's postulates.

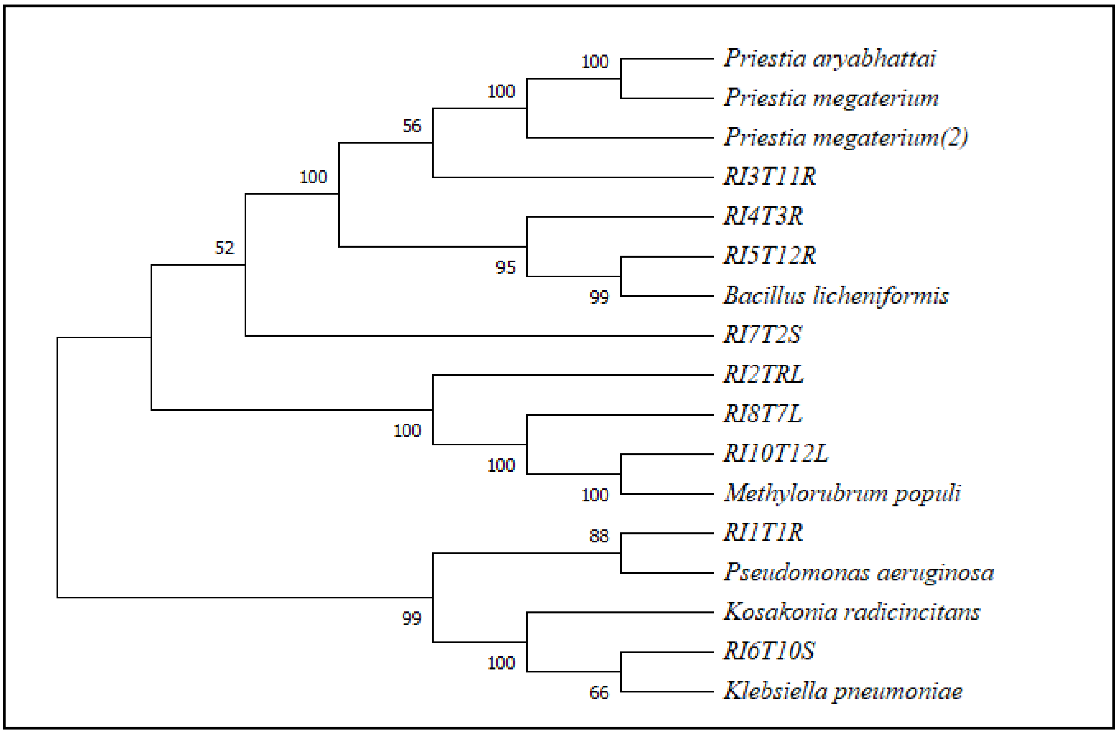


Fig. 4.4. Phylogenetic tree expressing the relationships of Re isolated bacterial endophytes to taxonomically similar inoculated bacterial endophytes based on the 16S rRNA gene sequences. (The positions of the endophyte strains are based on the best match for genus and species. The numbers at the nodes are bootstrap values based on 100 replications).

Table 4.16. Assay of Re-isolated endophytic bacteria against antibiotic sensitivity

S. No	Isolate code	Expected isolate	Ampicillin (10 mcg)	Chloramphenicol (25 mcg)	Penicillin-G (0.6 mcg)	Streptomycin (10 mcg)	Sulphatriad (300 mcg)	Tetracyclin (25 mcg)
1	RI$_{1}$T1R	*Pseudomonas aeruginosa*	R	S	R	R	R	S
2	RI$_{7}$T2S	*Priestia megaterium*	R	S	S	S	S	S
3	**RI$_{4}$T3R**	***Priestia aryabhattai***	R	S	S	**R**	S	S
4	RI$_{5}$T12R	*Bacillus licheniformis*	R	S	R	S	S	S
5	RI$_{6}$T10S	*Klebsiella pneumoniae*	R	S	R	S	S	S
6	RI$_{8}$T7L	*Methylorubrum populi*	S	S	S	R	S	R
7	RI$_{10}$T12L	*Methylorubrum populi*	S	S	S	R	S	R
8	**RI$_{9}$T5S**	***Pseudomonas aeruginosa***	S	**R**	S	R	S	R
9	**RI$_{11}$T9S**	***Klebsiella pneumoniae***	**S**	S	S	R	S	R
10	RI$_{2}$T9L	*Klebsiella pneumoniae*	R	S	R	S	S	S
11	RI$_{3}$T11R	*Priestia megaterium*	R	S	S	S	S	S
12	RI$_{12}$T4R	*Bacillus licheniformis*	R	S	R	S	S	S
13	**RI$_{13}$T10L**	***Kosakonia radicincitus***	R	S	R	**S**	**S**	S
14	**RI$_{14}$T9S**	***Priestia aryabhattai***	**S**	S	S	R	S	**R**
15	RI$_{15}$T12R	*Bacillus licheniformis*	R	S	R	S	S	S

Bold font- Recovered isolates showing dissimilarity in antibiotic sensitivity with inoculated isolates (see table 4.13)

Table 4.17. Assay of Re-isolated endophytic bacteria against various carbon source utilization

S. No	Isolate code	Expected isolate	Dextrose	Sucrose	Maltose	Lactose
1	RI$_1$T1R	*Pseudomonas aeruginosa*	+	+	+	-
2	RI$_7$T2S	*Priestia megaterium*	+	+	+	+
3	RI$_4$T3R	*Priestia aryabhattai*	+	+	+	-
4	RI$_5$T12R	*Bacillus licheniformis*	+	+	+	-
5	RI$_6$T10S	*Klebsiella pneumoniae*	+	+	+	+
6	RI$_8$T7L	*Methylorubrum populi*	+	+	+	-
7	RI$_{10}$T12L	*Methylorubrum populi*	+	+	+	-
8	RI$_9$T5S	*Pseudomonas aeruginosa*	+	+	-	-
9	**RI$_{11}$T9S**	***Klebsiella pneumoniae***	**+**	**+**	**+**	**-**
10	RI$_2$T9L	*Klebsiella pneumoniae*	+	+	+	+
11	RI$_3$T11R	*Priestia megaterium*	+	+	+	+
12	**RI$_{12}$T4R**	***Bacillus licheniformis***	**+**	**+**	**+**	**-**
13	**RI$_{13}$T10L**	***Kosakonia radicincitus***	**+**	**+**	**+**	**-**
14	**RI$_{14}$T9S**	***Priestia aryabhattai***	**+**	**+**	**-**	**-**
15	RI$_{15}$T12R	*Bacillus licheniformis*	+	+	+	-

Bold font- Recovered isolates showing dissimilarity in carbon source utilization with inoculated isolates (see table 4.5)

Table 4.18. Comparision of recovered isolates with inoculated endophytic bacterial isolates based on 16S rRNA gene sequence

S. No.	Isolate code	Expected bacterial strain	Identified bacteria (homologous with sequence run in NCBI database)	Similarity	NCBI Accession number
1	RI$_{1}$T1R	*Pseudomonas aeruginosa*	*Pseudomonas hibiscicola*	99.72%	NR_024709.1
2	RI$_{7}$T2S	*Priestia megaterium*	*Arthrobacter pascens*	98.13%	NR_02619.1
3	RI$_{4}$T3R	*Priestia aryabhattai*	*Bacillus subtilis*	100.0%	NR_118383.1
4	RI$_{5}$T12R	*Bacillus licheniformis*	*Bacillus licheniformis*	99.82%	NR_118996.1
5	RI$_{6}$T10S	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	98.92%	NR_113702.1
6	RI$_{8}$T7L	*Methylorubrum populi*	*Methylobacterium radiotolerance*	98.98%	NR_074244.1
7	RI$_{10}$T12L	*Methylorubrum populi*	*Methylorubrum populi*	99.40%	NR_074257.1
8	RI$_{2}$T9L	*Klebsiella pneumoniae*	*Ochrobactrumgrignonense*	99.41%	NR_028901.1
9	RI$_{3}$T11R	*Priestia megaterium*	*Priestia megaterium* *Priestia aryabhattai*	97.06% 97.07%	NR_117473.1 NR_115953.1

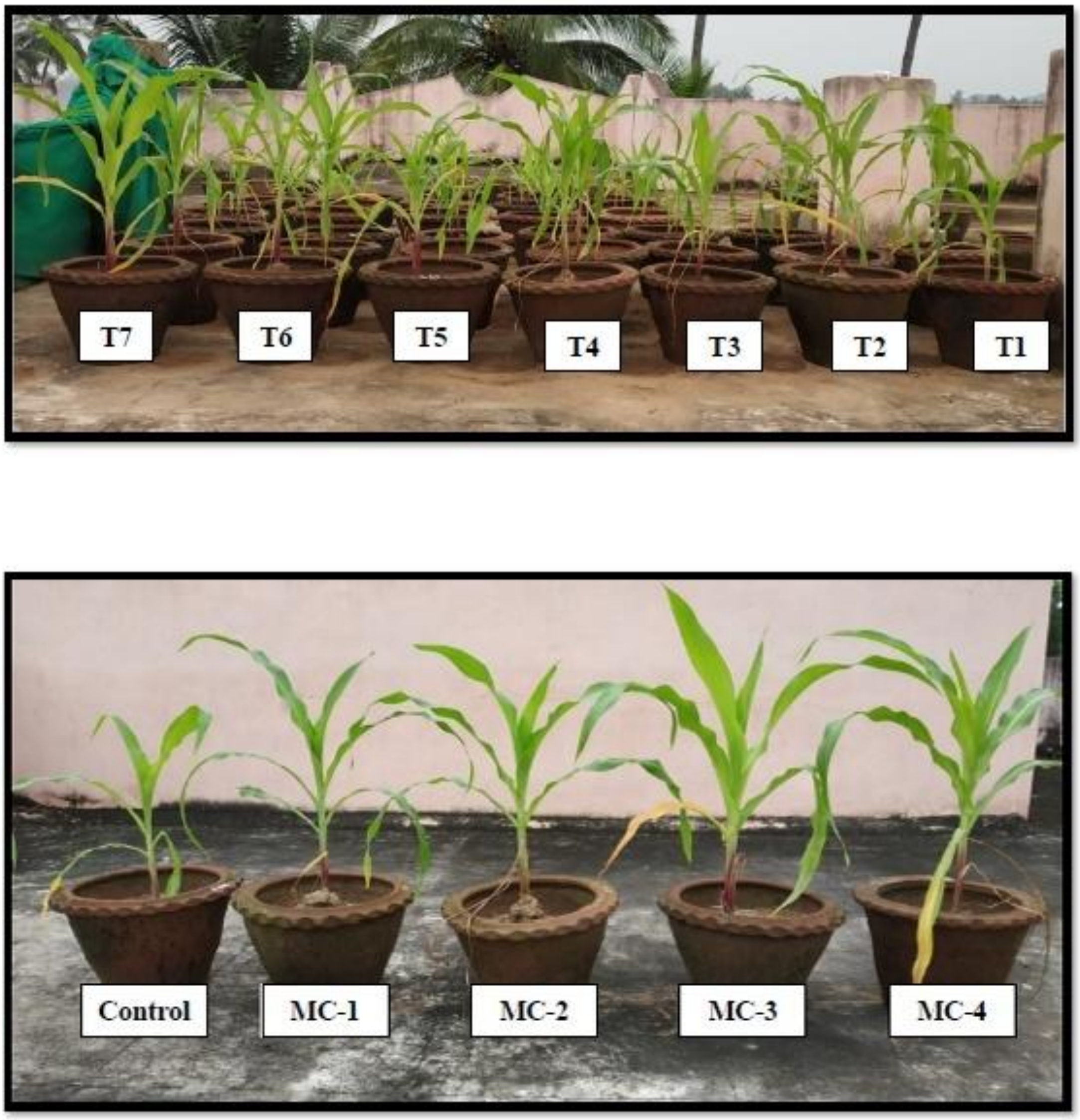
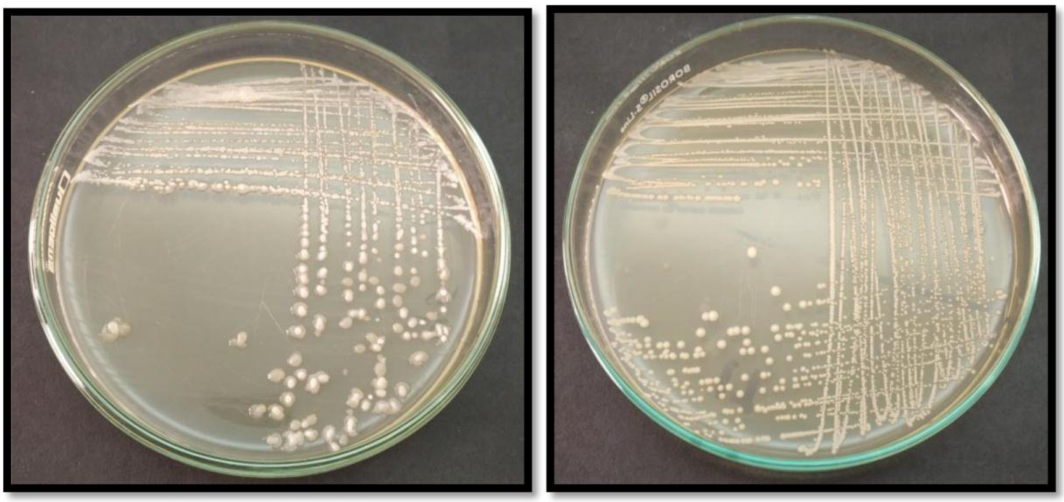
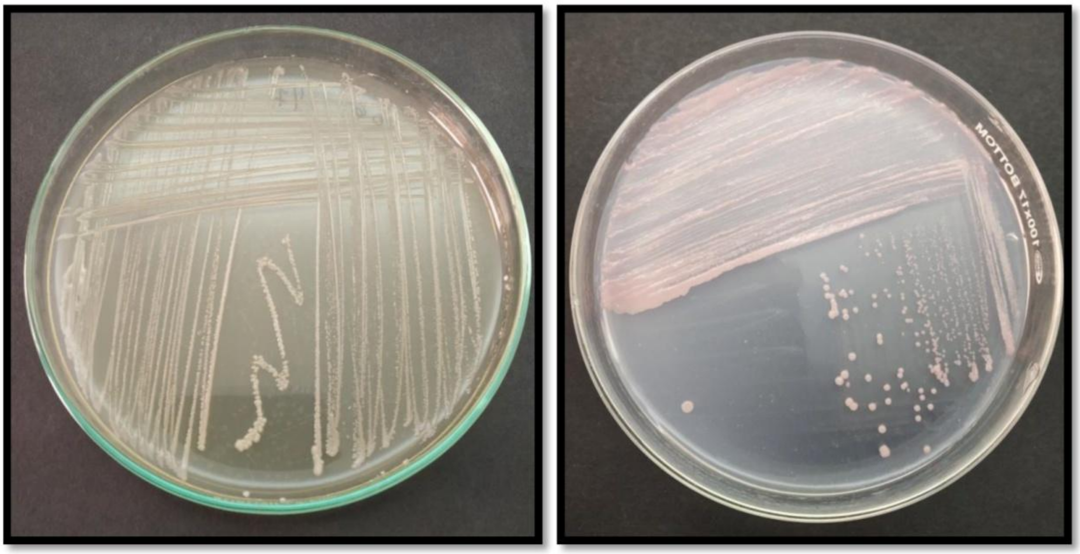


Plate 4.7. **Pot experiment for endophytic colonization studies and consortium screening**



a) Bacillus licheniformis *b) Priestia megaterium*



c) Klebsiella pneumoniae *d) Methylorubrum populi*

Plate 4.8. Efficient endophytic bacterial isolates used for microbial consortium

Results obtained were in correlation with the studies of Stoltzfus *et al.* (1997) that the analysis of bacteria re-isolated from rice seedlings inoculated with *Azorhizobium caulinodans* ORS571, R22(88), R100(64), R6a(126), R33(120), R45(42), R032.S2.3, and R061.S1.3 using rep-PCR genomic fingerprinting revealed that Koch's postulate was fulfilled, in that bacteria with the same fingerprint as the original inoculum strain could be re-isolated.

Similarly, Zinniel *et al.* (2002) has identified three prairies and three agronomic endophytes exhibiting the most promising levels of colonization and an ability to persist were identified as *Cellulomonas, Clavibacter, Curtobacterium,* and *Microbacterium* isolates by 16S rRNA gene sequencing.

Three novel endophytic rhizobial strains (RRE3, RRE5, and RRE6) were isolated from naturally growing surface sterilized rice roots. Polymerase chain reaction restriction fragment length polymorphism and sequencing of 16S rDNA of these isolates revealed that RRE3 and RRE5 are phylogenetically very close to *Burkholderia cepacia* complex, whereas RRE6 has affinity with *Rhizobium leguminosarum bv. phaseoli*. Plant infection test using gusA reporter gene tagged construct of these isolates indicated that bacterial cells can go inside and colonize the rice root interiors (Singh *et al.*, 2006).

Based on obtained results the endophytic microbial consortium of *Priestia megaterium* (NL3E3), *Bacillus licheniformis* (VaR3E1), *Klebsiella pneumonia* (LS3E3) and *Methylorubrum populi* (LL3E1) were selected (Plate 4.8) for further pot and field experimentation.

4.5 INFLUENCE OF ENDOPHYTIC MICROBIAL CONSORTIA ON MAIZE UNDER WATER STRESS UNDE POT CULTURE

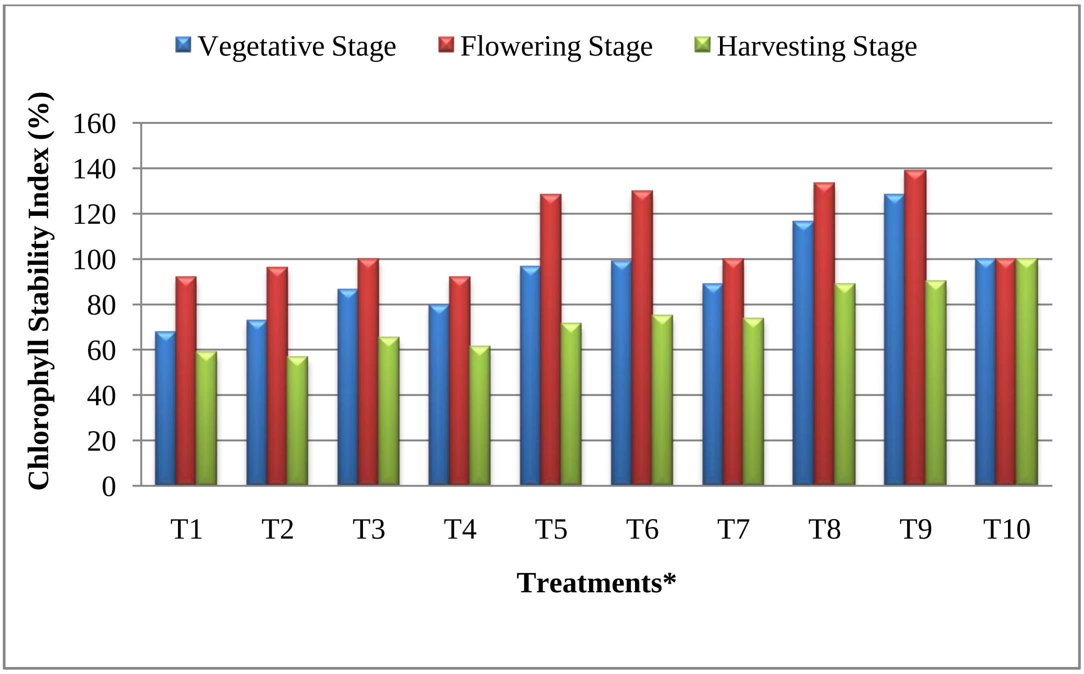
4.5.1 Influence of endophytic microbial consortia on plant biochemical parameters of maize

a) Chlorophyll Stability Index (CSI %)

Chlorophyll stability index is the most promising character to screen the plants under drought stress (Arunyanark *et al.*, 2008). At vegetative stage significantly higher CSI was found in T9:75 % WHC+ 100 % RDF + MC (128.33%) and T8:75 % WHC+ 75 % RDF + MC (116.33%) while the treatments T6:50% WHC + 100 % RDF + MC

(99.00%) and T5: 50% WHC + 75 % RDF + MC (96.33%) were found to have CSI on par with the control. At flowering stage four treatments were found to have CSI significantly more than the control T10:100% WHC+ 100 % RDF (100%) in the following order T9:75 % WHC+ 100 % RDF + MC (138.67%), T8:75 % WHC+ 75 % RDF + MC (133.33%), T6: 50% WHC + 100 % RDF + MC (129.67%) and T5: 50% WHC + 75 % RDF + MC (128.00%) and the treatment T7:75 % WHC+ 50 % RDF + MC was found to have CSI 99.67% which was on par with the control (Table 4.19).

At harvesting stage maximum CSI was found in T9:75 % WHC+ 100 % RDF + MC (90.00%) followed by T8:75 % WHC+ 75 % RDF + MC (89.00%). The CSI was observed to increase at flowering stage than the vegetative stage and was gradually reduced at harvesting stage (Fig. 4.5). And the treatments with water deficit conditions have shown less CSI than treatments given with full irrigations because drought impairs the photosynthesis by reversible inhibition of chlorophyll activity. However treatments T9, T8, T6 and T5 were found to have higher CSI though given lesser irrigations than the control which might be due to the application of osmotolerant and plant growth promoting microbial consortium in particular nitrogen fixing ability increases N content in plants enhancing the synthesis of chlorophyll than in control.



*Treatment details as given in table 3.3

Fig. 4.5. Influence of endophytic microbial consortia on chlorophyll stability index of maize grown in pot under water stress.

Sehgal *et al.* (2018) reported that the drought inversely affects the chlorophyll content, with increased drought intensity chlorophyll content decreases results in inhibition of photosynthesis and accelerating the senescence process in plants. But, studies have proved that inoculation of microbes have improved chlorophyll content even under drought conditions.

In maize inoculated with *Azospirillum* sp. and *Herbaspirillum* sp. under drought stress increased total chlorophyll content was observed suggesting that the available nitrogen in could have been used for chlorophyll synthesis (Curá *et al.*, 2017)

Inoculation of *Pseudomonas syringae, Enterobacter aerogenes* and *P. fluorescens* to maize increased the chlorophyll content under drought stress (Nadeem *et al.*, 2007).

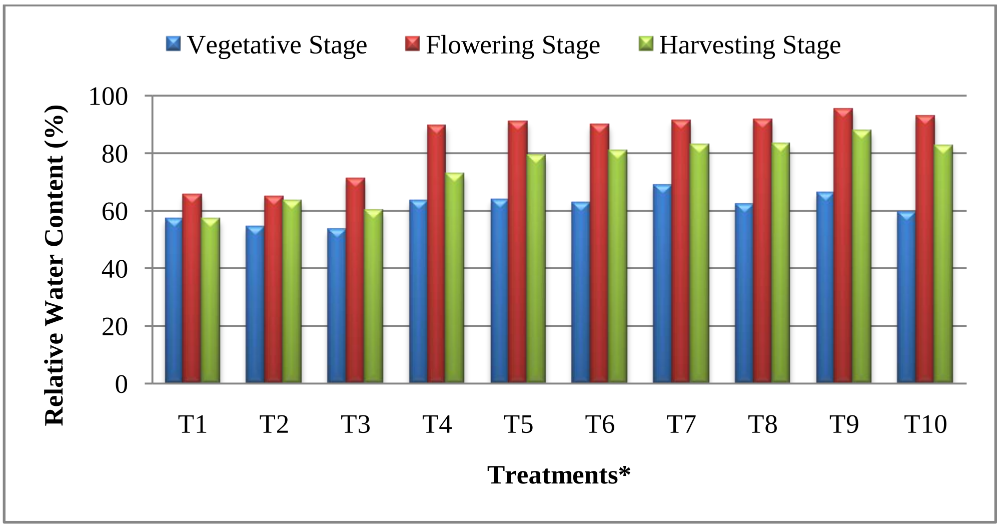
b) Relative Water Content (RWC %)

Relative water content (RWC) of leaf also known as relative water turgidity is a measurement of actual water content relative to its extreme water holding capacity. It shows the degree of drought and heat stress, and provides one of the best criteria for measuring water deficit in leaves (Mullan and Pietragalla 2012). Higher relative water content of a species shows that the species are better adapted to environmental stress, i.e., drought stress. Hence, a higher RWC is considered as an important strategy for higher drought tolerance compared to a lower RWC (Nounjan *et al.*, 2018). To check the potential of endophytic PGP bacteria as a drought stress–alleviating agent, RWC of plant leaves could be used as a parameter (Ngumbi and Kloepper 2016). In this regard, developed endophytic bacterial consortia have been investigated that alleviate drought stress in plants by increasing RWC in inoculated plants under given stress conditions.

At vegetative stage maximum and significantly higher RWC was observed in T7:75 % WHC+ 50 % RDF + MC (68.97%) followed by T9 :75 % WHC+ 100 % RDF + MC (66.27%) while T8 :75 % WHC+ 75 % RDF + MC (62.07%) and all the three treatments with 50% WHC T6 (63.03%), T5 (63.90%) and T4 (63.57%) were recorded to have RWC on par with the control T10:100% WHC+ 100 % RDF (59.63%). At flowering stage there was no significant difference among the treatments given with 50% WHC and 75% WHC and they are on par with the control given with 100% WHC and no microbial inoculums. The RWC was found maximum in T9:75 % WHC+ 100 % RDF + MC (95.20%) followed by T10:100% WHC+ 100 % RDF (92.97%) (Table 4.19).

At harvesting stage T9:75 % WHC+ 100 % RDF + MC (87.87%) was found to have significantly more RWC than the control T10:100% WHC+ 100 % RDF (82.70%) while the treatments T8:75 % WHC+ 75 % RDF + MC (83.37%), T7:75 % WHC+ 50 % RDF + MC (82.80%) and 50% WHC + 100 % RDF + MC (80.77%) were observed to have RWC on par with the control. RWC of all the treatments were observed to increase at flowering stage than vegetative stage and found to decrease at harvesting stage (Fig. 4.6). The higher RWC may protect plant from osmotic and oxidative stress triggered by water deficit conditions. The increased RWC under water deficit conditions is due to altered physiological processes in plants by microbes producing phytoharmones like Abscisic acid which induces closure of stomata thereby reducing transpirational losses.

Dodd *et al.* (2010) reported that the increased RWC in microbial treated plants could be due to altered physiological processes such as stomatal closure and Grover *et al.* (2014) has demonstrated that 24% increase RWC was observed in sorghum plants treated with *Bacillus* sp. strain KB 129 under drought stress.



*Treatment details as given in table 3.3

Fig. 4.6. Influence of endophytic microbial consortia on relative water content of maize grown in pot under water stress.

Ma *et al.* (2017) isolated an endophytic bacteria, *Pseudomonas azotoformans* from the leaves of *Alyssum serpyllifolium* and tested for enhanced drought tolerance. Inoculation of *Trifolium arvense* with *Pseudomonas azotoformans* increased leaf RWC in the absence as well as presence of drought stress.

c) H_2O_2 (μmol g^{-1}) content

Exposure of plants to abiotic stress leads to increased production of reactive oxygen species (ROS) by partial reduction of atmospheric O_2 leading to production of hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$) and superoxide anion radical ($O_2^{\,-}$) (Ullah *et al.,* 2017) causing oxidative damage to lipids, proteins, and other macro molecules (Mittler, 2002). However, the production ROS can be minimized in the plants by the application of endophytic bacteria under drought stress as reported (Khan *et al.,* 2014; Khan *et al.,* 2016). Hence, in present investigation H_2O_2 content was taken as parameter to evaluate the function of developed endophytic microbial consortia in decreasing ROS under water deficit stress conditions.

At vegetative stage the H_2O_2 content in control i.e., T10:100% WHC+ 100 % RDF given no moisture stress and microbial treatment was found to be 28.50 µmol g^{-1} and the treatments which were recorded lower H_2O_2 than the control even though provided with moisture stress are found to be T9: 75 % WHC + 100 % RDF + MC (23.97 µmol g^{-1}), T8: 75 % WHC + 75 % RDF + MC (25.20 µmol g^{-1}) and T7: 75 % WHC + 50 % RDF + MC (28.13 µmol g^{-1}). Maximum H_2O_2 content was found in T1: 25% WHC + 50 % RDF + MC (53.97 µmol g^{-1}) and T2: 25% WHC + 75 % RDF + MC (44.17 µmol g^{-1}) (Table 4.20).

At flowering stage minimal H_2O_2 content were found in treatments given with moisture stress are T9 (37.97 µmol g^{-1}), T8 (37.77 µmol g^{-1}) and T7 (36.97 µmol g^{-1}) while the treatment T6 :50 % WHC+ 100 % RDF + MC (43.83 µmol g^{-1}) was found to have H_2O_2 content on par with the control T10:100% WHC+ 100 % RDF (42.70 µmol g^{-1}). At harvesting stage lower content of H_2O_2 were found in treatments given with moisture stress are T9 (30.43 µmol g^{-1}), T8 (31.87 µmol g^{-1}), T7 (32.80 µmol g^{-1}) and treatment T10 given no moisture stress has recorded 33.40 µmol g^{-1} of H_2O_2 content. Maximum H_2O_2 content was observed in treatments given with moisture stress are T1 (56.07 µmol g^{-1}) and T2 (54.70 µmol g^{-1})

Table 4.19. Influence of endophytic microbial consortia on plant biochemical parameters of maize in pot culture

Treatments	Chlorophyll Stability Index (CSI %)			Relative Water Content (RWC %)		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T_1 : 25 % WHC + 50 % RDF + MC	67.67	92.00	58.67	57.20	65.60	57.33
T_2 : 25 % WHC + 75 % RDF + MC	72.67	96.00	56.67	54.37	65.03	63.63
T_3 : 25 % WHC+ 100 % RDF + MC	86.33	100.00	65.33	53.40	71.23	60.07
T_4 : 50 % WHC+ 50 % RDF + MC	79.67	92.00	61.33	63.57	89.53	72.97
T_5 : 50 % WHC+ 75 % RDF + MC	96.33	128.00	71.33	63.90	91.00	79.13
T_6 : 50 % WHC+ 100 % RDF + MC	99.00	129.67	75.00	63.03	89.80	80.77
T_7 : 75 % WHC+ 50 % RDF + MC	88.67	99.67	73.67	68.97	91.33	82.80
T_8 : 75 % WHC+ 75 % RDF + MC	116.33	133.33	89.00	62.07	91.50	83.37
T_9 : 75 % WHC+ 100 % RDF + MC	128.33	138.67	90.00	66.27	95.20	87.87
T_{10} : 100% WHC+ 100 % RDF (Control)	100.00	100.00	100.00	59.63	92.96	82.70
CD(P=0.05)	5.93	4.28	5.31	5.35	4.30	5.38
S.Em. ±	1.99	1.44	1.78	1.80	1.45	1.81
S.Ed	2.82	2.03	2.53	2.55	2.05	2.56

Table 4.20. Influence of endophytic microbial consortia on antioxidant enzymes and proline content in maize in pot culture

Treatments	H_2O_2 (µmol g^{-1})			Peroxidase (units min^{-1} g^{-1})			Proline (µg g^{-1})		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T_1: 25 % WHC + 50 % RDF + MC	53.96	71.37	56.07	35.83	76.73	44.57	65.27	67.63	69.97
T_2 : 25 % WHC + 75 % RDF + MC	44.16	69.10	54.70	35.27	75.23	43.33	66.77	65.03	73.17
T_3 : 25 % WHC+ 100 % RDF + MC	43.00	66.43	52.70	34.63	73.43	45.80	63.87	68.53	68.80
T_4 : 50 % WHC+ 50 % RDF + MC	34.26	46.13	45.50	30.03	68.77	37.57	35.60	34.43	40.20
T_5 : 50 % WHC+ 75 % RDF + MC	33.96	46.53	43.03	30.03	70.03	35.17	34.73	33.63	39.60
T_6 : 50 % WHC+ 100 % RDF + MC	32.00	43.83	40.97	30.37	66.57	34.17	33.80	35.13	37.77
T_7 : 75 % WHC+ 50 % RDF + MC	28.13	36.97	32.80	26.47	54.27	30.50	28.73	26.43	33.60
T_8 : 75 % WHC+ 75 % RDF + MC	25.20	37.77	31.87	26.97	56.47	29.63	30.33	29.40	31.97
T_9 : 75 % WHC+ 100 % RDF + MC	23.96	37.97	30.43	24.83	54.43	29.47	29.47	28.80	31.40
T_{10}: 100% WHC+ 100 % RDF (Control)	28.50	42.70	33.40	24.80	55.43	30.43	25.17	25.50	26.77
CD(P=0.05)	2.56	3.97	3.63	3.47	4.44	2.27	2.84	3.78	4.79
S.Em. ±	0.86	1.34	1.22	1.16	1.49	0.76	0.95	1.27	1.61
S.Ed	1.22	1.89	1.74	1.64	2.11	1.08	1.35	1.79	2.28
CV (%)	4.30	4.64	5.04	6.72	3.97	3.67	3.99	5.32	6.17

In a case study, 7 endophytic bacteria, i.e., *Acinetobacter calcoaceticus, Burkholderia vietnameinsis, Enterobacter asburiae, Rhizobium tropici, Sphingomonas yanoikuyae, Curtobacterium* sp., *Rhanella sp.,* and *Pseudomonas* sp., were isolated from stem of poplar and willow plants. Poplar plants were inoculated with these bacteria and evaluated for their drought tolerance as a result plants showed decreased ROS activity, in addition to other drought tolerance-enhancing parameters (Khan *et al.,* 2016).

d) Peroxidase (units min^{-1} g^{-1})

At vegetative stage highest peroxidase content was produced by T1: 25 % WHC + 50 % RDF + MC (35.83 µmol g^{-1}), T2 :25 % WHC + 75 % RDF + MC (35.27 µmol g^{-1}), T3 :25 % WHC+ 100 % RDF + MC (34.63 µmol g^{-1}) while lower content of peroxidase was observed in T7 :75 % WHC+ 50 % RDF + MC (26.47 µmol g^{-1}), T8 :75 % WHC+ 75 % RDF + MC (26.97 µmol g^{-1}), T9 :75 % WHC+ 100 % RDF + MC (24.83 µmol g^{-1}) which were found on par with the control given with no water stress T10:100% WHC+ 100 % RDF (24.80 µmol g^{-1})

At flowering stage highest peroxidase content was produced by T1 (76.73 µmol g^{-1}), T2 (75.23 µmol g^{-1}), T3 (73.43 µmol g^{-1}) while lower content of peroxidase was observed in T7 (54.27 µmol g^{-1}), T8 (56.47 µmol g^{-1}), T9 (54.43 µmol g^{-1}) which were found on par with the control given with no water stress T10 (55.43 µmol g^{-1})

At harvesting stage highest peroxidase content was produced by T1 (44.57 µmol g^{-1}) and T3 (45.80 µmol g^{-1}) while lower content of peroxidase was observed in T7 (30.50 µmol g^{-1}), T8 (29.63 µmol g^{-1}), T9 (29.47 µmol g^{-1}) which were found on par with the control given with no water stress T10 (30.43 µmol g^{-1}) (Table 4.20).

At all the growth stages peroxidase content is higher in treatments provided with 75% and 100% RDF under water deficit stress conditions. The results suggested that at optimum levels of fertilizer application could regulate increased tolerance to drought stress by improving levels of oxidative enzymes. As similar results were reported by Qu *et al.* (1996) that phosphorus in maize leaves efficiently kept higher RWC and also increased superoxide dismutase (SOD) and peroxidase (POD) enzyme activities. Sun *et al.* (2001) has observed increased activities of antioxidant enzymes SOD, POD, and CAT with a suitable nitrogen level under a water deficit stress.

The significant reduction of antioxidant enzyme activity was observed in the plants treated with the consortium T3 and T4 respectively, as compared to single bacterial strain inoculation (T1 and T2) under drought stress conditions (Joshi *et al.*, 2020). A drought-resistant endophytic bacteria *Pseudomonas azotoformans* ASS1 isolated from leaves of *Alyssum serpyllifolium* enhanced plant chlorophyll content, peroxidase, catalase, superoxide dismutase, and proline content under drought stress (Khan *et al.,* 2014)

e) Proline (µg g^{-1})

Drought stress is associated with an increase in a number of compatible solutes, specifically proline (Kiani *et al.,* 2007). The accumulation of compatiable solutes namely sugars, organic acids, inorganic ions and proline under water deficit stress conditions is termed as osmotic adjustment. Thus, influence of applied endophytic microbial consortium on accumulation of proline content under water deficit stress conditions was evaluated in the present study (Table 4.20).

At vegetative stage maximum proline content was found in T2 :25 % WHC + 75 % RDF + MC (66.77 µg g^{-1}) followed by T1: 25 % WHC + 50 % RDF + MC (65.27 µg g^{-1}) and minimum production of proline was observed in T7 :75 % WHC+ 50 % RDF + MC (28.73 µg g^{-1}), T8 :75 % WHC+ 75 % RDF + MC (30.33 µg g^{-1}), T9 :75 % WHC+ 100 % RDF + MC (29.47 µg g^{-1}) which were found on par with the control given with no water stress T10:100% WHC+ 100 % RDF (25.17 µg g^{-1})

At flowering stage highest proline content was found in by T1: 25 % WHC + 50 % RDF + MC (67.63 µg g^{-1}) followed by T3 :25 % WHC+ 100 % RDF + MC (68.53 µg g^{-1}) and lowest production of proline was observed in T7 (26.43 µg g^{-1}), T8 (29.40 µg g^{-1}) and T9 (28.80 µg g^{-1}) which were found on par with the control given with no water stress T10 (26.60 µg g^{-1}). At harvesting stage highest proline content was found in by T2 (73.17 µg g^{-1}) followed by T3 (73.00 µg g^{-1}) and lowest production of proline was observed in T7 (31.30 µg g^{-1}), T8 (31.97 µg g^{-1}) and T9 (29.33 µg g^{-1}) which were found on par with the control given with no water stress T10 (32.23 µg g^{-1})

At all the growth stages proline content was found highest in treatments (T1, T2 and T3) given with 25% WHC while the treatments provided with 75% WHC (T7, T8 and T9) were found to have lesser proline content than the control T10 provided with no water stress and no microbial inoculums, indicating that applied microbial consortia might have improved osmatic tolerance by reducing the compatiable solutes like proline. In study of Marulanda *et al.* (2009), *P. putida* and *B. megaterium* exhibited the highest osmotic tolerance, and both strains also showed increased proline content, involved in osmotic cellular adaptation

4.5.2 Effect of Endophytic Microbial Consortia on Physicochemical Properties of Maize Rhizospheric Soil

a) pH

The soil pH is a very crucial factor for determining various physiological properties of soil including nutrient availability and the solubility of different metal ions. The activity of soil microbes can also be determined by the soil pH. Hence pH of the rhizospheric soil was measured to determine the activity of applied microbial consortia under water deficit stress conditions and their activity in minerals solubilization.

At vegetative stage slightly acidic pH was observed in T1: 25 % WHC + 50 % RDF + MC (6.13) and highest pH was observed in T9:75 % WHC+ 100 % RDF + MC (7.00) followed by T10:100% WHC+ 100 % RDF (Control) (6.97). At flowering stage less pH was shown by T3:25 % WHC+ 100 % RDF + MC (7.20) and maximum pH was recorded in T8:75 % WHC+ 75 % RDF + MC (7.63) (Table 4.21). At harvesting stage there was no significant difference was observed among the treatments.

Initial pH of the pot soil was 7.9 (Table 3.2) and it got reduced at vegetative stage and flowering stage while pH of soil was observed to increase at harvesting stage. Results suggest that decreased pH may be due to the release of organic acids through the secretions of microbial inoculants into soil for solubilizing minerals into available forms.

According to Li *et al*. (2017) microbes lower the soil pH by releasing organic acids into soil and changes the pH values. Soil pH and moisture were found to greatly influence the solubilization of zinc and phosphate by endophytes (Ali *et al.*, 2018).

b) Electrical conductivity (EC)

Electrical conductivity is the measurement of the ability to transport electric charge. The EC of soil depends on salts and sodium content level. Electrical conductivity levels can serve as an indirect indicator of the amount of water and water-soluble nutrients available for plant uptake such as nitrate-N, phosphates etc., At vegetative stage maximum EC was observed in T1: 25 % WHC + 50 % RDF + MC (0.56 dS m^{-1}) followed by T2:25 % WHC + 75 % RDF + MC (0.44 dS m^{-1}) and lower EC was recorded in T8:75 % WHC+ 75 % RDF + MC (0.25 dS m^{-1}) and T9:75 % WHC+ 100 % RDF + MC (0.26 dS m^{-1}) while control T10:100% WHC+ 100 % RDF (0.31 dS m^{-1}) was found to have EC 0.313 dS m^{-1}. At flowering stage highest EC was recorded in T1 (0.60 dS m^{-1}) followed by T2 (0.51 dS m^{-1}) while lowest EC was recorded in T6 (0.35 dS m^{-1}) and T9 (0.35 dS m^{-1}). At harvesting stage highest EC was recorded in T1 (0.46 dS m^{-1}) followed by T2 (0.37 dS m^{-1}) while lowest EC was recorded in T7 (0.23 dS m^{-1}) and T8 (0.24 dS m^{-1}) (Table 4.21).

Initial EC of soil used for pot experiment was found as 0.18 dS m^{-1} (Table 3.2). An increase in EC at vegetative and flowering stages was observed indicating that microbial activity was higher at initial growth stages resulting active mineralization process and release of more available minerals like nitrates, phosphates thereby increasing EC of the soil. At all the growth stages EC is higher in treatments given with 25% WHC than the treatments provided with 50% WHC and 75% WHC because under moisture limited conditions mineral solutes concentration will be more than the sufficiently moisture available conditions where solutes will get dissolved rapidly and uptaken by plants.

Mathivanan *et al*. (2014) reported that application of bioinoculants increased the EC of PGPR treated soil to 0.99 dS m^{-1} from 0.40 dS m^{-1} in control due to increased solubility of many nutrients.

Increase EC with increased concentrations of soil nitrates and inorganic N was observed in treatments inoculated with PGPR due to the production of acid forming compounds and acids that reacted with soluble salts present in the soil (Ren *et al.*, 2020).

c) Organic carbon (OC %)

At vegetative stage highest OC was found in T8:75 % WHC+ 75 % RDF + MC (0.36%) and T9:75 % WHC+ 100 % RDF + MC (0.36%) followed by T6 :50 % WHC+ 100 % RDF + MC (0.35%) which were significantly more than the control T10:100% WHC+ 100 % RDF (0.25%). At flowering stage maximum OC was recorded in T5:50 % WHC+ 75 % RDF + MC (0.39%) followed by T8:75 % WHC+ 75 % RDF + MC (0.38%) and lowest percent of OC was observed in T1: 25 % WHC + 50 % RDF + MC (0.28%). At harvesting stage highest OC was found in T9 (0.36%) followed by T8 (0.35%) and T7 (0.31%) which was significantly more than control T10:100% WHC+ 100 % RDF (0.21%) (Table 4.21).

Initial OC content in soil used for pot experiment was found to be 0.12% (Table 3.2).and significant increase in OC in microbial treated pots even under water deficit stress than the control was observed. The developed endophytic microbial consortia has the ability of IAA production, mineral solubilization, N_2 fixation and ACC deaminase activity which all together enhances the release of root exudates consisting of photosynthetically fixed carbon into rhizosphere resulting in increased organic carbon level in microbial treated pots.

Obtained results in the present study were comparable with the studies of Walker *et al.* (2003) who demonstrated that applied microbial consortia producing phytoharmones including IAA and gibberellins which stimulate the production of root exudates which are reported to transfer about 5-21% of fixed carbon to the rhizosphere, resulting in a substantial increase of soil organic carbon content.

In the study of Curá *et al.* (2017), the maize was inoculated to study the role of *Azospirillum brasilense* SP-7 and *Herbaspirillum seropedicae* Z-152 under drought stress. The report concluded enhanced biomass production, higher carbon, nitrogen, and chlorophyll content.

Table 4.21. Influence of endophytic microbial consortia on soil physico-chemical properties of maize rhizosphere in pot culture

Treatments	pH			EC			OC (%)		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T_1: 25 % WHC + 50 % RDF + MC	6.13	7.30	7.80	0.56	0.60	0.46	0.25	0.28	0.17
T_2 : 25 % WHC + 75 % RDF + MC	6.63	7.53	7.77	0.44	0.51	0.37	0.26	0.30	0.18
T_3 : 25 % WHC+ 100 % RDF + MC	6.87	7.20	7.67	0.35	0.42	0.30	0.27	0.30	0.19
T_4 : 50 % WHC+ 50 % RDF + MC	6.97	7.33	7.73	0.39	0.42	0.27	0.27	0.36	0.23
T_5 : 50 % WHC+ 75 % RDF + MC	6.73	7.43	7.77	0.36	0.40	0.28	0.32	0.39	0.22
T_6 : 50 % WHC+ 100 % RDF + MC	6.73	7.50	7.77	0.28	0.35	0.25	0.35	0.37	0.25
T_7 : 75 % WHC+ 50 % RDF + MC	6.80	7.47	7.80	0.32	0.37	0.26	0.33	0.37	0.31
T_8 : 75 % WHC+ 75 % RDF + MC	6.90	7.63	7.80	0.25	0.38	0.23	0.36	0.38	0.35
T_9 : 75 % WHC+ 100 % RDF + MC	7.00	7.40	7.60	0.26	0.35	0.24	0.36	0.37	0.36
T_{10}: 100% WHC+ 100 % RDF (Control)	6.97	7.40	7.73	0.31	0.37	0.25	0.25	0.30	0.21
CD(P=0.05)	0.27	0.26	N/A	0.02	0.03	0.02	0.02	0.03	0.02
S.Em. ±	0.09	0.09	0.08	0.01	0.01	0.00	0.00	0.01	0.00
S.Ed	0.13	0.12	0.12	0.01	0.01	0.01	0.01	0.01	0.02
CV (%)	2.39	2.13	1.91	3.43	4.52	4.60	4.78	5.03	5.00

4.5.3 Effect of endophytic microbial consortia on available nutrients in maize rhizosphere

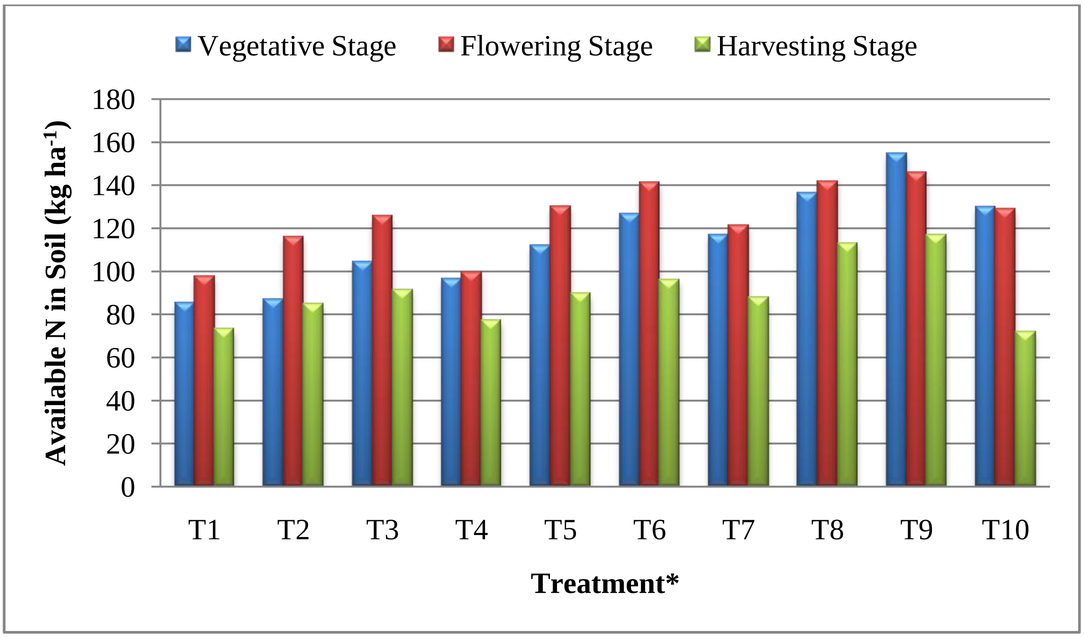
a) Available nitrogen in soil (Kg/ha)

Nitrogen is a prereqisite macro nutrient for plants for synthesizing proteins and nucleic acids including DNA. Although nitrogen is abundantly available in atmosphere (N_2), it cannot be utilized by plants. Available forms of nitrogenous compounds are added to the soil in the form of ammonium (NH_4^+) and nitrate (NO_3^-) by the process of biological nitrogen fixation. Plant growth promoting bacteria can fix atmospheric nitrogen and make available to plants. In the present investigation the endophytic bacterial isolates used in consortia were found to have nitrogen fixing ability and their influence on available nitrogen in rhizospheric soil under drought stress was studied.

At vegetative stage the available nitrogen in soil was found greater and significantly higher in T9:75 % WHC+ 100 % RDF + MC (154.71 Kg ha^{-1}). The treatments T8:75 % WHC+ 75 % RDF + MC (136.25 Kg ha^{-1}) and T6:50 % WHC+ 100 % RDF + MC (126.71 Kg ha^{-1}) were found to have available nitrogen in soil on par with the control T10:100% WHC+ 100 % RDF (129.62 Kg ha^{-1}) and lowest available nitrogen was found in T1: 25 % WHC + 50 % RDF + MC (85.16 Kg ha^{-1}) and T2: 25 % WHC + 75 % RDF + MC (86.86 Kg ha^{-1})

At flowering stage treatments T9 (145.61 Kg ha^{-1}), T8 (141.65 Kg ha^{-1}) and T6 (141.27 Kg ha^{-1}) were found to have significantly higher available nitrogen in soil where as T7:75 % WHC+ 50 % RDF + MC (121.26 Kg ha^{-1}), T5:50 % WHC+ 75 % RDF + MC (130.16 Kg ha^{-1}) and T3 :25 % WHC+ 100 % RDF + MC (125.68 Kg ha^{-1}) have recorded available nitrogen that are on par with the control T10 (129.02 Kg ha^{-1}) and lowest available nitrogen was found in T1 (97.61 Kg ha^{-1}) and T4 (99.57 Kg ha^{-1})

At harvesting stage available nitrogen in soil was found maximum in the treatments T9 (116.89 Kg ha^{-1}), T8 (113.02 Kg ha^{-1}), T6 (96.08 Kg ha^{-1}), T3 (91.54 Kg ha^{-1}) and T5 (89.49 Kg ha^{-1}) which are significantly higher than the control T10 (72.09 Kg ha^{-1}) (Table 4.22)



*Treatment details given in table 3.3

Fig. 4.7. Influence of endophytic microbial consortia on available N in maize rhizospheric soil in pot under water stress

At initial growth stages available nitrogen was found significantly higher in treatments given with 75% WHC provided 100% or 75% RDF (Fig. 4.7) indicating limited application of fertilizers by 25% didn't affect the nitrogen availability in soil. The results suggested that dose of chemical fertilizers can be minimized when applied in combination with nitrogen fixing bio inoculants; moreover the N availability can be increased than the non inoculant treatments though provided with 100% RDF.

Our results were comparable with Vali *et al.* (2020) who reported that application of inorganic fertilizers in combination with biofertilizers increased the available nitrogen in soil.

Similarly Anusha *et al.* (2017) observed that combined application of bio inoculants and recommended dose of chemical fertilizers enhanced the available nitrogen in soil by 63 %.

b) Available phosphorus in soil (Kg/ha)

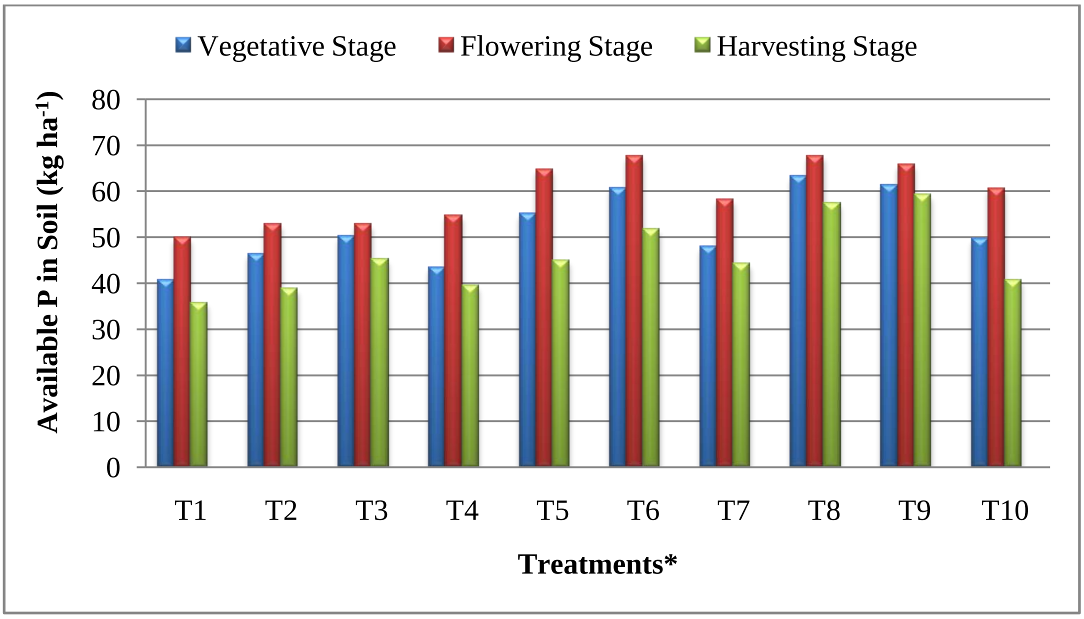
Phosphorus (P) is the primary macronutrient required by plants for their various metabolic processes, including energy transfer, signal transduction, macromolecular biosynthesis, photosynthesis, and respiration, but it is simultaneously the primary limiting mineral nutrient for plant growth due to its lower availability as well as reduced mobility. Soil constitutes about 0.5% of phosphorus; only a small amount of phosphorus is available for uptake by plants and others remain as insoluble salts. The concentration of phosphorus in most soils ranges from about 0.1 to 10 µM, while the concentration required for herbs is about 1 to 5 µM while nutrient exhaustive crops like tomato, pea and corn require about 5 to 60 µM. The available P in soil at different growth stages was estimated to evaluate the ability of developed microbial consortium to solubilize phosphorus under nutrient limiting and water deficit conditions.

At vegetative stage treatments with significantly higher available P were found to be T8:75 % WHC+ 75 % RDF + MC (63.26 Kg ha^{-1}), T9:75 % WHC+ 100 % RDF + MC (61.38 Kg ha^{-1}), T6:50 % WHC+ 100 % RDF + MC (60.82Kg ha^{-1}) and T5:50 % WHC+ 75 % RDF + MC (55.03 Kg ha^{-1}) when compared to control T10:100% WHC+ 100 % RDF (49.66 Kg ha^{-1}). At flowering stage available P in soil was significantly higher in T8 (67.68 Kg ha^{-1}), T6 (67.60 Kg ha^{-1}), T9 (65.79 Kg ha^{-1}), and T5 (64.75 Kg ha^{-1}) while the control T10 has recorded 60.59 Kg ha^{-1}of available P in soil. At harvesting stage T9 (59.19 Kg ha^{-1}), T8 (57.37 Kg ha^{-1}), T6 (51.67 Kg ha^{-1}), T3 (45.22 Kg ha^{-1}), T5 (44.95 Kg ha^{-1}) and T7 (44.18 Kg ha^{-1}) were found to have significantly higher available P (Table 4.22) in soil and the control T10 was found to have 40.81 Kg ha^{-1} of available P in soil.

The initial soil used for pot experiment was estimated to have 36.67 Kg ha^{-1} of available P (Table 3.2). Inoculation of plants with microbial consortia has abundantly improved the levels of available P (Fig. 4.8) because developed consortium consists of efficiently phoshate solubilizing bacteria *Klebsiella pneumonia* (LS3E3) with ability of 512 µg/mL of soluble P_2O_5 and *Methylorubrum populi* and *Bacillus licheniformis* with 347 µg/mL and 230 µg/mL of phosphate solubilization ability respectively. At vegetative and flowering stage the available P was higher but gradually declined at harvesting stage. The results may suggest that the inoculated endophytic bacteria could efficiently solubilize P in soil until before evading the plant tissues and most of the solubilized P must be uptaken by plants at initial stages of crop growth and flowering stage.

Table 4.22. Influence of endophytic microbial consortia on available nutrients in maize rhizosphere soil in pot culture

Treatments	Nitrogen (Kg/ha)			Phosphorus (Kg/ha)			Potassium (Kg/ha)		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T1: 25 % WHC + 50 % RDF + MC	85.16	97.61	73.08	40.69	49.82	35.80	201.29	203.81	169.11
T_2 : 25 % WHC + 75 % RDF + MC	86.86	116.09	84.75	46.37	52.87	38.75	207.49	225.79	170.23
T_3 : 25 % WHC+ 100 % RDF + MC	104.60	125.68	91.54	50.28	52.83	45.22	211.64	245.26	189.75
T_4 : 50 % WHC+ 50 % RDF + MC	96.17	99.57	77.15	43.51	54.76	39.46	226.52	216.49	213.35
T_5 : 50 % WHC+ 75 % RDF + MC	112.05	130.16	89.49	55.03	64.75	44.95	236.38	266.68	245.54
T_6 : 50 % WHC+ 100 % RDF + MC	126.71	141.27	96.08	60.82	67.60	51.67	252.99	310.16	266.69
T_7 : 75 % WHC+ 50 % RDF + MC	117.08	121.26	87.81	47.94	58.25	44.18	223.88	291.05	217.79
T_8 : 75 % WHC+ 75 % RDF + MC	136.25	141.65	113.02	63.26	67.68	57.37	275.53	314.44	269.06
T_9 : 75 % WHC+ 100 % RDF + MC	154.71	145.61	116.89	61.38	65.79	59.19	289.69	345.86	276.04
T_{10}: 100% WHC+ 100 % RDF (Control)	129.62	129.02	72.09	49.66	60.59	40.81	218.40	222.04	233.17
CD(P=0.05)	10.31	12.57	6.00	3.11	2.27	3.05	17.81	23.32	15.71
S.Em. ±	3.47	4.23	2.02	1.04	0.76	1.02	5.98	7.85	5.28
S.Ed	4.91	5.98	2.85	1.48	1.08	1.45	8.48	11.11	7.48
CV (%)	5.232	5.87	3.88	3.50	2.22	3.89	4.43	5.15	4.07



*Treatment details given in table 3.3

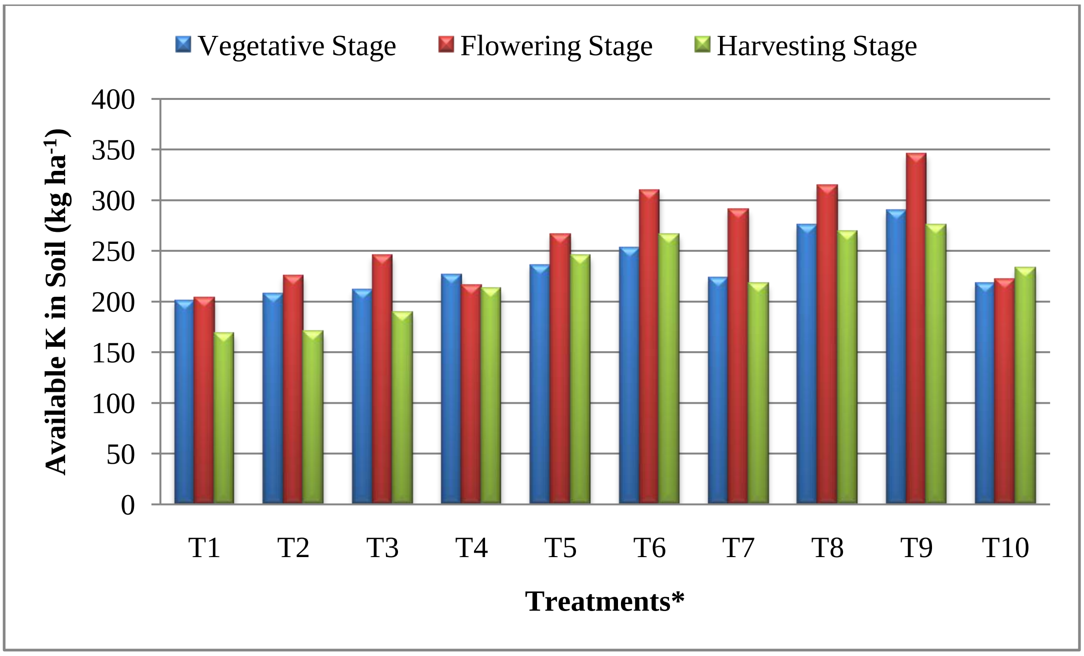
Fig. 4.8. Influence of endophytic microbial consortia on available P in maize rhizospheric soil in pot under water stress

Bacillus licheniformis RC08 and *Bacillus megaterium* RC07 were identified as efficient phosphate solubilizers in wheat and spinach (Çakmakçı *et al.,* 2007). The drought-tolerant phosphate solubilizing bacteria having multifunctional PGP traits which may be used as biofertilizers to replace chemical fertilizers are *Bacillus subtilis* IARI-IIWP-2, *Bacillus halodenitrificans* PU62; *Pseudomonas thivervalensis* IHD-3, *Pseudomonas monteilii*, IARI-IIWP-27 and *Pseudomonas* sp. which reported to exhibit multiple PGP attributes including phosphate solubilization, IAA production, siderophore production, and ACC deaminase activity (Poonguzhali *et al.* 2008; Ramadoss *et al.* 2013; Verma *et al.* 2014).

c) Availble potassium in soil (Kg/ha)

At vegetative stage treatments with significantly higher available K were found to be T9:75 % WHC+ 100 % RDF + MC (289.69 Kg ha^{-1}), T8:75 % WHC+ 75 % RDF + MC (275.53 Kg ha^{-1}), T6:50 % WHC+ 100 % RDF + MC (252.99 Kg ha^{-1}) and T5:50 % WHC+ 75 % RDF + MC (236.38 Kg ha^{-1}) when compared to control T10:100% WHC+ 100 % RDF (218.40 Kg ha^{-1}).

At flowering stage available K in soil was significantly higher in T9 (345.86 Kg ha^{-1}), T8 (314.44 Kg ha^{-1}), T6 (310.16 Kg ha^{-1}), and T7 (291.05 Kg ha^{-1}) while the control T10 has recorded 222.04 Kg ha^{-1}of available K in soil. At harvesting stage T9 (276.04 Kg ha^{-1}), T8 (269.06 Kg ha^{-1}) and T6 (266.69 Kg ha^{-1}) were found to have significantly higher available K in soil and the control T10 was found to have 233.17 Kg ha^{-1} of available K in soil (Table 4.22).



*Treatment details given in table 3.3

Fig. 4.9. Influence of endophytic microbial consortia on available K in maize rhizospheric soil in pot under water stress

The applied microbial consortia was developed with isolates with multi trait plant growth promoting traits of which *P. megateriuma* and *K. pneumonia* were efficient K solubilizers with 39 and 34 mg/mL of potassium solubilizing ability. In addition enhance microbial activity increases root exudates which can generate polysaccharides and carboxylic acids. Potassium solubilization by microbial consortia and carboxylic acids increases the available potassium to the plants (Fig. 4.9).

Similar results were obtained with the application of rhizospheric bacteria *Enterobacter cloacae* Rhizo33 in wheat cultivation, a considerably higher water soluble potassium (21.3 %) was found even without the application of chemical fertilizer when compared to the non inoculated control (Ghadam *et al.*, 2019).

Sindhu *et al.* (2016) reported significant increases in available potassium by applying KSB along with rock K and the microbial inoculants to solubilize the unsolubilized K available to plants.

4.5.4 Effect of endophytic microbial consortia on macro nutrient concentration of maize in pot culture

a) Nitrogen (%) in plant

At vegetative stage significantly higher concentration of nitrogen in plants was found (Table 4.23) in treatments T9:75 % WHC+ 100 % RDF + MC (5.28 %), T6:50 % WHC+ 100 % RDF + MC (4.92 %) and T8:75 % WHC+ 75 % RDF + MC (4.79 %) while T7:75 % WHC+ 50 % RDF + MC (4.20 %) was found to have N concentration in plants on par with the control T10:100% WHC+ 100 % RDF(4.43 %)

At flowering stage significantly higher concentration of nitrogen in plants was found in treatments T9 (5.39 %), T6 (5.23 %) and T8 (5.11 %) while T5:50 % WHC+ 75 % RDF + MC (4.61 %) was found to have N concentration in plants on par with the control T10 (4.65 %). At harvesting stage significantly higher concentration of nitrogen in plants was found in treatments T6 (3.69 %), T9 (3.49 %) and T8 (3.44 %) when compared to control T10 (0.32 %).

b) Phosphorus (%) in plant

At vegetative stage maximum percent of phosphorus concentration in plants was found (Table 4.23) in treatments T9:75 % WHC+ 100 % RDF + MC (0.64 %), T8:75 % WHC+ 75 % RDF + MC (0.64 %) and T6:50 % WHC+ 100 % RDF + MC (0.61 %) while the control T10:100% WHC+ 100 % RDF has 0.58 % of P in plants. Lowest concentration of P in plants was found in T1: 25 % WHC + 50 % RDF + MC (0.34 %).

At flowering stage maximum concentration of P in plants was recorded in T9 (1.30 %) followed by T8 (1.12 %) while lowest P concentration in plants was found in T4 (0.55 %) and T1 (0.57 %). At harvesting stage higher concentration of P in plants was found in T9 (0.72 %) followed by T8 (0.69 %) while lowest P concentration in plants was found in T4 (0.38 %) and T1 (0.38 %).

c) Potassium (%) in plants

At vegetative stage significantly higher potassium concentration in plants was found (Table 4.23) in T9:75 % WHC+ 100 % RDF + MC (6.28 %) while T6:50 % WHC+ 100 % RDF + MC (5.61 %) and T8:75 % WHC+ 75 % RDF + MC (5.57 %)

were found on par with the control T10:100% WHC+ 100 % RDF (5.99 %) while the minimum K concentration in plants was observed in T1: 25 % WHC + 50 % RDF + MC (4.53 %)

At flowering stage in plants significantly more concentration of K was found in the treatments T9 (6.67 %), T6 (6.67 %), T8 (6.44 %) and T5 (6.19 %) when compared to control T10 (5.65 %) and lowest concentration of K in plants was observed in T1 (4.31 %).

At harvesting stage significantly greater concentration of K in plants was observed in T9 (6.67 %) and T6 (6.67 %) while T8 (6.44 %) was found have K concentration on par with the control T10 (6.10 %).

Application of microbial consortium to maize has improved uptake of N, P and K even under drought stress which might be due to the ability of endophytic bacterial consortium to produce IAA efficiently and ACC deaminase activity that reduces ethylene levels all together enhancing the root growth and root hair production that helps the plants to uptake relatively more water and minerals available thereby increasing concentration of N, P and K in plant.

Endophytic bacterial strains *Bacillus* and *Brevibacillus* isolated from the leaves two medicinal plants *Fagonia mollis* and *Achillea fragrantissima* had significant positive effects on plant growth and shoot nitrogen (N) and phosphorus (P) contents (ALKahtani *et al.,* 2020)

By inoculating plants with endophytic bacteria, an increase in the lateral roots and root hairs were observed which lead to the increased surface area and consequently enhanced moisture and mineral uptake from the soil (Spaepen and Vanderleyden 2011).

In the study of Danish *et al.* (2020b) a significant improvement in shoot N, P and K, was recorded which might be due to increased root elongation. According to Zahir *et al.* (2008) the improved root elongation helps the plants to uptake relatively more water thus increasing water use efficiency under drought stress. Better uptake of nutrients was also observed in pea plants treated with ACC deaminase containing PGPR *P. brassicacearum* and *P. marginalis.*

Table 4.23. Influence of endophytic microbial consortia on macro nutrient concentration of maize in pot culture

Treatments	N Content (%) in plant			P Content (%) in plant			K Content (%) in plant		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T_1: 25 % WHC + 50 % RDF + MC	3.70	3.64	2.65	0.34	0.57	0.38	4.53	5.24	5.31
T_2 :25 % WHC + 75 % RDF + MC	3.88	4.15	3.04	0.44	0.63	0.43	4.73	6.17	5.72
T_3 :25 % WHC+ 100 % RDF + MC	4.07	4.23	2.92	0.47	0.69	0.45	4.79	6.48	6.22
T_4 :50 % WHC+ 50 % RDF + MC	3.51	3.81	2.36	0.45	0.55	0.38	4.10	4.58	5.32
T_5 :50 % WHC+ 75 % RDF + MC	4.06	4.61	3.17	0.53	0.73	0.56	4.96	6.12	6.19
T_6 :50 % WHC+ 100 % RDF + MC	4.92	5.23	3.69	0.61	0.87	0.65	5.61	6.51	6.67
T_7 :75 % WHC+ 50 % RDF + MC	4.20	4.40	3.17	0.47	0.75	0.62	4.67	5.60	5.41
T_8 :75 % WHC+ 75 % RDF + MC	4.79	5.11	3.44	0.64	1.12	0.69	5.57	6.82	6.44
T_9 :75 % WHC+ 100 % RDF + MC	5.28	5.39	3.49	0.64	1.30	0.72	6.28	6.29	6.67
T_{10}:100% WHC+ 100 % RDF (Control)	4.43	4.65	3.20	0.58	0.84	0.64	5.52	5.99	6.10
CD(P=0.05)	0.33	0.34	0.16	0.02	0.06	0.04	1.03	0.46	0.47
S.Em. ±	0.11	0.11	0.05	0.01	0.02	0.01	0.35	0.16	0.16
S.Ed	0.16	0.16	0.07	0.01	0.03	0.0	0.49	0.22	0.22
CV (%)	4.60	4.41	3.11	2.69	4.58	4.41	11.81	4.49	4.56

4.5.5 Effect of endophytic microbial consortia on uptake of macro nutrients by maize in pot culture

a) Uptake of nitrogen (g plant^{-1})

At vegetative stage significantly higher uptake of nitrogen by maize was found (Table 4.23) in treatments T9:75 % WHC+ 100 % RDF + MC (5.33 g plant^{-1}), T6:50 % WHC+ 100 % RDF + MC (4.73 g plant^{-1}) and T8:75 % WHC+ 75 % RDF + MC (4.68 g plant^{-1}) while the control T10:100% WHC+ 100 % RDF has shown 4.01 g plant^{-1} of nitrogen uptake. At flowering stage significantly higher amounts of nitrogen uptake was found in treatments T9 (13.98 g plant^{-1}), T8 (12.26 g plant^{-1}) and T6 (10.24 g plant^{-1}) while the control T10 has shown 9.96 g plant^{-1} of nitrogen uptake. At harvesting stage significantly higher nitrogen uptake by plants was found in treatments T9 (10.56 g plant^{-1}), T8 (10.28 g plant^{-1}) and T6 (10.08) when compared to control T10 (8.45 g plant^{-1})

b) Uptake of phosphorus (g plant^{-1})

At vegetative stage maximum uptake of phosphorus by plants was found (Table 4.23) in treatments T9:75 % WHC+ 100 % RDF + MC (0.65 g plant^{-1}), T8:75 % WHC+ 75 % RDF + MC (0.62 g plant^{-1}) and T6:50 % WHC+ 100 % RDF + MC (0.59 g plant^{-1}) while the control T10:100% WHC+ 100 % RDF has shown 0.52 g plant^{-1} of P uptake by plants. At flowering stage maximum P uptake by plants was recorded in T9 (3.38 g plant^{-1}) followed by T8 (2.69 g plant^{-1}) while the control T10 has shown 1.79 g plant^{-1} of P uptake by plants. At harvesting stage higher uptake of P by plants was found in T9 (2.19 g plant^{-1}) followed by T8 (2.06 g plant^{-1}) and T6 (1.78 g plant^{-1}) while lowest the control T10 has shown 1.69 g plant^{-1} of P uptake.

c) Uptake of potassium (g plant^{-1})

At vegetative stage significantly high quantity of potassium uptake by plants was found (Table 4.23) in T9:75 % WHC+ 100 % RDF + MC (6.36 g plant^{-1}) while T8:75 % WHC+ 75 % RDF + MC (5.44 g plant^{-1}), and T6:50 % WHC+ 100 % RDF + MC (5.40 g plant^{-1}) were found to have K uptake on par with the control T10:100% WHC+ 100 % RDF (4.99 g plant^{-1}). At flowering stage in plants significantly more K uptake was found in the treatments T9 (16.33 g plant^{-1}) and T8 (16.36 g plant^{-1}) when compared to control T10 (12.80 g plant^{-1}). At harvesting stage significantly higher uptake of K by plants was observed in T9 (20.14 g plant^{-1}) followed by T8 (19.23 g plant^{-1}) and T6 (18.19 g plant^{-1}) while the control T10 has shown 16.07 g plant^{-1} of K uptake by plants.

The similar results were observed by Raúl *et al*. (2009) and Krishnaveni (2010) that the application of microbial inoculants increased the uptake of both macro- and micronutrients. It was observed that microbial inoculants application had enhanced the uptake of all plant nutrients especially phosphorus content in the plant tissue.

4.5.6 Effect of endophytic microbial consortia on maize rhizosheric and endospheric microflora under drought stress

a) Total bacteria

The population of total bacteria in the initial soil used for pot experiment was 5.23 Log CFU g^{-1} of soil (Table 3.2). An abundant increase in the bacterial population was found at vegetative stage recoding maximum of 9.50 Log CFU g^{-1} of soil in the treatment T8:75 % WHC+ 75 % RDF + MC followed by 9.41 Log CFU g^{-1} of soil in T9:75 % WHC+ 100 % RDF + MC and 9.39 Log CFU g^{-1} of soil in T7:75 % WHC+ 50 % RDF + MC while the lowest bacterial population was found in treatment with no inoculum T10:100% WHC+ 100 % RDF (Control) measuring 8.93 Log CFU of bacteria per gram of soil and T1: 25% WHC + 50% RDF with 8.84 Log CFU of bacteria per gram of soil.

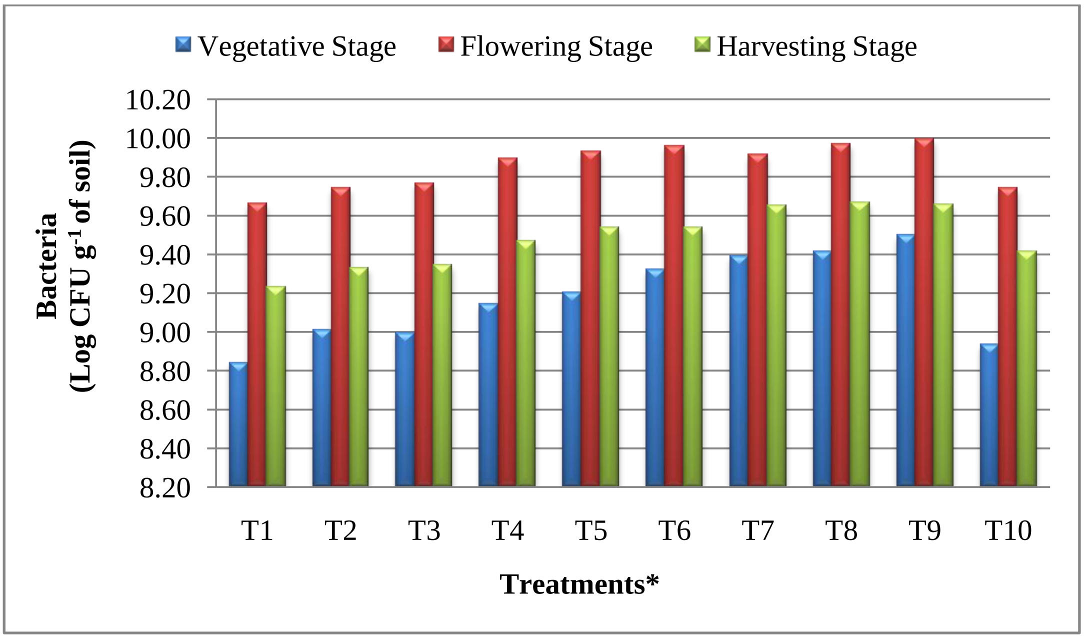
Further increase in bacterial population was observed at flowering stage recording highest in T9 (9.99 Log CFU g^{-1} of soil), T8 (9.97 Log CFU g^{-1} of soil), T6 (9.96 Log CFU g^{-1} of soil), T5 (9.93 Log CFU g^{-1} of soil) and T7 (9.91 Log CFU g^{-1} of soil) while the control was found to have 9.74 Log CFU g^{-1} of soil (Table 4.24). All the treatments inoculated with microbial consortia were found to have significantly higher bacteria population than T1 (9.66 Log CFU g^{-1} of soil) when compared to un inoculated treatment T10 with 9.74 Log CFU g^{-1} of soil (Fig. 4.10). At harvesting stage population of bacteria were found to get decreased of which maximum count was observed in T8 (9.67 Log CFU g^{-1} of soil) followed by T9 (9.66 Log CFU g^{-1} of soil) and T7 (9.65 Log CFU g^{-1} of soil).

Table 4.24. Influence of endophytic microbial consortia on uptake of macro nutrients by maize in pot culture

Treatments	Nitrogen uptake (g plant^{-1})			Phosphorus uptake (g plant^{-1})			Potassium uptake (g plant^{-1})		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T_1: 25 % WHC + 50 % RDF + MC	2.55	4.88	5.08	0.23	0.77	0.72	3.13	7.02	10.18
T_2 : 25 % WHC + 75 % RDF + MC	2.92	6.03	5.90	0.33	0.91	0.84	3.55	8.98	11.09
T_3 : 25 % WHC+ 100 % RDF + MC	3.11	6.33	6.59	0.36	1.03	1.03	3.65	9.68	14.03
T_4 : 50 % WHC+ 50 % RDF + MC	2.42	5.57	4.57	0.31	0.81	0.74	2.83	6.69	10.27
T_5 : 50 % WHC+ 75 % RDF + MC	3.49	7.27	7.89	0.46	1.15	1.38	4.27	9.65	15.38
T_6 : 50 % WHC+ 100 % RDF + MC	4.73	10.24	10.08	0.59	1.71	1.78	5.40	12.75	18.19
T_7 : 75 % WHC+ 50 % RDF + MC	3.49	7.18	7.14	0.39	1.23	1.39	3.89	9.15	12.16
T_8 : 75 % WHC+ 75 % RDF + MC	4.68	12.26	10.28	0.62	2.69	2.06	5.44	16.36	19.23
T_9 : 75 % WHC+ 100 % RDF + MC	5.33	13.98	10.56	0.65	3.38	2.19	6.36	16.33	20.14
T_{10}: 100% WHC+ 100 % RDF (Control)	4.01	9.93	8.45	0.52	1.79	1.69	4.99	12.80	16.07
CD(P=0.05)	0.38	0.88	0.57	0.03	0.19	0.14	1.02	1.08	0.93
S.Em. ±	0.13	0.30	0.19	0.01	0.06	0.05	0.34	0.36	0.31
S.Ed	0.18	0.42	0.27	0.01	0.09	0.07	0.48	0.51	0.44
CV (%)	6.01	6.11	4.31	3.54	7.00	6.08	13.60	5.75	3.68

pH of the soil has great impact on the microbial population in soil. In response water deficit stress the applied endophytic bacterial consortium induces plants to develop elongated roots with more root hairs and accelerates more of exudates secretions containing carboxylic acids, sugars etc., which favors and attracts the growth of microbes in rhizosphere. Hence, in the present study higher bacterial population was observed at 50% and 75% WHC and uninoculated control though provided with 100% WHC have very less microbial population compared to inoculated treatments.

Muhammad *et al.* (2017) reported that the mean bacterial population was enhanced by 32.5 % in microbes inoculated treatments due to the production of root exudates and availability of nutrients than the uninocuated control.



*Treatments details are given in table 3.3

Fig. 4.10. Influence of endophytic microbial consortia on total bacteria of maize rhizosphere in pot under water stress

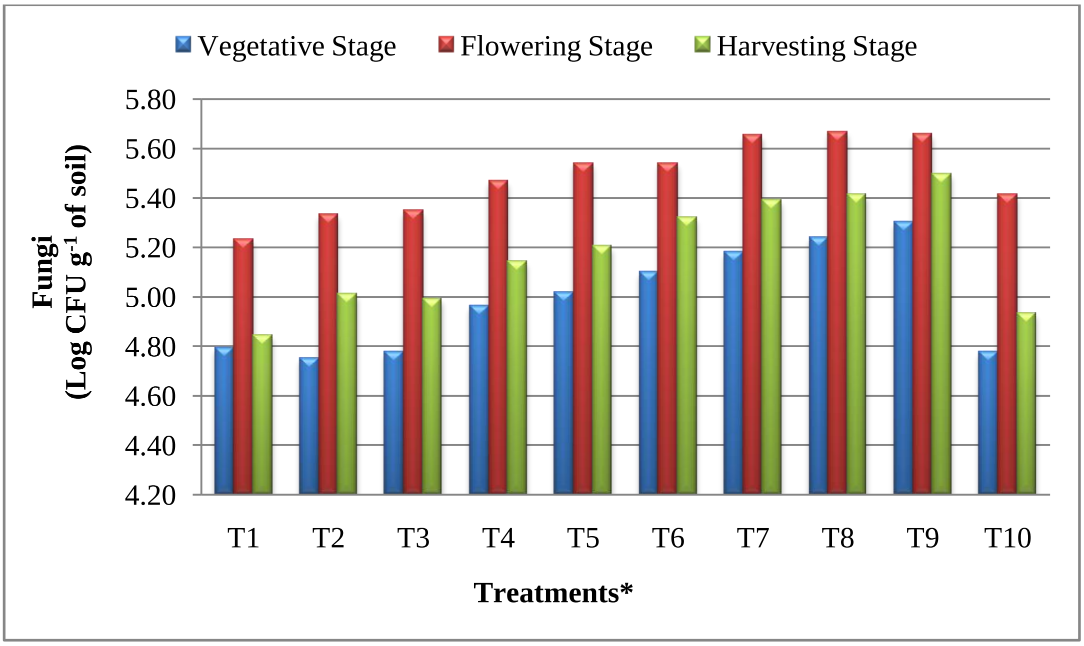
a) Total fungi

The population of total fungi in the initial soil used for pot experiment was 4.11 Log CFU g^{-1} of soil (Table 3.2). An abundant increase in the fungal population was found at vegetative stage recoding maximum of 5.30 Log CFU g^{-1} of soil in the treatment T9:75 % WHC+ 100 % RDF + MC followed by 5.24 Log CFU g^{-1} of soil in T8:75 % WHC+ 75 % RDF + MC and 5.18 Log CFU g^{-1} of soil in T7:75 % WHC+

50 % RDF + MC while the lowest fungal population was found in treatment with no inoculum T10:100% WHC+ 100 % RDF (Control) measuring 4.78 Log CFU of fungi per gram of soil.

Further increase in fungal population (Table 4.25) was observed at flowering stage recording highest in T8 (5.67 Log CFU g^{-1} of soil) followed by T9 (5.66 Log CFU g^{-1} of soil), T7 (5.65 Log CFU g^{-1} of soil). The fungal population of the un inoculated treatment T10 was found as 5.41 Log CFU g^{-1} of soil. At harvesting stage population of fungi were found to get decreased of which maximum count was observed in T9 (5.50 Log CFU g^{-1} of soil) followed by T8 (5.41 Log CFU g^{-1} of soil) and T7 (5.39 Log CFU g^{-1} of soil).

Population of fungi was found comparatively less than bacteria. For fungal growth optimum pH is 4-6, whereas pH recorded in the present study was greater than 6 in all the treatments and thereby less growth of fungi. But relatively higher fungal population was observed in consortium inoculated pots than the uninoculated control (Fig. 4.11).



*Treatment details were given in table 3.3

Fig. 4.11 Influence of endophytic microbial consortia on total fungi of maize rhizosphere in pot under water stress

Table 4.25. Influence of endophytic microbial consortia on microflora of maize rhizosphere in pot culture experiment

Treatments	Bacteria (Log CFU g^{-1} of soil)			Fungi (Log CFU g^{-1} of soil)			Actinomycetes (Log CFU g^{-1} of soil)		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T_1: 25 % WHC + 50 % RDF + MC	8.84	9.66	9.23	4.79	5.23	4.84	3.66	4.11	3.98
T_2 : 25 % WHC + 75 % RDF + MC	9.01	9.74	9.33	4.75	5.33	5.01	3.58	4.18	4.08
T_3 : 25 % WHC+ 100 % RDF + MC	8.99	9.77	9.35	4.78	5.35	4.99	3.74	4.24	4.00
T_4 : 50 % WHC+ 50 % RDF + MC	9.14	9.89	9.47	4.96	5.47	5.14	3.96	4.29	4.14
T_5 : 50 % WHC+ 75 % RDF + MC	9.20	9.93	9.54	5.02	5.54	5.20	3.82	4.28	4.25
T_6 : 50 % WHC+ 100 % RDF + MC	9.32	9.96	9.54	5.10	5.54	5.32	3.92	4.39	4.25
T_7 : 75 % WHC+ 50 % RDF + MC	9.39	9.91	9.65	5.18	5.65	5.39	4.08	4.41	4.24
T_8 : 75 % WHC+ 75 % RDF + MC	9.50	9.97	9.67	5.24	5.67	5.41	4.19	4.47	4.35
T_9 : 75 % WHC+ 100 % RDF + MC	9.41	9.99	9.66	5.30	5.66	5.50	4.24	4.41	4.38
T_{10}: 100% WHC+ 100 % RDF (Control)	8.93	9.74	9.41	4.78	5.41	4.93	3.66	4.06	3.98
CD(P=0.05)	0.10	0.05	0.07	0.21	0.07	0.10	0.33	0.14	0.13
S.Em. ±	0.03	0.02	0.02	0.07	0.02	0.03	0.11	0.05	0.04
S.Ed	0.05	0.03	0.03	0.10	0.03	0.05	0.16	0.07	0.06
CV (%)	0.61	0.31	0.44	2.47	0.77	1.08	4.87	1.87	1.78

Similar results were reported by Shivkumar *et al.* (2006) that higher fungal population was found in the consortia treated plots than non inoculated plots and fungi comprised less component out of total microbial population in soil. In studies of Chander *et al.* (2018) decreased fungal population was observed over the crop growth stage and contrary to bacterial population lesser fungal growth was observed.

b) Total actinomycetes

The population of actinomycetes was found maximum at flowering stage and gradually decreased at harvesting stage (Table 4.25). At initial growth stage maximum actinomycete count was found in T9 (4.24 Log CFU g^{-1} of soil) followed by T8 (4.19 Log CFU g^{-1} of soil) and T7 (4.08 Log CFU g^{-1} of soil). At flowering stage significantly higher population of actinomycetes was found in T8 (4.47 Log CFU g^{-1} of soil) followed by T9 and T7 with 4.41 Log CFU of actinomycetes per gram of soil. At harvesting stage population of actinomycetes were found to get decreased of which maximum count was observed in T9 (4.38 Log CFU g^{-1} of soil) followed by T8 (4.35 Log CFU g^{-1} of soil)

The initial population of actinomycetes in soil used for pot experiment was found as 3.66 Log CFU g^{-1} of soil (Table 3.2). An abundant increase in population of actinomycetes was observed in all treatments inoculated with microbial consortia when compared to uninoculated control. This may be due the increased bacterial activity, accelerated root exudates of plants, and pH alteration in soil has influenced population of actinomycetes. Similar results were reported by Brar *et al*. (2017) that population of actinomycetes was observed to get increased with the application of farm yard manure and microbial inoculants.

c) Total endophytic bacteria

At vegetative stage significantly higher endophytic population was found in T8 :75 % WHC+ 75 % RDF + MC with 4.18 Log CFU per gram of root tissue followed by T9 :75 % WHC+ 100 % RDF + MC with 4.17 Log CFU per gram of root tissue and 4.11 Log CFU per gram of stem tissue. At flowering stage highest endophytic bacterial population was shown by T5 :50 % WHC+ 75 % RDF + MC with 4.56 Log CFU per gram of root tissue, T8 :75 % WHC+ 75 % RDF + MC with 4.50 Log CFU per gram of stem tissue and 4.18 Log CFU per gram of leaf tissue. At harvesting stage maximum endophytic bacterial population was found in T9 with 4.28 Log CFU per gram of root tissue, T8 with 4.16 Log CFU per gram of stem tissue, T5 with 3.98 Log CFU per gram of leaf tissue and T9 with 4.01 Log CFU per gram of kernel (Table 4.26).

The present study suggested that inoculation of microbial consortium has improved endophytic population significantly at all the growth stages. At vegetative stage maximum colonization of endophytic bacteria was found in roots followed by stem and least colonization was found in leaves. Contradictory to that at flowering stage maximum endophytic bacterial population was observed in stem followed by roots and leaves. At the harvesting stage equal probability of colonization in all plant parts was observed but comparatively less population in kernels. At flowering stage highest population of endophytic colonizers was observed than the vegetative and harvesting stage. The variation in endophytic bacterial population and their colonization in different tissues will depend on stage of crop, intensity of drought stress and competition with indigenous microbes. It is likely that plant endophytic microbiota will adjust in accordance of water deficit stress and limiting nutrients thereby protecting plant by contributing to the plant’s physiology and performance.

Our results were in accordance with Jones *et al.* (2019) who demonstrated that the intensity and duration of water deficit stress plays vital role in growth, development, physiological metabolism of plants and also affects the plant-associated microbiota. In the present study initial growth stages were found to have more root colonizing endophytes but at later stages where drought was imposed the population in roots was decreased and more endophytic microbes were found in stem followed by leaf. Similar results were also reported by Fitzpatrick *et al.* (2018) that in angiosperms water stress reduced bacterial population and diversity in root endosphere and rhizosphere rather increased proportion of Proteobacteria and Actinobacteria in above ground parts. Similarly in sorghum, the lack of irrigation causes alterations in the root-associated microbiota, reducing its diversity, but leading to a considerable increase in the relative abundance of Actinobacteria and Firmicutes (Xu *et al.*, 2018).

Table 4.26. Influence of endophytic microbial consortia on microflora of maize endosphere in pot culture experiment

Treatments	Vegetative stage (Log CFU g^{-1} of Tissue)			Flowering stage (Log CFU g^{-1} of Tissue)			Harvesting stage (Log CFU g^{-1} of Tissue)			
	Root	**Stem**	**Leaf**	**Root**	**Stem**	**Leaf**	**Root**	**Stem**	**Leaf**	**Kernel**
T_1: 25 % WHC + 50 % RDF + MC	4.01	3.76	3.38	4.25	4.38	3.89	4.22	4.03	3.69	3.79
T_2 : 25 % WHC + 75 % RDF + MC	4.05	3.66	3.65	4.33	4.26	3.92	3.94	3.88	3.66	3.43
T_3 : 25 % WHC+ 100 % RDF + MC	4.02	3.73	3.63	4.38	4.44	3.88	4.11	3.98	3.68	3.62
T_4 : 50 % WHC+ 50 % RDF + MC	4.03	3.89	3.75	4.35	4.29	4.04	4.14	4.10	3.72	3.80
T_5 : 50 % WHC+ 75 % RDF + MC	4.04	3.95	3.66	4.56	4.45	4.06	4.26	4.15	3.98	3.90
T_6 : 50 % WHC+ 100 % RDF + MC	4.09	3.95	3.76	4.47	4.39	3.98	4.17	4.03	3.84	3.92
T_7 : 75 % WHC+ 50 % RDF + MC	4.10	3.91	3.70	4.47	4.34	3.99	4.19	3.99	3.60	3.86
T_8 : 75 % WHC+ 75 % RDF + MC	4.18	4.09	3.54	4.51	4.50	4.18	4.19	4.16	3.90	3.94
T_9 : 75 % WHC+ 100 % RDF + MC	4.17	4.11	3.62	4.54	4.49	4.10	4.28	4.12	3.95	4.01
T_{10}: 100% WHC+ 100 % RDF (Control)	3.76	3.50	3.42	4.22	4.24	3.76	4.09	3.84	3.66	3.63
CD(P=0.05)	0.16	0.28	N/A	0.12	0.09	0.17	N/A	0.20	N/A	0.30
S.Em. ±	0.05	0.10	0.14	0.04	0.03	0.06	0.07	0.07	0.11	0.10
S.Ed	0.08	0.13	0.19	0.06	0.04	0.08	0.09	0.09	0.16	0.14
CV (%)	2.27	4.27	6.53	1.52	1.21	2.51	2.75	2.85	5.16	4.65

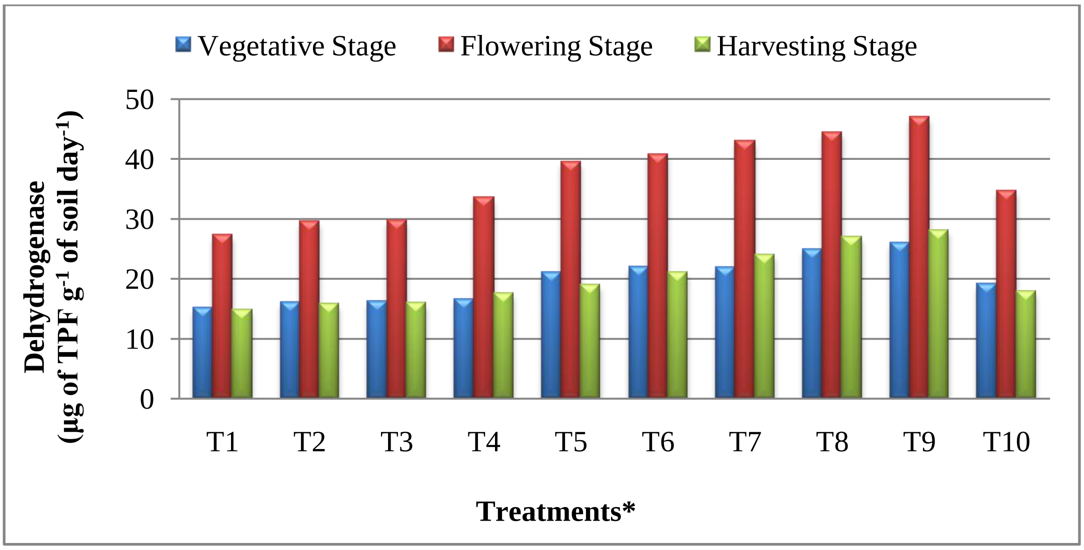
4.5.7 Effect of endophytic microbial consortia on soil enzyme activity in maize rhizosphere

a) Dehydrogenase (µg of TPF g^{-1} of soil day^{-1})

At vegetative stage significantly higher dehydrogenase activity was found (Table 4.27) in the treatments in the following order T9 :75 % WHC+ 100 % RDF + MC (26.10 µg of TPF g^{-1} of soil day^{-1}), T8 :75 % WHC+ 75 % RDF + MC (24.93 µg of TPF g^{-1} of soil day^{-1}), T6 :50 % WHC+ 100 % RDF + MC (22.05 µg of TPF g^{-1} of soil day^{-1}), T7 :75 % WHC+ 50 % RDF + MC (21.83 µg of TPF g^{-1} of soil day^{-1}) and T5 :50 % WHC+ 75 % RDF + MC (21.00 µg of TPF g^{-1} of soil day^{-1}) while in control where 100% WHC and 100% RDF were maintained and without any microbial treatment was found to have 19.14 µg of TPF g^{-1} of soil day^{-1} of soil dehydrogenase activity. Lowest activity was found in treatments given with only 25%WHC i.e., T1 (15.13 µg of TPF g^{-1} of soil day^{-1}), T2 (16.04 µg of TPF g^{-1} of soil day^{-1}) and T3 (16.29 µg of TPF g^{-1} of soil day^{-1}).

At flowering stage also similar trend was followed as of vegetative stage with significantly greater activity of dehydrogenase in the treatments as follows T9 (46.93 µg of TPF g^{-1} of soil day^{-1}), T8 (44.43 µg of TPF g^{-1} of soil day^{-1}), T7 (43.03 µg of TPF g^{-1} of soil day^{-1}), T6 (40.8340.83 µg of TPF g^{-1} of soil day^{-1}) and T5 (39.43 µg of TPF g^{-1} of soil day^{-1}) while control has dehydogenase activity of 34.73 µg of TPF g^{-1} of soil day^{-1}. At harvesting stage the maximum dehydrogensae activity in rhizhospheric soil was found in the treatments T9 (28.10 µg of TPF g^{-1} of soil day^{-1}), T8 (26.93 µg of TPF g^{-1} of soil day^{-1}), T7 (24.03 µg of TPF g^{-1} of soil day^{-1}), T6 (21.13 µg of TPF g^{-1} of soil day^{-1}) and T5 (18.97 µg of TPF g^{-1} of soil day^{-1}) while control has dehydogenase activity of 17.93 µg of TPF g^{-1} of soil day^{-1}.

Dehydrogenase activity can be considered as a good measure of microbial activity in soil. Moreover, soil organic matter content affects the soil enzyme activities. Organic matter can be improved by the application of optimum dose of nutrients through inorganic fertilizers and manures through organic fertilizers which induces the microbes to enhance soil enzyme activities (Gao *et al.,* 2020). In the present study enhance soil dehydrogenase activity was found (Fig. 4.12) in treatments provided with 100% and 75% RDF even under water deficit conditions and the enzyme activity was found prominent at flowering stage.



*Treatment details were given in table 3.3

Fig. 4.12. Influence of endophytic microbial consortia on dehydrogenase activity of maize rhizosphere soil in pot under water stress

Sireesha *et al.* (2017) reported that inoculation of biofertilizers, organic manures along with inorganic fertilizer has profoundly increased soil enzyme activities compared to control treated with no bio inoculums.

Similar results were also obtained by Khati *et al.* (2017), showing significant increase in soil dehydrogenase activity in all treatments inoculated with plant growth promoting rhizobacteria when compared to untreated soil.

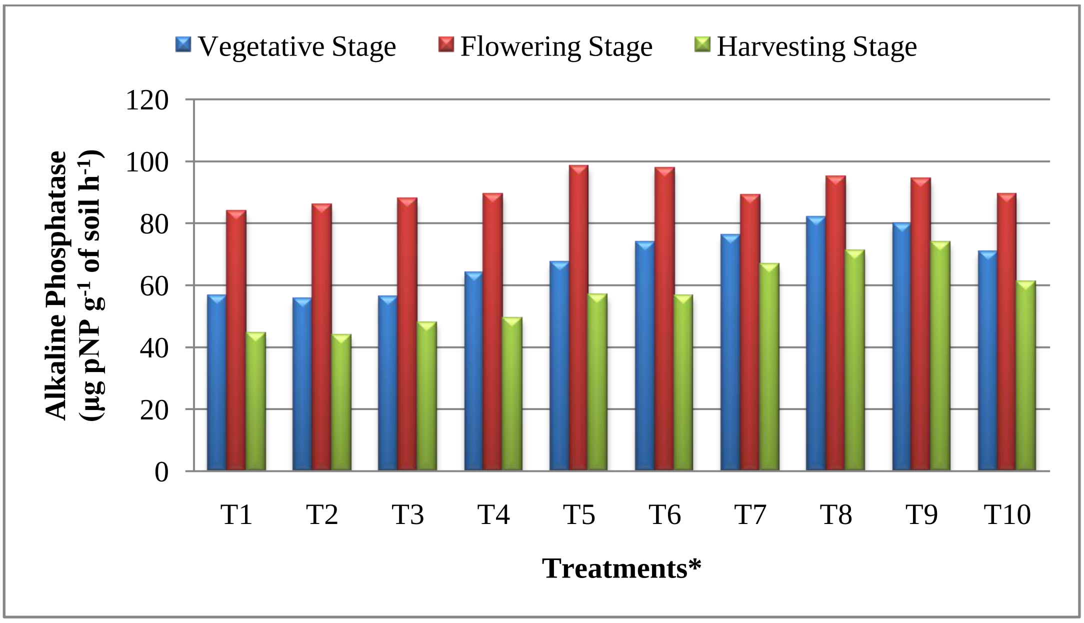
b) Phosphatase (µg pNP g^{-1} of soil h^{-1})

At vegetative stage significantly highest alkaline phosphatase activity in soil was observed in T8 :75 % WHC+ 75 % RDF + MC (82.13 µg pNP g^{-1} of soil h^{-1}) followed by T9 :75 % WHC+ 100 % RDF + MC (79.73 µg pNP g^{-1} of soil h^{-1}), T7 :75 % WHC+ 50 % RDF + MC (76.00 µg pNP g^{-1} of soil h^{-1}) and T6 :50 % WHC+ 100 % RDF + MC (74.14 µg pNP g^{-1} of soil h^{-1}). While the treatment T5:50 % WHC+ 75 % RDF + MC (67.43 µg pNP g^{-1} of soil h^{-1}) was found to have phosphatase activity on par with the control T10:100% WHC+ 100 % RDF (70.87 µg pNP g^{-1} of soil h^{-1}).

At flowering stage significantly greater alkaline phosphatase activity was observed in T5 (98.43 µg pNP g^{-1} of soil h^{-1}) followed by T6 (97.77 µg pNP g^{-1} of soil h^{-1}), T8 (94.90 µg pNP g^{-1} of soil h^{-1}) and T9 (94.33 µg pNP g^{-1} of soil h^{-1}) while treatments T7 (89.20 µg pNP g^{-1} of soil h^{-1}), T4 (89.33 µg pNP g^{-1} of soil h^{-1}) and T3

(87.80 µg pNP g^{-1} of soil h^{-1}) were found to have phosphatase activity on par with the control T10 (89.50 µg pNP g^{-1} of soil h^{-1}). At harvesting stage highest alkaline phosphatase activity T9 (73.83 µg pNP g^{-1} of soil h^{-1}), T8 (71.13 µg pNP g^{-1} of soil h^{-1}) and T7 (66.83 µg pNP g^{-1} of soil h^{-1}) while the control T10 (61.13 µg pNP g^{-1} of soil h^{-1}) (Table 4.27).

In the present study alkaline phosphatase activity in soil was found to increase in all treatments provided with 100%, 75% or 50% RDF along with microbial consortia at 75% and 50% WHC, the enzyme activity was found prominent at flowering stage (Fig. 4.13).



*Treatment details were given in table 3.3

Fig. 4.13. Influence of endophytic microbial consortia on alkaline phosphotase activity of maize rhizosphere soil in pot under water stress

Laxman *et al.* (2017) observed the comparable results in both acid and alkaline phosphatase activites in soils treated with microbial consortia particularly increased activities were found high at flowering stage of crop. Similarly Khati *et al.* (2017) has confirmed that one to two folds increase in the alkaline phosphatase activity in soil in all treatments inoculated with PGPR strain in comparison to untreated soil.

Table 4.27. Influence of endophytic microbial consortia on soil enzymes activity of maize rhizosphere of pot culture

Treatments	Dehydrogenase (μg of TPF g^{-1} of soil day^{-1})			Phosphatase (μg pNP g^{-1} of soil h^{-1})		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T1: 25 % WHC + 50 % RDF + MC	15.13	27.30	14.90	56.77	84.00	44.63
T_2 :25 % WHC + 75 % RDF + MC	16.04	29.50	15.80	55.60	86.10	44.03
T_3 :25 % WHC+ 100 % RDF + MC	16.29	29.70	15.90	56.27	87.80	48.10
T_4 :50 % WHC+ 50 % RDF + MC	16.63	33.53	17.53	64.00	89.33	49.53
T_5 :50 % WHC+ 75 % RDF + MC	21.00	39.43	18.97	67.43	98.43	56.90
T_6 :50 % WHC+ 100 % RDF + MC	22.05	40.83	21.13	74.10	97.77	56.77
T_7 :75 % WHC+ 50 % RDF + MC	21.83	43.03	24.03	76.00	89.20	66.83
T_8 :75 % WHC+ 75 % RDF + MC	24.93	44.43	26.93	82.13	94.90	71.13
T_9 :75 % WHC+ 100 % RDF + MC	26.10	46.93	28.10	79.73	94.33	73.83
T_{10}:100% WHC+ 100 % RDF (Control)	19.14	34.73	17.92	70.87	89.50	61.13
CD(P=0.05)	1.53	1.95	1.51	3.63	4.44	3.85
S.Em. ±	0.51	0.66	0.51	1.22	1.49	1.29
S.Ed	0.73	0.93	0.72	1.73	2.11	1.83
CV (%)	4.51	3.10	4.41	3.11	2.84	3.92

Table 4.28. Influence of endophytic microbial consortia on plant growth parameters of maize in pot culture

Treatments	Plant height (cm)			Biomass (g)		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T1: 25 % WHC + 50 % RDF + MC	85.00	132.67	141.33	69.00	134.00	191.67
T_2 : 25 % WHC + 75 % RDF + MC	88.33	134.67	155.33	75.00	145.33	194.00
T_3 : 25 % WHC+ 100 % RDF + MC	92.67	167.00	165.00	76.33	149.67	225.67
T_4 : 50 % WHC+ 50 % RDF + MC	89.33	133.33	159.00	69.00	146.00	193.33
T_5 : 50 % WHC+ 75 % RDF + MC	105.67	140.33	161.67	86.00	157.67	248.67
T_6 : 50 % WHC+ 100 % RDF + MC	96.33	144.33	176.33	96.00	196.00	273.00
T_7 : 75 % WHC+ 50 % RDF + MC	111.67	140.33	167.33	83.00	163.33	225.33
T_8 : 75 % WHC+ 75 % RDF + MC	116.33	166.67	180.67	97.67	240.00	298.67
T_9 : 75 % WHC+ 100 % RDF + MC	124.33	176.00	188.00	101.00	259.67	302.67
T_{10}: 100% WHC+ 100 % RDF (Control)	113.00	163.33	174.33	90.33	213.67	263.67
CD(P=0.05)	4.29	5.49	13.58	3.51	12.06	17.08
S.Em. ±	1.44	1.85	4.57	1.18	4.06	5.74
S.Ed	2.04	2.61	6.46	1.67	5.74	8.13
CV (%)	2.44	2.13	4.74	2.43	3.89	4.12

4.5.8 Effect of endophytic microbial consortia on plant growth parameters of maize

a) Plant height (cm)

At vegetative stage significantly higher plant height was measured in T9:75 % WHC+ 100 % RDF + MC (124.33 cm) while the treatments T8 :75 % WHC+ 75 % RDF + MC (116.33 cm) and T7 :75 % WHC+ 50 % RDF + MC (111.67 cm) were found to record plant height on par with the control T10:100% WHC+ 100 % RDF (113.00 cm). Lowest plant height was measures in T1: 25 % WHC + 50 % RDF + MC (85.00 cm) followed by T2:25 % WHC + 75 % RDF + MC (88.33 cm).

At flowering stage maximum and significantly higher plant height was recorded in T9 (176.00 cm) while the treatment T8 (166.67 cm) was found to have plant height on par with the control T10 (163.33 cm). Treatments with minimum plant height were found to be T1 (132.67 cm) and T4 (133.33 cm). At harvesting stage significantly higher plant height was recorded in T9 while the treatments T8 (180.67 cm), T6 (176.33 cm) and T7 (167.33 cm) were found to have plant height on par with the control T10 (174.33 cm) (Table 4.28).

In the present study increased plant height in inoculated plants was observed at all growth stages than the control which might be due to the efficient IAA production, nutrient mineralization and other plant growth promoting activities of applied microbial consortia.

An endophytic bacterium *Pantoea alhagi* isolated from wheat had the ability to improve plant growth, root length, and drought tolerance by producing IAA, siderophores, ammonia, and protease. In addition, phosphate solubilization activity was also reported (Chen *et al.*, 2017). Plants inoculated with PGP endophytic bacteria might sustain (or nearly sustain) shoot growth and enhance yield under drought stress conditions (Egamberdieva *et al.*, 2017a).

Azospirillum sp. and *Bacillus thuringiensis* are capable of synthesizing IAA and evidently causing the enhancement of the formation of lateral roots and root hairs, thereby helping plants to grow under water deficit conditions (Armada *et al.*, 2014)

Inoculation of PGPR mixture 1 (P1) significantly influenced corn plant height, on average, with an increase of 4.6 and 8.1% at 45 and 57 DAP, respectively.

Inoculation of PGPR strains improved plant growth of corn under limited and deficit irrigation conditions. There were no irrigation × fertility × PGPR inoculants interaction or any combination of the effects observed between treatments (Lin *et al.*, 2020).

b) Biomass (g)

At vegetative stage significantly higher biomass was produced by T9:75 % WHC+ 100 % RDF + MC (101.00 g) followed by T8:75 % WHC+ 75 % RDF + MC (97.67 g) and T6 :50 % WHC+ 100 % RDF + MC (96 g) when compared to control T10:100% WHC+ 100 % RDF (90.33 g) and lowest biomass production was observed in T1: 25 % WHC + 50 % RDF + MC (69.00 g) and T4 :50 % WHC+ 50 % RDF + MC (69.00 g).

At flowering stage maximum and significantly higher biomass was produced by T9 (259.67 g) followed by T8 (240.00 g) when compared to control T10 (213.67 g) while the lowest biomass production was recorded in T1 (134.00 g) and T2:25 % WHC + 75 % RDF + MC (145.33 g). At harvesting stage significantly higher biomass production was recorded in T9 (302.67 g) followed by T8 (298.67 g) and T6 (273.00 g) while treatment T5:50 % WHC+ 75 % RDF + MC (248.67 g) was found to have biomass on par with the control T10 (263.67 g) (Table 4.28).

Inoculation of endophytic microbial consortia has improved biomass of maize plants when compared to uninoculated control. Several studies have shown that endophytic bacteria increased plant biomass and yield under water deficit conditions. For example, In the study of Curá *et al.* (2017), the maize was inoculated to study the role of *Azospirillum brasilense* SP-7 and *Herbaspirillum seropedicae* Z-152 under drought stress and reported enhanced biomass production

Naveed *et al.* (2014a) studied the inoculation of maize plants with the *Burkholderia phytofirmans* improved shoot growth. The inoculated plants showed higher root and shoot biomass compared to non-inoculated plants under drought stress. In addition, higher yield of inoculated plants was also recorded.

Vardharajula *et al.* (2011) reported that corn plants inoculated with plant growth-promoting *Bacillus* sp. under drought stress conditions showed improvement in the shoot growth as well as the dry biomass. In the study of Marulanda *et al.*

(2009), *Trifolium repens* plants were treated with *Pseudomonas putida* and *Bacillus megaterium* under drought stress; the study concluded the increase in root-shoot biomass and water content was due to IAA production

4.5.9 Effect of developed endophytic microbial consortia on yield attributes of maize in pot culture

a) Cob weight (g/ pot)

Significantly more cob weight was obtained (Fig. 4.14a) in the treatments T9:75 % WHC+ 100 % RDF + MC (163.67 g), T8:75 % WHC+ 75 % RDF + MC (160.33 g), T6:50 % WHC+ 100 % RDF + MC (152.00 g), T5:50 % WHC+ 75 % RDF + MC (146.00 g) and T7:75 % WHC+ 50 % RDF + MC (131.33 g). While the control T10:100% WHC+ 100 % RDF has 127.00 g and lowest cob weight was recorded in T1: 25 % WHC + 50 % RDF + MC (95.00 g) and T2:25 % WHC + 75 % RDF + MC (98.00 g) (Table 4.28)

b) Grain yield (g/ pot)

Significantly higher grain yield was recorded in the treatments T9:75 % WHC+ 100 % RDF + MC (53.56 g), T8:75 % WHC+ 75 % RDF + MC (51.39 g), T6:50 % WHC+ 100 % RDF + MC (49.62 g), T5:50 % WHC+ 75 % RDF + MC (49.01 g) and T7:75 % WHC+ 50 % RDF + MC (48.70 g) when compared to T10:100% WHC+ 100 % RDF (37.10 g) while lowest grain yield was obtained in T1: 25 % WHC + 50 % RDF + MC (25.47 g) and T2 :25 % WHC + 75 % RDF + MC (29.54 g) (Fig. 4.14b)

Three novel endophytic rhizobial strains (RRE3, RRE5, and RRE6) were isolated from naturally growing surface sterilized rice roots. These isolates had the ability to nodulate common bean (*Phaseolus vulgaris*). A significant increase in biomass and grain yield was also recorded in greenhouse-grown rice plants inoculated with these isolates (Singh *et al.,* 2006)

Worldwide, there is a huge growing market of microbial community for plant growth improvement and higher yield under biotic and abiotic stresses with an annual growth rate of approximately 10% (Timmusk *et al.,* 2017)

c) Test weight (g/100 seed)

The treatments which obtained statistically significant amount of kernel test weight are T9:75 % WHC+ 100 % RDF + MC (31.73 g), T8:75 % WHC+ 75 % RDF + MC (30.67 g), T6:50 % WHC+ 100 % RDF + MC (26.88 g), T5:50 % WHC+ 75 % RDF + MC (25.68 g) and T7:75 % WHC+ 50 % RDF + MC (25.52 g). And lowest kernel test weight was recorded in T4:50 % WHC+ 50 % RDF + MC (18.82 g) and the control T10:100% WHC+ 100 % RDF has 25.14 g of kernel test weight. (Fig. 4.14c)

Application of ACC deaminase producing *Enterobacter cloacae* and *Achromobacter xylosoxidans* supplemented with 1.50% biochar has improved 100 seed weight and grain yield of maize even under drought stress (Danish *et al.,* 2020a).

d) Protein (%)

The protein content in the maize kernels were found to be significantly higher in T9:75 % WHC+ 100 % RDF + MC (5.96 %), T8:75 % WHC+ 75 % RDF + MC (5.75 %), T6:50 % WHC+ 100 % RDF + MC (5.64 %), T5:50 % WHC+ 75 % RDF + MC (5.20 %) and T7:75 % WHC+ 50 % RDF + MC (5.04 %) when compared to control T10:100% WHC+ 100 % RDF (4.81 %) and minimum protein content was observed in T1: 25 % WHC + 50 % RDF + MC (4.14 %) and T2 :25 % WHC + 75 % RDF + MC (4.39 %) (Fig. 4.15).

Emami *et al.* (2018) has demonstrated that increased protein content in wheat cultivars by 11.3% (Marvdasht) and 9% (Roshan) due to co inoculation of pot cultures with the rhizospheric and endophytic plant growth promoting bacteria. Similarly Shehata and Khawas (2003) also reported that application of bio inoculation of sunflower crop has increased protein content in seed.

e) Starch (%)

The starch content in the maize kernels were found to be significantly higher in T9:75 % WHC+ 100 % RDF + MC (61.45 %), T8:75 % WHC+ 75 % RDF + MC (58.24 %), T6:50 % WHC+ 100 % RDF + MC (57.55 %), T5:50 % WHC+ 75 % RDF + MC (51.65 %) and T7 :75 % WHC+ 50 % RDF + MC (45.49 %) when compared to control T10:100% WHC+ 100 % RDF (46.37 %) and minimum starch

Table 4.29. Influence of developed endophytic microbial consortia on yield attributes of maize in pot culture

Treatments	Cob weight (g/ pot)	Grain yield (g/ pot)	Test weight (g/100 seed)	Protein (%)	Starch (%)	Stover yield (g/ pot)
T1: 25 % WHC + 50 % RDF + MC	95.00	25.47	19.57	4.14	39.59	82.12
T_2 :25 % WHC + 75 % RDF + MC	98.00	29.54	19.75	4.39	42.48	83.91
T_3 :25 % WHC+ 100 % RDF + MC	115.67	35.10	21.49	4.67	47.34	92.95
T_4 :50 % WHC+ 50 % RDF + MC	103.33	35.52	18.82	4.95	41.44	81.53
T_5 :50 % WHC+ 75 % RDF + MC	146.00	49.01	25.68	5.20	51.65	110.87
T_6 :50 % WHC+ 100 % RDF + MC	152.00	49.62	26.88	5.64	57.55	122.27
T_7 :75 % WHC+ 50 % RDF + MC	131.33	48.70	25.52	5.04	45.49	104.82
T_8 :75 % WHC+ 75 % RDF + MC	160.33	51.39	30.67	5.75	58.24	130.41
T_9 :75 % WHC+ 100 % RDF + MC	163.67	53.56	31.73	5.96	61.45	141.26
T_{10}:100% WHC+ 100 % RDF (Control)	127.00	37.10	25.14	4.81	46.37	107.17
CD(P=0.05)	7.66	2.50	2.37	0.17	2.91	9.31
S.Em. ±	2.58	0.84	0.79	0.05	0.98	3.13
S.Ed	3.64	1.19	1.12	0.08	1.38	4.43
CV (%)	3.45	3.51	5.63	2.01	3.45	5.13

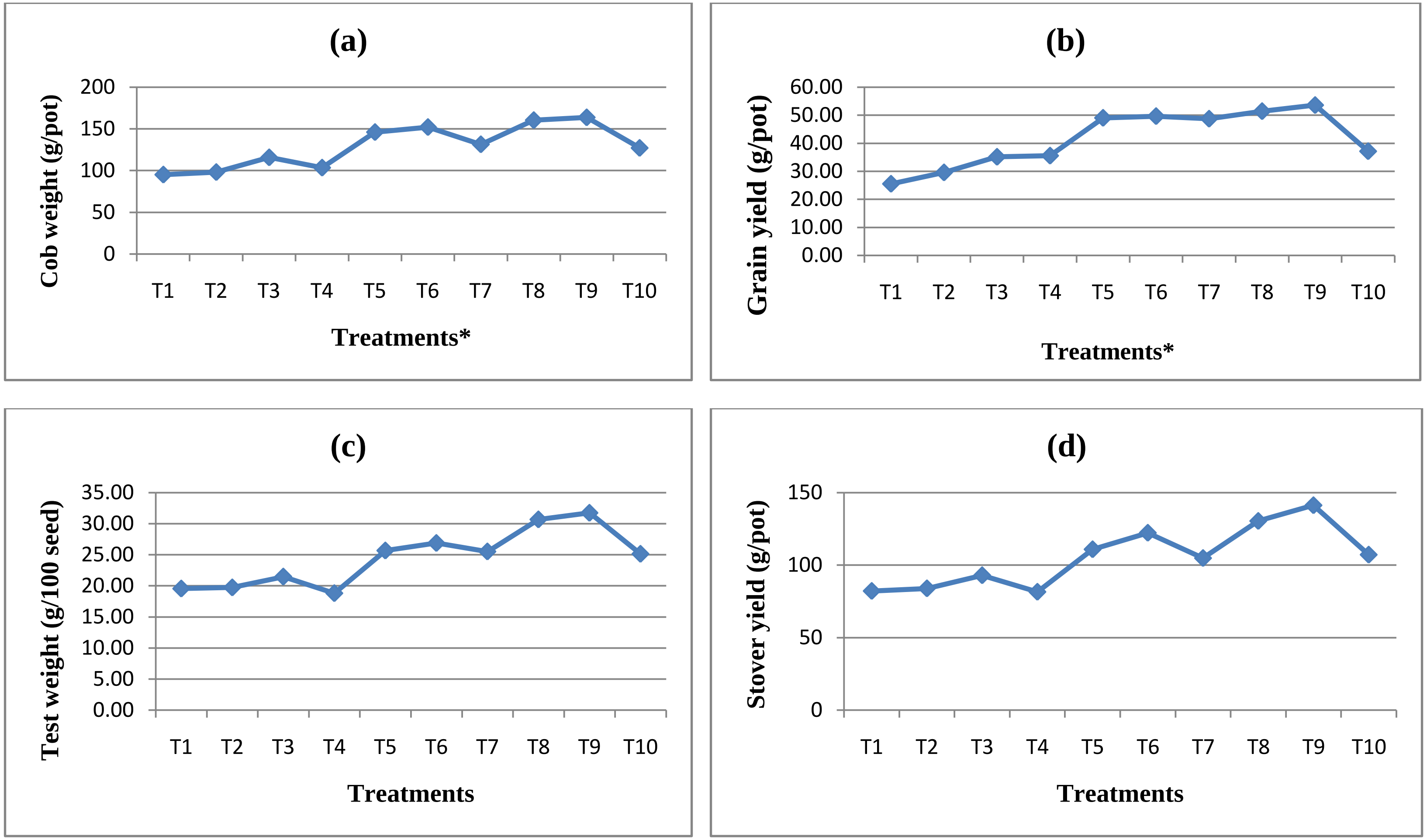


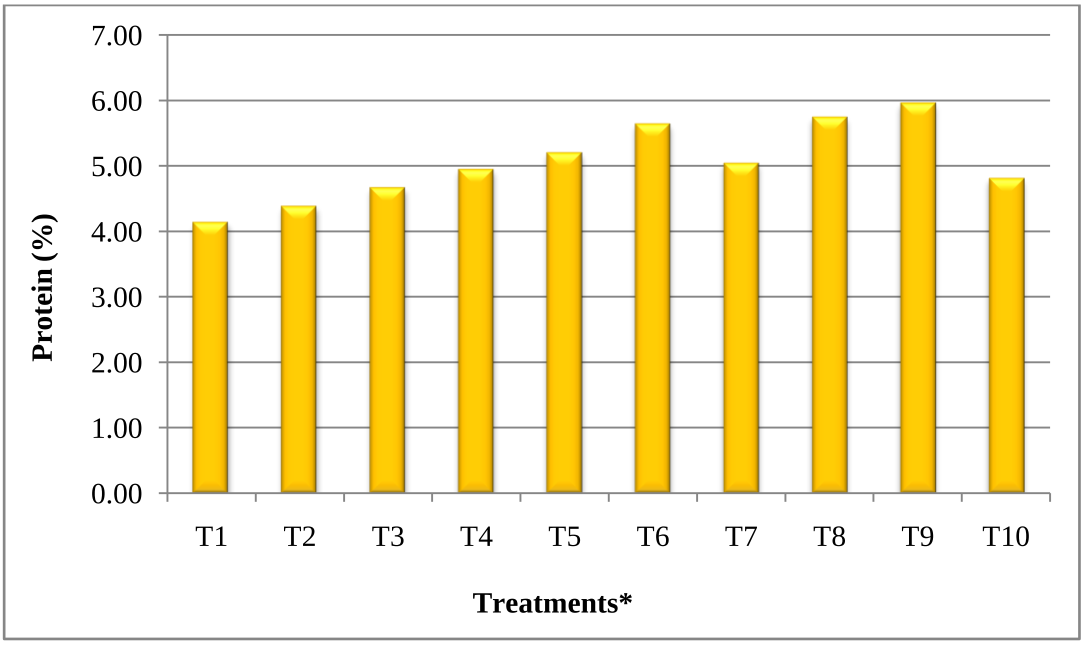
Fig. 4.14. Influence of endophytic microbial consortia on yield attributes maize grown in pot under water stress

a) Cob Weight b) Grain Yield c) Test Weight d) Stover Yield

content was observed in T1: 25 % WHC + 50 % RDF + MC (82.12 %) and T4:50 % WHC+ 50 % RDF + MC (41.44 %) (Fig. 4.16)

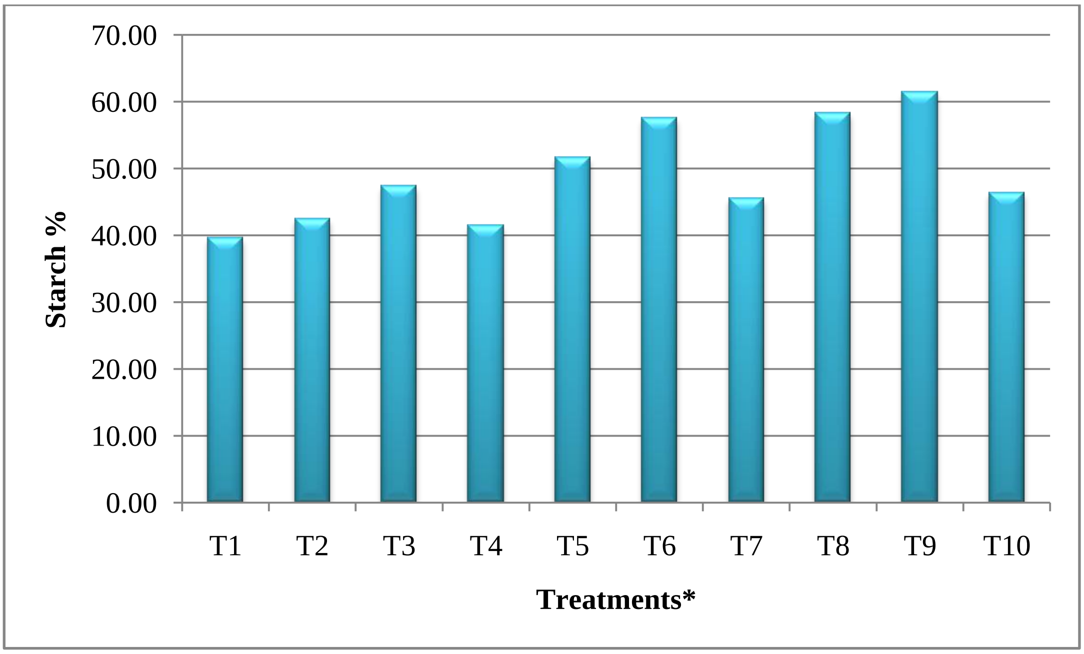
f) Stover yield (g/ pot)

Maximum and statistically higher stover yield was obtained in the treatments T9:75 % WHC+ 100 % RDF + MC (141.23 g) followed by T8:75 % WHC+ 75 % RDF + MC (130.41 g) and T6:50 % WHC+ 100 % RDF + MC (122.27 g), while the treatments T5:50 % WHC+ 75 % RDF + MC (110.87 g) and T7:75 % WHC+ 50 % RDF + MC (104.82 g) have recorded stover yield on par with the control T10:100% WHC+ 100 % RDF (107.17 g). Lowest stover yield was obtained in treatments T4:50 % WHC+ 50 % RDF + MC (81.53 g) and T1: 25 % WHC + 50 % RDF + MC (82.12 g) (Fig. 4.14d).



*Treatment details were given in table 3.3

Fig. 4.15. Influence of endophytic microbial consortia on protein content in maize grains grown in pot under water stress



*Treatment details were given in table 3.3

Fig. 4.16. Influence of endophytic microbial consortia on starch content in maize grains grown in pot under water stress

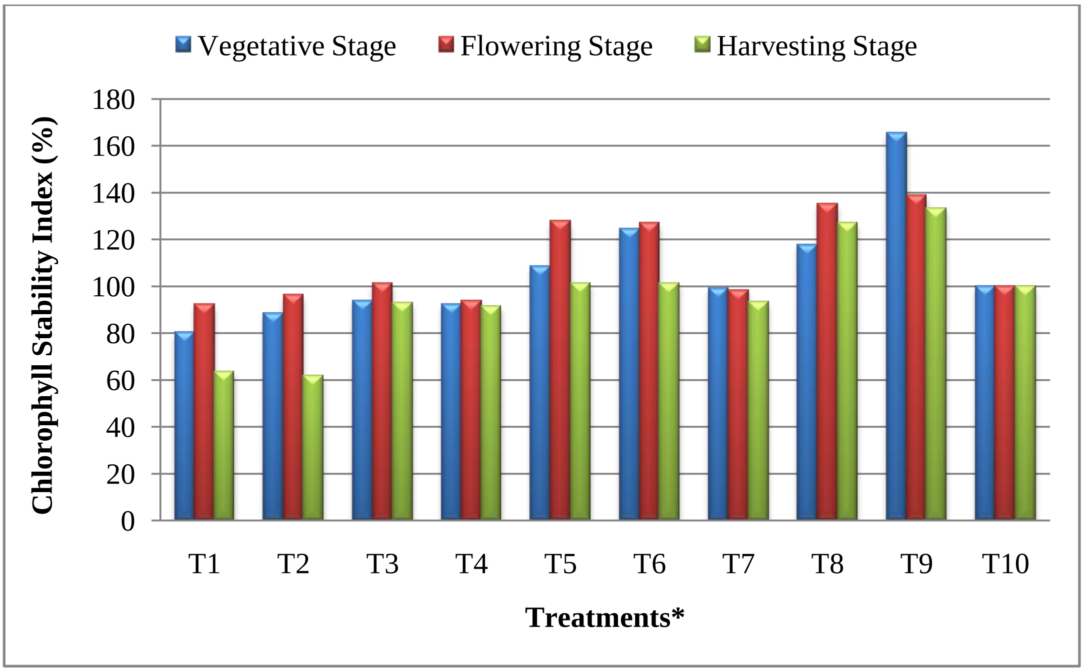
4.6 INFLUENCE OF ENDOPHYTIC MICROBIAL CONSORTIA ON MAIZE AGAINST WATER STRESS UNDER FIELD CONDITIONS

4.6.1 Influence of endophytic microbial consortia on plant biochemical parameters of maize under water stress imposed field

a) Chlorophyll stability index (CSI %)

Chlorophyll is one of the major chloroplast components for photosynthesis, and chlorophyll stability index has a positive relationship with photosynthetic rate. At vegetative stage when compared to control T10: Full Irrigations + 100 % RDF three treatments have shown significantly higher CSI namely T9:7 Irrigations + 100 % RDF + MC with maximum CSI (165.67%) followed by T8:7 Irrigations + 75 % RDF + MC (117.67%) and T6:5 Irrigations + 100 % RDF + MC (124.33%). Treatments T5:5 Irrigations + 75 % RDF + MC and T7:7 Irrigations + 50 % RDF + MC were on par with the control with 108.67% and 99.33% of CSI respectively. Minimum CSI 80.33% and 88.67% was observed in the treatments T1:3 Irrigations + 50 % RDF + MC and T2:3 Irrigations + 75 % RDF + MC respectively (Table 4.30)

At flowering stage four treatments were found to be with significantly higher CSI compared to control. Maximum CSI was observed for T9:7 Irrigations + 100 % RDF + MC with 138.67% followed by T8:7 Irrigations + 75 % RDF + MC with 135.33%, T5:5 Irrigations + 75 % RDF + MC and T6:5 Irrigations + 100 % RDF + MC with 127% of CSI. The CSI was on par with T3:3 Irrigations + 100 % RDF + MC (101%). Minimum CSI was observed in T1:3 Irrigations + 50 % RDF + MC (92.00%). At harvest stage maximum and significant CSI 133.33 % was observed in T9:7 Irrigations + 100 % RDF + MC followed by T8:7 Irrigations + 75 % RDF + MC with 127 % of CSI. Treatments T5:5 Irrigations + 75 % RDF + MC and T6:5 Irrigations + 100 % RDF + MC were found to have CSI of 101% which was on par with the control. Minimum CSI 61.67% was observed in T2:3 Irrigations + 75 % RDF + MC (Fig. 4.17).



*Treatment details were given in table 3.5

Fig. 4.17. Influence of endophytic microbial consortia on chlorophyll stability index of maize grown in field under water stress.

The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Farooq *et al.*, 2009). Loss of chlorophyll contents under water stress is considered a main cause of inactivation of photosynthesis. Low concentrations of photosynthetic pigments can directly limit photosynthetic potential and hence primary production (Anjum *et al.*, 2011b). But the application of maize with developed endophytic microbial consortia improved chlorophyll stability index even under water stress conditions provided with 100% and 75% RDF.

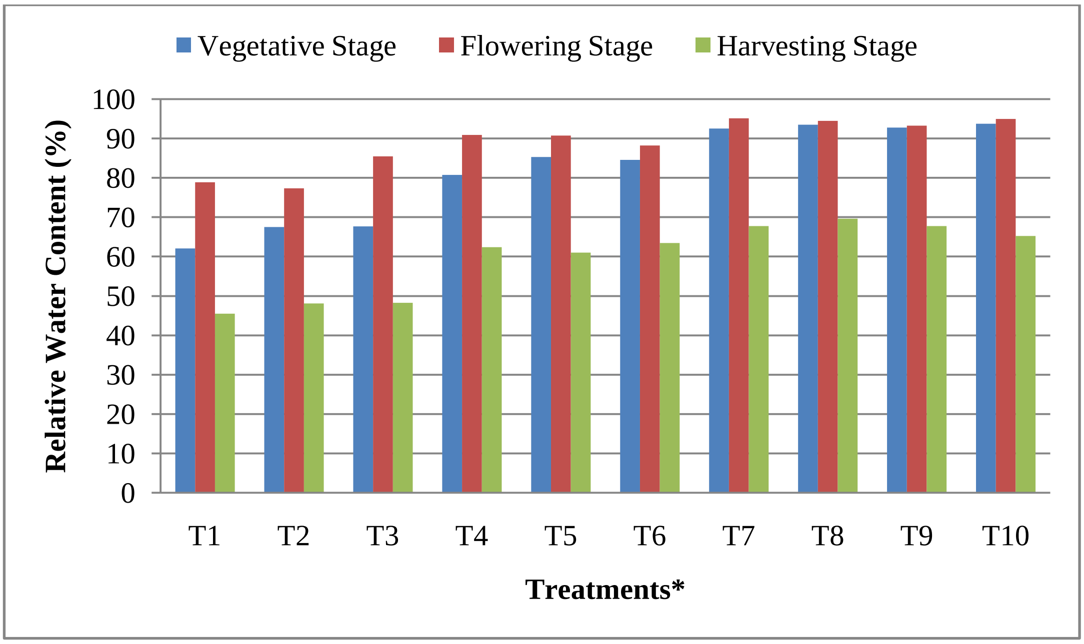
Our results suggest that biological nitrogen fixation ability and IAA production by bacterial isolates of consortia has induced the improved chlorophyll synthesis and the results were comparable with Stefan *et al.* (2013) demonstrated that the activity of IAA may act as an allied factor for increasing synthesis of chlorophyll.

Inoculation of *Pseudomonas syringae*, *Enterobacter aerogenes* and *P. fluorescens* to maize increased the chlorophyll content under drought stress (Nadeem *et al.*, 2007). Previous studies have shown a significant effect of PGPB on the chlorophyll content in maize (Almaghrabi *et al.*, 2014).

Bacillus subtilis was found as the most prominent in enhancing the N accumulation and, consequently, the chlorophyll content in maize was found increased (Pupathy and Radziah, 2015). Similarly, for maize and sorghum inoculation of IPACC26 (*B. subtilis*) and IPACF66 (*Pseudomonas* sp.) significantly increased the chlorophyll content (de Aquino *et al.*, 2019)

b) Relative water content (RWC %)

Relative water content is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance. Maximum RWC of 93.71% was observed in T10: Full Irrigations + 100 % RDF (Control) at vegetative stage and three treatments were on par with the control namely in the descending order are T8:7 Irrigations + 75 % RDF + MC (93.50%), T9:7 Irrigations + 100 % RDF + MC (92.73%) and T7:7 Irrigations + 50 % RDF + MC (92.53%). Minimum RWC was observed in treatments T1 (62.09%), T2 (67.52%) and T3 (67.68%) treated with 3 irrigations and 50%, 75% and 100% of RDF respectively along with developed microbial consortium.



*Treatment details were given in table 3.5

Fig. 4.18. Influence of endophytic microbial consortia on relative water content of maize grown in field under water stress.

At flowering stage maximum RWC was shown by T7:7 Irrigations + 50 % RDF + MC (95.13%) followed by T10: Full Irrigations + 100 % RDF (Control) (95.00%). The treatments on par with the control are in the following descending order T8:7 Irrigations + 75 % RDF + MC (94.46%), T9:7 Irrigations + 100 % RDF + MC (93.23%), T4:5 Irrigations + 50 % RDF + MC (90.93%) and T5:5 Irrigations + 75 % RDF + MC (90.73%). Minimum RWC was shown by T2:3 Irrigations + 75 % RDF + MC (77.23%).

At harvesting stage maximum RWC was found in T8:7 Irrigations + 75 % RDF + MC (69.63%), followed by T7:7 Irrigations + 50 % RDF + MC (67.78%) and T9:7 Irrigations + 100 % RDF + MC (67.76%) and were recorded on par with T10: Full Irrigations + 100 % RDF (Control) (65.21%). Minimum RWC was shown by T1:3 Irrigations + 50 % RDF + MC (45.51%) (Table 4.30, Fig. 4.18)

RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures. RWC related to water uptake by the roots as well as water loss by transpiration. A decrease in the relative water content in response to drought stress has been noted in wide variety of plants as reported by

Nayyar and Gupta (2006) that when leaves are subjected to drought, leaves exhibit large reductions in RWC and water potential.

c) H_2O_2 (µmol g^{-1})

The production of ROS (H_2O_2, O_2^- and $^\bullet OH$ radicals) in plants, known as the oxidative burst, is an early event of plant defense response to water-stress and acts as a secondary massager to trigger subsequent defense reaction in plants. During environmental stress such as drought, ROS levels increase dramatically resulting in oxidative damage to proteins, DNA and lipids (Apel and Hirt, 2004). Being highly reactive, ROS can seriously damage plants by increasing lipid peroxidation, protein degradation, DNA fragmentation and ultimately cell death (Mittler, 2002).

At vegetative stage the H_2O_2 content in control i.e., T10: Full Irrigations + 100 % RDF was found to be 24.80 µmol g^{-1} and the treatments which were on par with the control are found to be T9:7 Irrigations + 100 % RDF + MC (24.83 µmol g^{-1}), T8:7 Irrigations + 75 % RDF + MC (26.97 µmol g^{-1}) and T7:7 Irrigations + 50 % RDF + MC (26.46 µmol g^{-1}). Maximum H_2O_2 content was found in T1:3 Irrigations + 50 % RDF + MC (35.83 µmol g^{-1}) and T2:3 Irrigations + 75 % RDF + MC (35.26 µmol g^{-1})

At flowering stage the H_2O_2 content was found minimum in T9:7 Irrigations + 100 % RDF + MC (54.43 µmol g^{-1}) and T7:7 Irrigations + 50 % RDF + MC (54.26 µmol g^{-1}). Control *i.e.,* T10: Full Irrigations + 100 % RDF was found to have 55.43 µmol g^{-1} of H_2O_2 content and the treatment T8:7 Irrigations + 75 % RDF + MC (56.46 µmol g^{-1}) was observed to be on par with the control. Maximum H_2O_2 content was found in T1:3 Irrigations + 50 % RDF + MC (76.73 µmol g^{-1}) and T2:3 Irrigations + 75 % RDF + MC (75.23 µmol g^{-1})

At harvesting stage the H_2O_2 content was found minimum in T9:7 Irrigations + 100 % RDF + MC (29.46 µmol g^{-1}) and T8:7 Irrigations + 75 % RDF + MC (29.63 µmol g^{-1}). The treatments T7:7 Irrigations + 50 % RDF + MC and T10: Full Irrigations + 100 % RDF (control) were found to be on par with each other with 30.50 and 30.63 µmol g^{-1} of H_2O_2 content respectively. Maximum H_2O_2 content was found in T1:3 Irrigations + 50 % RDF + MC (44.56 µmol g^{-1}) and T2:3 Irrigations + 75 % RDF + MC (43.33 µmol g^{-1}) (Table 4.31).

The production of H_2O_2 was higher in treatments given with lower number of irrigations. The H_2O_2 production was increased in flowering stage and then reduced at harvesting stage but not lower than the vegetative stage and lower levels of H_2O_2 was found in microbial treated plants even under drought stress when compared to non inoculated treatments. Lowering of H_2O_2 contents in microbial treated stress plants can be considered as an prominent mechanism to attenuate the activation of plant defenses.

This ameliorated effect on H_2O_2 accumulation is directly linked with increased accumulation of nitrogen containing compatiable solutes such as proline, which is involved in scavenging free radicals, stabilizesb sub-cellular structures and improved cellular redox potential under stress conditions (KaviKishor *et al.* 2005; Yang *et al.* 2008).

In the study of Islam *et al*. (2015) the bacterial inoculation to both stress and un-stress conditions showed lowering the levels of H_2O_2 and MDA contents indicating a decreased accumulation of reactive oxygen species and reduces membrane damage in inoculated plants.

d) Peroxidase (units min^{-1} g^{-1})

At vegetative stage maximum peroxidase content (Table 4.31) was produced by T1:3 Irrigations + 50 % RDF + MC (46.70 units min^{-1} g^{-1}), T3:3 Irrigations + 100 % RDF + MC (46.03 units min^{-1} g^{-1}) and followed by T2:3 Irrigations + 75 % RDF + MC (45.73 units min^{-1} g^{-1}). Minimum content of peroxidase was found in T9:7 Irrigations + 100 % RDF + MC (23.53 units min^{-1} g^{-1}) and T10: Full Irrigations + 100 % RDF (control) (23.70 units min^{-1} g^{-1}).

At flowering stage maximum peroxidase conent was produced by T1:3 Irrigations + 50 % RDF + MC (81.33 units min^{-1} g^{-1}), T2:3 Irrigations + 75 % RDF + MC (79.76 units min^{-1} g^{-1}) and followed by T3:3 Irrigations + 100 % RDF + MC (78.66 units min^{-1} g^{-1}). Minimum content of peroxidase was found in T9:7 Irrigations + 100 % RDF + MC (43.30 units min^{-1} g^{-1}) and T10: Full Irrigations + 100 % RDF (control) (41.00 units min^{-1} g^{-1}).

At harvesting stage maximum peroxidase conent was produced by T1:3 Irrigations + 50 % RDF + MC (35.83 units min^{-1} g^{-1}), T2:3 Irrigations + 75 % RDF + MC (35.26 units min^{-1} g^{-1}) and followed by T3:3 Irrigations + 100 % RDF + MC (34.63 units min^{-1} g^{-1}). Minimum content of peroxidase was found in T9:7 Irrigations + 100 % RDF + MC (24.83 units min^{-1} g^{-1}) and T10: Full Irrigations + 100 % RDF (control) (24.80 units min^{-1} g^{-1}).

The increased activity of peroxidase in microbial inoculated treatments under drought stress conditions suggests that microbial inoculation protects plant from stress by scavenging reactive oxygen species like H_2O_2 by increased production of peroxidase leading to improved plant growth under stress. The triggering effect of PGPB on genes encoding for antioxidative enzymes in plants under stress conditions, increases the ROS scavenging enzymes activities and protects the plant (Gururani *et al.,* 2012)

In severely drought-stressed plants, free radical accumulation leads to the damage of cell membranes and other cellular machinery (Munns, 2002). Antioxidant enzymes, like catalase (CAT) and peroxidase (POD), have the ability to eliminate free radicals and prevent cell membranes and DNA content from further damage (Scandalios, 1994). Efficient destruction of O_2^- and H_2O_2 in plant cells requires the concerted action of antioxidants. POD plays a key role in scavenging H_2O_2 which was produces through dismutation of O_2^- catalyzed by super oxide dismutase. Maintaining a higher level of antioxidative enzyme activities may contribute to drought induction by increasing the capacity against oxidative damage (Sharma and Dubey, 2005).

e) Proline (μg g^{-1})

Under drought, the maintenance of leaf turgor may also be achieved by the way of osmotic adjustment in response to the accumulation of proline, sucrose, soluble carbohydrates, glycinebetaine, and other solutes in cytoplasm improving water uptake from drying soil. The process of accumulation of such solutes under drought stress is known as osmotic adjustment which strongly depends on the rate of plant water stress. Of these solutes, proline is the most widely studied because of its considerable importance in the stress tolerance.

Table 4.30. Influence of endophytic microbial consortia on plant biochemical parameters of maize under water stress imposed field conditions

Treatments	Chlorophyll Stability Index (CSI %)			Relative Water Content (RWC %)		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T_1:3 Irrigations + 50 % RDF + MC	80.33	92.00	63.67	62.09	78.90	45.51
T_2:3 Irrigations + 75 % RDF + MC	88.67	96.33	61.67	67.52	77.30	48.10
T_3:3 Irrigations + 100 % RDF + MC	94.00	101.00	93.00	67.68	85.43	48.29
T_4:5 Irrigations + 50 % RDF + MC	92.33	94.00	91.33	80.77	90.93	62.37
T_5:5 Irrigations + 75 % RDF + MC	108.67	127.67	101.33	85.30	90.73	61.03
T_6:5 Irrigations + 100 % RDF + MC	124.33	127.00	101.33	84.53	88.23	63.43
T_7:7 Irrigations + 50 % RDF + MC	99.33	98.33	93.33	92.53	95.13	67.78
T_8:7 Irrigations + 75 % RDF + MC	117.67	135.33	127.00	93.50	94.47	69.63
T_9:7 Irrigations + 100 % RDF + MC	165.67	138.67	133.33	92.73	93.23	67.76
T_{10}: Full Irrigations + 100 % RDF (Control)	100.00	100.00	100.00	93.71	95.00	65.21
CD(P=0.05)	10.52	3.71	4.78	8.38	7.81	8.45
S.Em. ±	3.51	1.24	1.59	2.82	2.62	2.84
S.Ed	4.97	1.75	2.26	3.99	3.71	4.02
CV (%)	5.68	1.93	2.86	5.96	5.11	8.22

Table 4.31. Influence of endophytic microbial consortia on antioxidant enzymes and proline content in maize under water stress imposed field conditions

Treatments	**H_2O_2 (µmol g^{-1})**			**Peroxidase (units min^{-1} g^{-1})**			**Proline (µg g^{-1})**		
	Vegetative Stage	**Flowering Stage**	**Harvest Stage**	**Vegetative Stage**	**Flowering Stage**	**Harvest Stage**	**Vegetative Stage**	**Flowering Stage**	**Harvest Stage**
T_1: 3 Irrigations + 50 % RDF + MC	35.83	76.73	44.57	46.70	81.33	35.83	74.70	75.30	72.03
T_2: 3 Irrigations + 75 % RDF + MC	35.27	75.23	43.33	45.73	79.77	35.27	71.57	75.20	73.40
T_3: 3 Irrigations + 100 % RDF + MC	34.63	73.43	45.80	46.03	78.67	34.63	72.50	71.70	72.40
T_4: 5 Irrigations + 50 % RDF + MC	30.03	68.77	37.57	34.83	70.70	30.03	62.73	45.77	52.97
T_5: 5 Irrigations + 75 % RDF + MC	30.03	70.03	35.17	36.03	67.50	30.03	43.87	42.23	51.87
T_6: 5 Irrigations + 100 % RDF + MC	30.37	66.57	34.17	35.60	66.63	30.37	32.80	41.23	44.93
T_7: 7 Irrigations + 50 % RDF + MC	26.47	54.27	30.50	29.37	46.83	26.47	32.37	31.83	36.30
T_8: 7 Irrigations + 75 % RDF + MC	26.97	56.47	29.63	27.03	44.70	26.97	33.40	31.23	34.97
T_9: 7 Irrigations + 100 % RDF + MC	24.83	54.43	29.47	23.53	43.30	24.83	32.07	28.63	35.27
T_{10}: Full Irrigations + 100 % RDF (Control)	24.80	55.43	30.63	23.70	41.00	24.80	29.93	24.97	31.50
CD(P=0.05)	3.57	4.32	2.29	3.13	3.81	3.57	7.02	6.46	7.51
S.Em. ±	1.19	1.44	0.76	1.04	1.27	1.19	2.34	2.16	2.51
S.Ed	1.68	2.04	1.08	1.47	1.80	1.68	3.32	3.05	3.55
CV (%)	6.90	3.84	3.67	5.19	3.55	6.90	8.35	7.98	8.59

At vegetative stage maximum proline content was produced by T1:3 Irrigations + 50 % RDF + MC (74.70 µg g^{-1}), T3:3 Irrigations + 100 % RDF + MC (72.50 µg g^{-1}) and followed by T2:3 Irrigations + 75 % RDF + MC (71.56 µg g^{-1}). Minimum content of proline was found in T9:7 Irrigations + 100 % RDF + MC (32.06 µg g^{-1}), T8:7 Irrigations + 75 % RDF + MC (33.40 µg g^{-1}) and T10: Full Irrigations + 100 % RDF (control) (29.93 µg g^{-1}).

At flowering stage maximum proline conent was produced by T1:3 Irrigations + 50 % RDF + MC (75.30 µg g^{-1}), T2:3 Irrigations + 75 % RDF + MC (75.20 µg g^{-1}) and followed by T3:3 Irrigations + 100 % RDF + MC (71.70 µg g^{-1}). Minimum content of proline was found in T9:7 Irrigations + 100 % RDF + MC (28.63 µg g^{-1}) and T10: Full Irrigations + 100 % RDF (control) (24.97 µg g^{-1}).

At harvesting stage maximum proline conent was produced by T1:3 Irrigations + 50 % RDF + MC (72.03 µg g^{-1}), T2:3 Irrigations + 75 % RDF + MC (73.40 µg g^{-1}) and followed by T3:3 Irrigations + 100 % RDF + MC (72.40 µg g^{-1}). Minimum content of proline was found in T9:7 Irrigations + 100 % RDF + MC (35.270 µg g^{-1}) and T8:7 Irrigations + 75 % RDF + MC (34.96 µg g^{-1}) (Table 4.31)

The higher proline accumulation in microbial consortia inoculated treatments (Plate 4.10c) may be due to the increased uptake of nutrients which results in a greater biosynthesis rate. The increased proline content in plants under stress conditions indicates its accumulation as a protective mechanism that keeps positive water potential for improved water availability from soil resulting in lower stress damage (Porcel and Ruiz-Lozano 2004). Thus, a high concentration of proline accumulation by microbial activity is to create osmotic pressure under stress conditions and to scavange free radicals that damages plant. In consortium inoculated treatments provided with 7 irrigations were found to have lesser proline content than the control (non inoculated) indicating that these treatments were less affected by induced water deficit stress.

Similar findings were reported by Jha *et al.* (2011) that inoculation of PGPR and endophytic bacteria to rice growing under saline-alkali stress reduced proline content significantly while non inoculated plants shown increased proline content with the increasing salt concentration. The free proline contents in PGPR and AMF inoculated oats induced with saline alkali and petroleum stress were found significantly lower than uninoculated control, suggesting that these plants were less affected by the saline-alkali and petroleum induced stress (Xun *et al.*, 2015)

Wheat is marked by low level of compatible solutes and the accumulation and mobilization of proline was observed to enhance tolerance to water stress (Nayyar and Walia, 2003). Progressive drought stress induced a considerable accumulation of proline in water stressed maize plants. The proline content increase as the drought stress progressed and reached a peak as recorded after 10 days stress, and then decreased under severe water stress as observed after 15 days of stress (Anjum *et al.*, 2011a). PGP *pseudomonas* spp. strains inoculation also increased proline content under drought stress, which may be due to up regulation of proline biosynthesis pathway to keep proline in high levels, which helps in maintaining cell water status, protects membranes and proteins from stress (Yoshiba *et al.*, 1997)

4.6.2 Influence of endophytic microbial consortia on soil physico-chemical properties of maize rhizosphere under field conditions

a) pH

In vegetative and flowering stage pH of the rhizospheric soil was found to be slightly acidic (< 7) while at harvesting stage the pH has slightly increase to basic (>7). The minimum pH at vegetative stage was found in rhizospheric soil of T2:3 Irrigations + 75 % RDF + MC (6.53) while the control treatment without microbial consortium T10: Full Irrigations + 100 % RDF was found to have pH of 6.86. At flowering stage minimum pH 6.36 was observed in T6:5 Irrigations + 100 % RDF + MC, maximum pH 7.23 was shown by T2:3 Irrigations + 75 % RDF + MC and the control T10: Full Irrigations + 100 % RDF has shown pH 6.87. At harvesting stage minimum pH 7.53 was shown by T1:3 Irrigations + 50 % RDF + MC and there was no significant difference among the treatments (Table 4.32)

Initial pH of the field soil where the present investigation was conducted was measured as 7.8 (Table 3.4). Inoculation of microbial consortium has decreased the pH which might be due to the production of organic acids. At maximum vegetative stage slightly acidic pH was observed at all the treatments and the pH was observed to increase slightly as the crop proceeds to flowering and then harvesting stage. The addition of microbial inoculants and organic manures to the soil is known to bring about changes in pH which might be due to release of H^+ ions from the organic manures during mineralization (Yadav *et al.*, 2000)

Application of the bacterial bioformulations influences the physicochemical properties of soil (Posso *et al.,* 2017). Decrease in pH was also recorded in rice and soybean rhizospheric soils due to endophytic bacterial inoculation (Adhikari and Pandey, 2020).

b) Electrical conductivity (EC)

Electrical conductivity (EC) of soil is a measure of the amount of salts in soil. Electrical conductivity levels can serve as an indirect indicator of the amount of water and water-soluble nutrients available for plant uptake such as nitrate-N. Initial electrical conductivity of the field soil was 0.17 dS m^{-1} (Table 3.4). EC was found increased at vegetative and flowering stage compared to initial EC while at harvesting stage EC was found to reduce. At vegetative stage maximum EC (0.63) was found in T3:3 Irrigations + 100 % RDF + MC and minimum EC (0.41) was shown by T7:7 Irrigations + 50 % RDF + MC. At flowering stage maximum EC (0.63) was found in T3:3 Irrigations + 100 % RDF + MC and minimum EC (0.44) was shown by T7:7 Irrigations + 50 % RDF + MC. At harvesting stage maximum EC (0.50) was found in T3:3 Irrigations + 100 % RDF + MC and minimum EC (0.28) was shown by both T8:7 Irrigations + 75 % RDF + MC and T9:7 Irrigations + 100 % RDF + MC (Table 4.32).

The treatments given with three irrigations were found to have more EC at all growth stages of crop that may be due to the accumulation of more salts in less irrigated treatments. However application of developed endophytic bacterial consortia was observed to maintain the concentration of salts due to process of mineralization and thus less EC was observed in treatments given with 7 irrigations than the control which was given with full irrigations and no microbial inoculants. Concentration of soil ions directly affect the EC of soil which is an essential physicochemical property. From the experimental data, we can suggest that endophytic microbial consortia have the potential property to improve the EC of soil.

In agreement with our results significant levels of EC in soils of pot and field grown chickpea treated with two seed endophytic bacteria inoculated as individual isolates and as a consortium. EC ranged from 25.00 to 28.14 (µScm^{-1}) 15.04 to 19.12 (µScm^{-1}) and in field and pot soils respectively (Mukherjee *et al.*, 2021).

c) Organic carbon (OC)

At vegetative stage maximum organic carbon was reported in T5:5 Irrigations + 75 % RDF + MC (0.41%) followed by T7:7 Irrigations + 50 % RDF + MC (0.40%) which are significantly higher than the control T10: Full Irrigations + 100 % RDF (0.30%) and minimum percent of OC was reported in T3:3 Irrigations + 100 % RDF + MC (0.30%). At flowering stage significantly higher OC was observed in T7:7 Irrigations + 50 % RDF + MC followed by (0.45%) T8:7 Irrigations + 75 % RDF + MC (0.43%) when compared to control T10: Full Irrigations + 100 % RDF (0.32%). Minimum percent of OC was reported by T3:3 Irrigations + 100 % RDF + MC (0.35%). At harvesting stage significantly higher OC was reported in T7:7 Irrigations + 50 % RDF + MC followed by (0.37%) T8:7 Irrigations + 75 % RDF + MC (0.36%) when compared to control T10: Full Irrigations + 100 % RDF (0.23%). Lower percent of OC was observed in T3:3 Irrigations + 100 % RDF + MC (0.26%) (Table 4.32)

The initial OC content of field soil was found to be 0.22% (Table 3.4) and there was rapid increase in OC at vegetative stage in all treatments. Among the crop stages OC has slightly increased in flowering stage and got reduced at harvesting stage. At vegetative stage T5 was found to have maximum OC while at both flowering and harvesting stage T7 and T8 were observed to have higher OC content. At all growth stages T3 was found to have minimum OC but not less than the control (T10).

Increased soil carbon due to application of bioagents and FYM sustains soil health for a longer period than the chemical fertilization (Jeyabal and Kuppuswamy, 2003; Amlinger et al., 2003). Wu et al. (2005) have shown that the dual inoculation of rhizobacteria and mycorrhizae with manure resulted in a significant increase of soil organic matter content in the maize rhizosphere. The significant increase in per cent organic matter and per cent OC were shown in chickpea soil inoculated with seed endophytic bacteria *Enterobacter cloacae* BHUJPCS-21 and *Enterobacter hormaechei* BHUJPCS-15 as compared to uninoculated control and (Mukherjee *et al.*, 2021).

Table 4.32. Influence of endophytic microbial consortia on soil physico-chemical properties of maize rhizosphere under field conditions

Treatments	pH			EC (dS m^{-1})			OC (%)		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T_1: 3 Irrigations + 50 % RDF + MC	6.63	7.20	7.53	0.57	0.61	0.41	0.38	0.40	0.32
T_2: 3 Irrigations + 75 % RDF + MC	6.53	7.23	7.67	0.60	0.62	0.43	0.38	0.42	0.31
T_3: 3 Irrigations + 100 % RDF + MC	6.70	7.13	7.77	0.63	0.63	0.51	0.30	0.35	0.26
T_4: 5 Irrigations + 50 % RDF + MC	6.60	6.73	7.63	0.53	0.53	0.42	0.34	0.38	0.31
T_5: 5 Irrigations + 75 % RDF + MC	6.67	6.80	7.73	0.55	0.56	0.42	0.41	0.43	0.33
T_6: 5 Irrigations + 100 % RDF + MC	6.70	6.37	7.67	0.55	0.56	0.38	0.36	0.42	0.35
T_7: 7 Irrigations + 50 % RDF + MC	6.63	6.47	7.63	0.41	0.44	0.34	0.40	0.45	0.37
T_8: 7 Irrigations + 75 % RDF + MC	6.80	6.77	7.73	0.45	0.49	0.28	0.38	0.43	0.36
T_9: 7 Irrigations + 100 % RDF + MC	6.80	7.03	7.73	0.47	0.52	0.28	0.37	0.39	0.35
T_{10}:Full Irrigations + 100 % RDF (Control)	6.87	6.87	7.77	0.45	0.49	0.33	0.30	0.32	0.23
CD(P=0.05)	N/A	0.272	N/A	0.038	0.034	0.024	0.035	0.057	0.035
S.Em. ±	0.082	0.091	0.105	0.013	0.011	0.008	0.012	0.019	0.012
S.Ed	0.116	0.129	0.149	0.018	0.016	0.011	0.017	0.027	0.016
CV (%)	2.125	2.298	2.371	4.181	3.631	3.618	5.659	8.322	6.290

4.6.3 Influence of endophytic microbial consortia on available nutrients in maize rhizosphere soil under water stress imposed field conditions

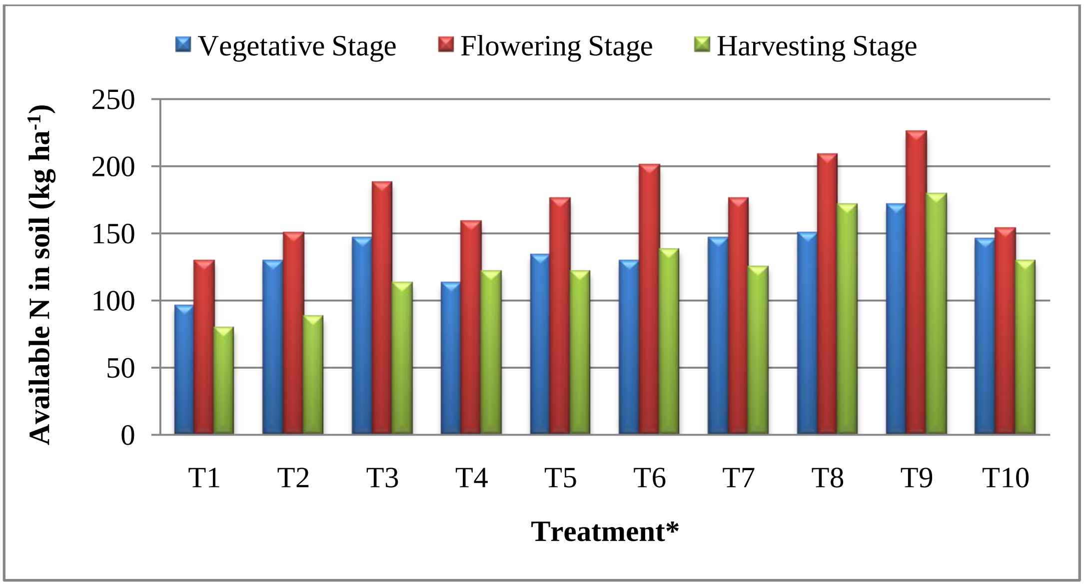
a) Available nitrogen in Soil:

At vegetative stage the available nitrogen was found highest in T9:7 Irrigations + 100 % RDF + MC (171.43 kg ha^{-1}), T8:7 Irrigations + 75 % RDF + MC (150.53 kg ha^{-1}), T7:7 Irrigations + 50 % RDF + MC (146.35 kg ha^{-1}) and T3:3 Irrigations + 100 % RDF + MC (146.34 kg ha^{-1}). But, all the above treatments are on par with the control T10: Full Irrigations + 100 % RDF (145.65 kg ha^{-1}).

At flowering stage significantly higher amount of available nitrogen was recorded in T9:7 Irrigations + 100 % RDF + MC (225.29 kg ha^{-1}) followed by T8:7 Irrigations + 75 % RDF + MC (209.09 kg ha^{-1}) and T6:5 Irrigations + 100 % RDF + MC (200.70 kg ha^{-1}) when compared to control T10: Full Irrigations + 100 % RDF (154.03 kg ha^{-1}).

At harvesting stage significantly higher amount of available nitrogen was recorded in T9:7 Irrigations + 100 % RDF + MC (179.80 kg ha^{-1}) followed by T8:7 Irrigations + 75 % RDF + MC (171.44 kg ha^{-1}) when compared to control T10: Full Irrigations + 100 % RDF (129.62 kg ha^{-1}) and T6:5 Irrigations + 100 % RDF + MC (137.98 kg ha^{-1}) was found on par with control (Table 4.34).

Available nitrogen (Table 3.4) in initial field soil (83.46 kg ha^{-1}) has increased prominently at vegetative stage of the crop and further increase of available N was recorded at flowering stage but reduction of available N was observed as the crop proceed to harvesting stage (Fig. 4.19). At all the growth stages available nitrogen in soil is significantly more in treatments given with 7 irrigations. While in treatments given with 5 irrigations (100 % RDF) available nitrogen in soil was found on par with control (full irrigations). This implies that even under water stress conditions the developed microbial consortia could able to improve soil available nitrogen. The nitrogen fixing ability of bacterial isolates applied as consortia to the crop might has improved available nitrogen content in soil though provided with 25% lesser RDF.



Treatment details were given in table 3.5

Fig. 4.19. Influence of endophytic microbial consortia on available N in maize rhizosphere soil in field under water stress

The results were in agreement with the studies of Ahmad *et al.* (2008) who reported that the increase in available nitrogen and phosphorus in soil was due to biological nitrogen fixation and mineralization of organic matter by microbial inoculants. Inoculation of *Azospirillum* spp. in sugarcane contributed 26.7 kg N ha^{-1} or 70% of total required N by plant by process of nitrogen fixation (Boddey *et al.*, 1995).

Complete uptake of fixed ammonia with no losses to environment is the main advantage of N derived from biological nitrogen fixation. Because 50–70% of N applied as inorganic fertiliser in soil is loosed through natural processes such as denitrification, volatilization and nutrient leaching thereby causing environmental pollution, limited nutrient availability and application of more dose of fertilizers than required (Hodge *et al.*, 2000).

A significant increase in available N, P and K in soils treated with microbial consortium was observed which positively correlated with increase in plant dry weight, plant height, pigment synthesis and seed production (Raklami *et al.*, 2019)

b) Available phosphorus in soil (kg ha^{-1})

At all the growth stages of the crop T9:7 Irrigations + 100 % RDF + MC, T8:7 Irrigations + 75 % RDF + MC, T6:5 Irrigations + 100 % RDF + MC and T5:5

Irrigations + 75 % RDF + MC were found to have significantly higher amount of available phosphorus in the rhizospheric soil when compared to control without microbial inoculum T10: Full Irrigations + 100 % RDF.

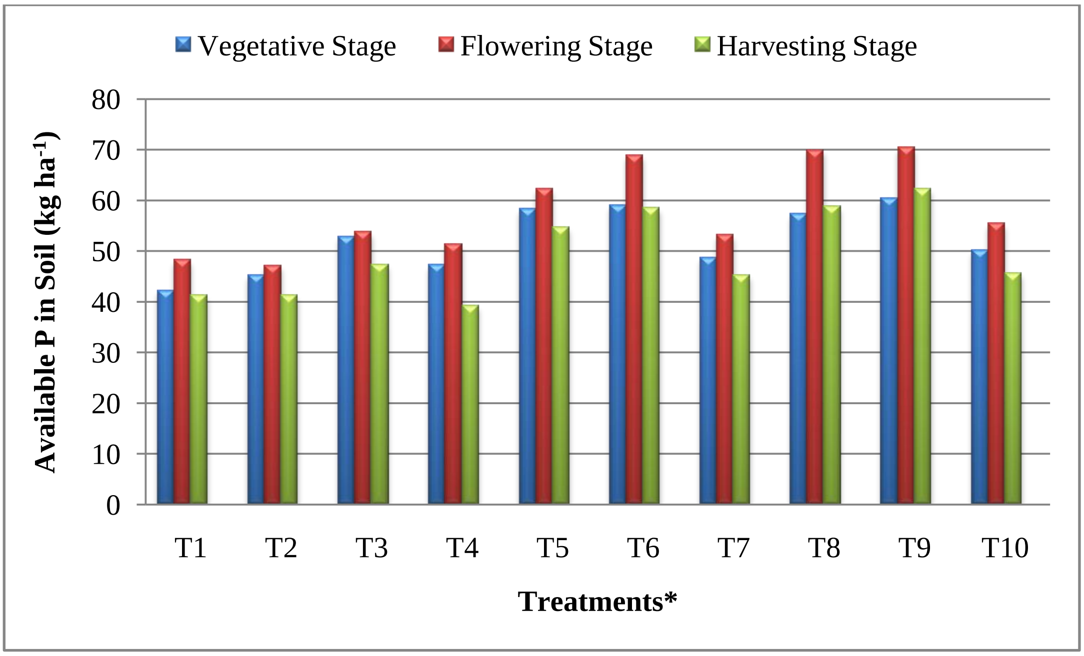
At vegetative stage maximum available P was recorded in T9 (60.46 kg ha^{-1}), T6 (59.04 kg ha^{-1}), T5 (58.23 kg ha^{-1}) and T8 (57.30 kg ha^{-1}), while minimum available P was found in T1:3 Irrigations + 50 % RDF + MC with 42.28 kg ha^{-1}. At flowering stage maximum available P was recorded in T9 (70.34 kg ha^{-1}), T8 (69.92 kg ha^{-1}), T6 (68.96 kg ha^{-1}) and T5 (62.31 kg ha^{-1}) while minimum available P was found in T2: 3 Irrigations + 75 % RDF + MC with 47.20 kg ha^{-1}.

At harvesting stage maximum available P was recorded in T9 (62.23 kg ha^{-1}), T8 (58.81 kg ha^{-1}), T6 (58.47 kg ha^{-1}) and T5 (54.71 kg ha^{-1}). The available P in the treatments T3:3 Irrigations + 100 % RDF + MC (47.40 kg ha^{-1}) and T7:7 Irrigations + 50 % RDF + MC (45.24 kg ha^{-1}) were on par with control T10: Full Irrigations + 100 % RDF (45.62 kg ha^{-1}) (Table 4.33).

The initial available phosphorus in soil was found to be 40.21 kg ha^{-1} (Table 3.4) and it was greately increased at vegetative and further at flowering stage. At harvesting stage reduce available P was observed (Fig. 4.20). The treatments given with water stress (7 and 5 irrigations) and limited nutrients (75% RDF) were found to have significantly higher available P in soil when treated with developed endophytic microbial consortia.

Mehta *et al.* (2014) reported that microbes solubilize P by producing metabolites like organic acids that aid in decreasing the pH of the culture media. Application of endophytic bacteria increases acidification of soil due to production of organic acids attributing to convert non-available phosphorus to available P form in soil (Posso *et al.*, 2017).

Our results were in accordance with the results of Sood *et al.* (2018) in terms of soil properties including available N, P, K, soil enzyme activities and phosphate-solubilising bacterial population were significantly increased in the wheat crop inoculated with bacterial inoculants with 80% of recommended dose of N and P in consecutive years over the uninoculated control.



*Treatment details were given in table 3.5

Fig. 4.20. Influence of endophytic microbial consortia on available P in maize rhizosphere soil in field under water stress

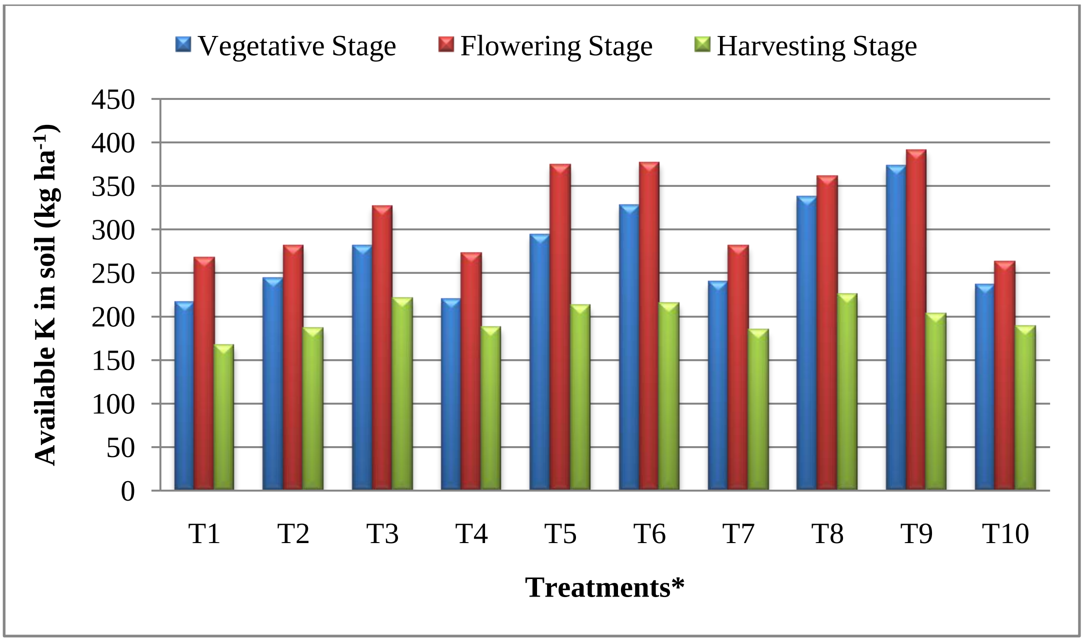
c) Available potassium in soil (kg ha^{-1})

At vegetative and flowering growth stages of the crop T9:7 Irrigations + 100 % RDF + MC, T8:7 Irrigations + 75 % RDF + MC, T6:5 Irrigations + 100 % RDF + MC, T5:5 Irrigations + 75 % RDF + MC and T3:3 Irrigations + 100 % RDF + MC were found to have significantly higher amount of available potassium in the rhizospheric soil when compared to control T10: Full Irrigations + 100 % RDF. At harvesting stage there was no significant difference was recorded among the treatments.

At vegetative stage maximum available K was recorded in T9 (372.16 kg ha^{-1}), T8 (337.71 kg ha^{-1}), T6 (327.87 kg ha^{-1}), T5 (293.17 kg ha^{-1}) and T3 (280.89 kg ha^{-1}) while minimum available K was found in T1:3 Irrigations + 50 % RDF + MC with 216.62 kg ha^{-1}. At flowering stage maximum available K was recorded in T9 (390.62 kg ha^{-1}), T8 (360.59 kg ha^{-1}), T6 (376.37 kg ha^{-1}), T5 (373.88 kg ha^{-1}) and T3 (326.72) while minimum available K was found in T1:3 Irrigations + 50 % RDF + MC with 267.02 kg ha^{-1}. At harvesting stage maximum available K was recorded in T9 (203.01 kg ha^{-1}) (Table 4.33).

The initial availability of potassium in soil is 172.83 kg ha^{-1} (Table 3.4) and it was prominently increased at vegetative and flowering stage while the available K at harvesting stage was observed to get reduced (Fig. 4.21). The microbial consortia could able to compensate 25% of potassium in treatments given with 75 % RDF even at water stress conditions (7 and 5 irrigations) as applied consortia was developed with efficient K solubilizers along with other PGP characters which converts unavailable forms of K into available forms and further the K ion concentration protects plants under stress conditions by maintaining membrane integrity.

By increasing available potassium in soil and enhanced K uptake with the inoculation of *Bacillus mucilaginosus* has promoted the growth of eggplant (Han *et al.*, 2006). Soil essential nutrients such as available N (49.47 kg ha^{-1}) , available P (25.41 kg ha^{-1}), and available K (43.01 kg ha^{-1}) observed to increase significantly with inoculation of endophytic microbial consortia in both pot and field soil of chickpea than untreated control soil (Mukherjee *et al.*, 2021)



Treatment details were given in table 3.5

Fig. 4.21. Influence of endophytic microbial consortia on available K in maize rhizosphere soil in field under water stress

Table 4.33. Influence of endophytic microbial consortia on available nutrients in maize rhizosphere soil under water stress imposed field conditions

Treatments	Available N in soil (kg ha^{-1})			Available P in soil (kg ha^{-1})			Available K in soil (kg ha^{-1})		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T_1: 3 Irrigations + 50 % RDF + MC	96.17	129.62	79.44	42.28	48.22	41.28	216.62	267.02	166.48
T_2: 3 Irrigations + 75 % RDF + MC	129.62	150.53	87.81	45.33	47.20	41.29	243.96	281.11	186.64
T_3: 3 Irrigations + 100 % RDF + MC	146.34	188.16	112.90	52.89	53.74	47.40	280.89	326.72	221.00
T_4: 5 Irrigations + 50 % RDF + MC	112.90	158.89	121.26	47.27	51.29	39.24	219.77	272.92	187.29
T_5: 5 Irrigations + 75 % RDF + MC	133.80	175.62	121.26	58.23	62.31	54.71	293.17	373.88	212.45
T_6: 5 Irrigations + 100 % RDF + MC	129.62	200.70	137.98	59.04	68.96	58.47	327.87	376.37	215.42
T_7: 7 Irrigations + 50 % RDF + MC	146.35	175.62	125.44	48.74	53.18	45.24	239.11	280.86	183.88
T_8: 7 Irrigations + 75 % RDF + MC	150.53	209.07	171.44	57.30	69.92	58.81	337.71	360.59	224.96
T_9: 7 Irrigations + 100 % RDF + MC	171.43	225.79	179.80	60.46	70.34	62.24	372.16	390.62	203.01
T_{10}: Full Irrigations + 100 % RDF (Control)	145.65	154.03	129.62	50.08	55.37	45.62	236.04	262.72	189.16
CD(P=0.05)	29.55	40.00	35.48	7.28	5.80	7.28	37.56	36.78	N/A
S.Em. ±	9.86	13.36	11.85	2.43	1.93	2.43	12.54	12.28	16.22
S.Ed	13.95	18.89	16.76	3.44	2.74	3.43	17.74	17.37	22.94
CV (%)	12.54	13.08	16.20	8.08	5.78	8.52	7.85	6.66	14.11

4.6.4 Influence of endophytic microbial consortia on macro nutrient concentration of maize under water stress imposed field conditions

a) Nitrogen in plant (%)

At vegetative stage significantly higher concentration of nitrogen in plant was found in treatment T8:7 Irrigations + 75 % RDF + MC (5.89%) while T9:7 Irrigations + 100 % RDF + MC (5.61%),T7:7 Irrigations + 50 % RDF + MC (5.56%), T6:5 Irrigations + 100 % RDF + MC (5.57%) and T5:5 Irrigations + 75 % RDF + MC (5.00%) were found to have N concentration in plant on par with the control T10: Full Irrigations + 100 % RDF (5.07%).

At flowering stage maximum N concentration in plant was found in T8:7 Irrigations + 75 % RDF + MC and T9:7 Irrigations + 100 % RDF + MC with 6.14% followed by T7:7 Irrigations + 50 % RDF + MC (5.73%), T6:5 Irrigations + 100 % RDF + MC (5.52%) and T5:5 Irrigations + 75 % RDF + MC (5.49%) the former two treatments are significantly higher while the later three treatments were on par with the control T10: Full Irrigations + 100 % RDF (5.18%).

At harvesting stage T9:7 Irrigations + 100 % RDF + MC have recorded significantly higher N concentration (4.26%) in plant while T8:7 Irrigations + 75 % RDF + MC (4.04%) and T6:5 Irrigations + 100 % RDF + MC (4.18%) were found to have N concentration in plant on par with the control T10: Full Irrigations + 100 % RDF (3.71%) (Table 4.34)

In present investigation nitrogen concentration in maize plants was more in vegetative and flowering stage and there was no significant difference between the two mentioned growth stages while the N content was observed to get reduced at harvesting stage. Treatments given with water stress (7 and 5 irrigations) and limited nutrients (50% and 75% RDF) were found to have significantly higher nitrogen content or on par with the control.

Similar results were demonstrated by Kuan *et al.* (2016) that the majority (70–81%) of N content in maize plant top was accumulated prior to anthesis, particularly *B. pumilus* S1r1 which fixed a significant amount of atmospheric N_2, promoted vegetative growth and delayed plant senescence of maize, thereby produced a higher N content and yield of maize ear.

A significant increase in nitrogen (33%), phosphorus (48%) and potassium (50%) concentrations in *Bacillus cereus* inoculated stress plants was observed as compared to the un-inoculated salt stress plants (Islam *et al.,* 2016).

Inoculation of maize palnts with *K. pneumoniae* significantly increased uptake of N at the rate of 61.4% and 48.4% at D50 and D65, respectively followed by *Bacillus pumilus* S1r1 was observed to show similar increment of N uptake by 55.1% and 50.1% at D50 and D65, respectively (Kuan *et al.,* 2016).

b) Phosphorus in plant (%)

At vegetative stage significantly higher concentration of phosphorus (P) in plant was found in treatment T9:7 Irrigations + 100 % RDF + MC (1.49%) while T8:7 Irrigations + 75 % RDF + MC (1.39%), T6:5 Irrigations + 100 % RDF + MC (1.33%) and T5:5 Irrigations + 75 % RDF + MC (1.28%) were found to have P concentration in plant on par with the control T10: Full Irrigations + 100 % RDF (1.27%).

At flowering stage maximum and significantly higher P concentration in plant was found in T9:7 Irrigations + 100 % RDF + MC (1.77%) while T8:7 Irrigations + 75 % RDF + MC (1.68%) and T6:5 Irrigations + 100 % RDF + MC (1.65%) were found to have P concentration in plants on par with the control T10: Full Irrigations + 100 % RDF (1.48%).

At harvesting stage maximum and significantly higher P concentration in plant was found in T6:5 Irrigations + 100 % RDF + MC (0.62) while T5:5 Irrigations + 75 % RDF + MC (0.59%), T9:7 Irrigations + 100 % RDF + MC (0.56%) and T8:7 Irrigations + 75 % RDF + MC (0.56%) were observed to have P concentration in plant on par with the control T10: Full Irrigations + 100 % RDF (0.52%) (Table 4.34).

The phosphorus content in maize plants was increased slightly in flowering stage than the vegetative stage while it got reduced significantly at harvesting stage. Water stress (5 irrigations) has no effect on uptake P while microbial consortium treated plants could compensate only 25 % nutrients as at 50% RDF concentration of P in plants was observed to be statistically lesser than plants with no microbial treatment. The increase in uptake of phosphorus in inoculated treatments was due to enhanced plant growth stimulating mechanisms by microbes as a result of phosphate solubilization which can positively affect the plant growth and crop yields.

The results were in correlation with Sood *et al.* (2018) who reported that increase in N and P contents of plant was attributed to enhanced microbial activity by combined application of bio inoculants with chemical fertilizers (integrated nutrient management) that favoured the conversion of atmospheric N_2 to nitrates and mineralization of organically bound N to inorganic plant available forms and the phosphate solubilizing microbes might have converted unavailable form of phosphates to available form by releasing phosphatase enzyme and organic acids ultimately increasing the uptake of available forms of phosphates by plants.

Under field condition, inoculation of endophytic bacterial isolate BS-290 to maize has significantly increased the phosphorus and nitrogen concentrations in shoot dry matter, phosphorus concentration in dry soil and also rhizospheric bacterial population when compared to the uninoculated control (Lobo *et al.,* 2019)

c) Potassium in plants (%)

At vegetative stage maximum and significantly higher concentration of potassium in plants was found in T9:7 Irrigations + 100 % RDF + MC (12.18%) and T8:7 Irrigations + 75 % RDF + MC (9.07%) while T6:5 Irrigations + 100 % RDF + MC was found to have 6.68% of K concentration in plants which was on par with the control T10: Full Irrigations + 100 % RDF (7.74%).

At flowering stage maximum and significantly higher concentration of potassium in plants was found in T9:7 Irrigations + 100 % RDF + MC (6.28%) while T8:7 Irrigations + 75 % RDF + MC and T6:5 Irrigations + 100 % RDF + MC were found to have 5.57% and 5.61% of K concentration in plants respectively which was on par with the control T10: Full Irrigations + 100 % RDF (5.17%).

At harvesting stage T9:7 Irrigations + 100 % RDF + MC (6.82 %) was found to have significantly higher K in plants while T8:7 Irrigations + 75 % RDF + MC (5.98 %) and T6:5 Irrigations + 100 % RDF + MC (5.94 %) were found have K concentration in plant on par with the control T10: Full Irrigations + 100 % RDF (5.87 %) (Table 4.34)

Potassium concentration in maize plants was found high at vegetative stage and slightly got reduced at flowering stage and further statistically less K concentration in plants was observed at harvesting stage. Potassium concentration was maintained significantly higher in treatments with 100% and 75% RDF only when provided with 7 irrigations or more along with microbial treatment. While treatments with water stress (5 irrigations) maintained K concentration in plants on par with control only at 100% RDF and at 75 and 50% RDF statistically lesser K in plants was observed though treated with microbial consortia. This may be due to reduced K solubilization activity of microbes at higher water potential.

Under abiotic stress conditions it is essential for maintenance of memberane permeability and turgidity. The inoculated endophytic bacteria has improved RWC (Sec 4.6.1.a) in water deficit conditions and increased compatiable solutes like proline (Sec 4.6.1.e) that helps to maintain membrane integrity under stress conditions. In addition to that maintenance of K concentration in plants also has impact in sustaining stress conditions as K-ions help in maintenance of membrane integrity. The K solubilization ability of inoculated microbial consortia improves the uptake of K in plants.

The Co-inoculation of *Rhizobium* and *Pseudomonas* having multi trait plant growth promoting abilities has increased the accumulation of proline content in stressed plants, decreased leakage of electrolytes and maintained RWC of leaves and uptake of K ions to improve salt tolerance in *Zea mays* (Bano and Fatima, 2009).

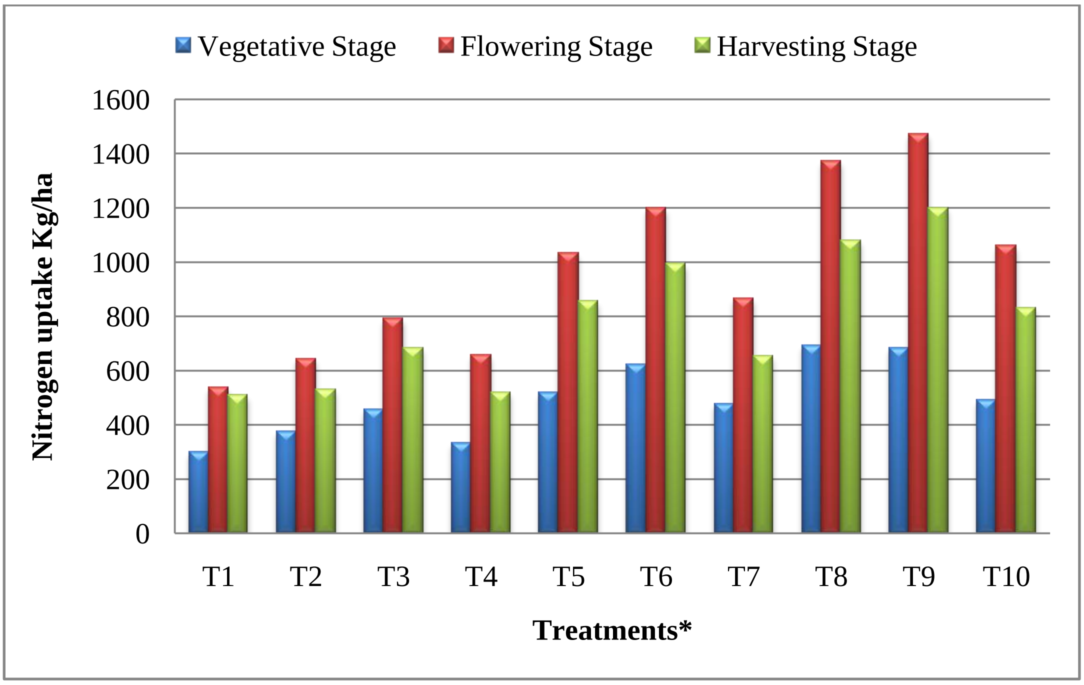
Table 4.34. Influence of endophytic microbial consortia on macro nutrient concentration of maize under water stress imposed field conditions

Treatments	N Content (%) in plant			P Content (%) in plant			K Content (%) in plant		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T_1: 3 Irrigations + 50 % RDF + MC	4.10	4.03	3.34	0.95	1.08	0.35	3.87	4.53	4.79
T_2: 3 Irrigations + 75 % RDF + MC	4.39	4.73	3.00	1.03	1.17	0.44	4.25	4.73	4.87
T_3: 3 Irrigations + 100 % RDF + MC	4.95	5.19	3.28	1.15	1.25	0.46	5.05	4.79	5.05
T_4: 5 Irrigations + 50 % RDF + MC	4.31	4.93	3.15	0.92	1.26	0.38	4.17	4.10	4.28
T_5: 5 Irrigations + 75 % RDF + MC	5.00	5.49	3.86	1.28	1.41	0.59	6.68	4.96	5.11
T_6: 5 Irrigations + 100 % RDF + MC	5.56	5.52	4.19	1.33	1.65	0.62	7.72	5.61	5.94
T_7: 7 Irrigations + 50 % RDF + MC	5.57	5.73	3.47	0.90	1.05	0.39	5.57	4.67	4.85
T_8: 7 Irrigations + 75 % RDF + MC	5.89	6.14	4.04	1.39	1.68	0.56	9.07	5.57	5.98
T_9: 7 Irrigations + 100 % RDF + MC	5.61	6.14	4.26	1.49	1.77	0.57	12.18	6.28	6.82
T_{10}: Full Irrigations + 100 % RDF (Control)	5.07	5.18	3.71	1.27	1.48	0.52	7.74	5.17	5.87
CD(P=0.05)	0.64	0.84	0.55	0.26	0.30	0.10	1.08	0.79	0.69
S.Em. ±	0.21	0.28	0.18	0.09	0.10	0.03	0.36	0.26	0.23
S.Ed	0.30	0.39	0.26	0.12	0.14	0.05	0.51	0.37	0.33
CV (%)	7.35	9.17	8.79	13.39	13.08	12.82	9.45	9.05	7.45

4.6.5 Influence of endophytic microbial consortia on nutrient uptake by maize under drought stress

a) Uptake of nitrogen (Kg ha^{-1})

At vegetative stage significantly higher uptake of nitrogen by maize was found (Table 4.35) in treatments T8:7 Irrigations + 75 % RDF + MC (693 Kg ha^{-1}) followed by T9:7 Irrigations + 100 % RDF + MC (683 Kg ha^{-1}) and T6:5 Irrigations + 100 % RDF + MC (620 Kg ha^{-1}) while T5:5 Irrigations + 75 % RDF + MC has shown 518 Kg ha^{-1} of total nitrogen uptake on par with the control T10: full irrigations + 100 % RDF (491 Kg ha^{-1}). At flowering stage significantly higher amounts of nitrogen uptake was found in treatments T9 (1472 Kg ha^{-1}) and T8 (1372 Kg ha^{-1}) while T6 has shown 1197 Kg ha^{-1} of nitrogen uptake which was on par with the control T10 (1060 Kg ha^{-1}). At harvesting stage significantly higher nitrogen uptake by plants was found in treatments T9 (1200 Kg ha^{-1}), T8 (1079 Kg ha^{-1}) when compared to control T10 (832 Kg ha^{-1}) (Fig. 4.22)

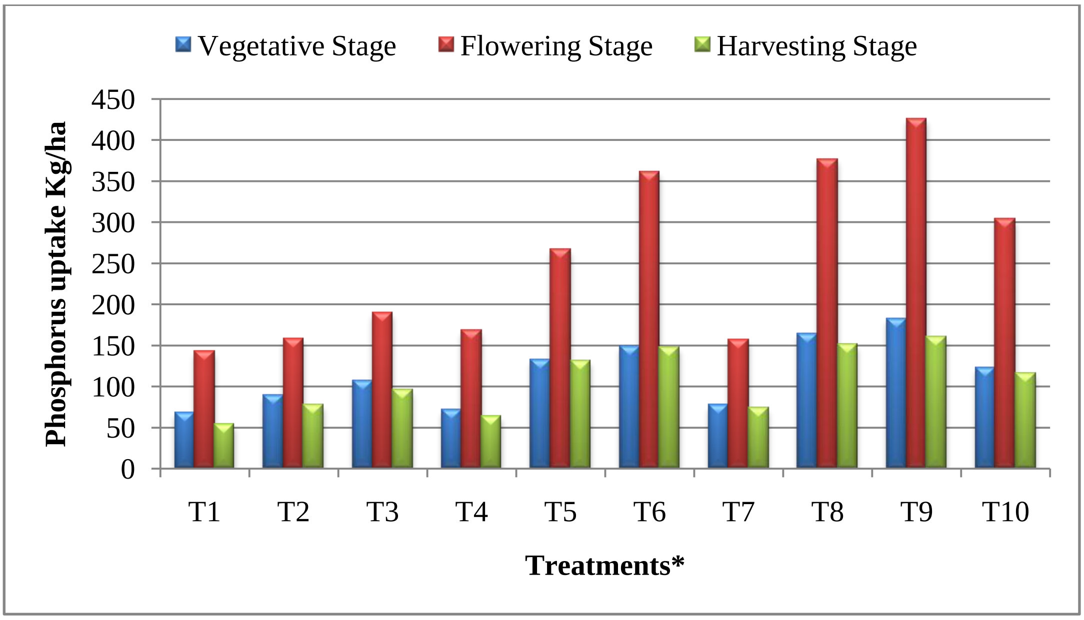


*Treatment details were given in table 3.5

Fig. 4.22. Influence of endophytic microbial consortia on uptake of nitrogen by maize in field under water stress

b) Uptake of phosphorus (Kg ha^{-1})

At vegetative stage significantly higher uptake of phosphorus by maize was found (Table 4.35) in treatments T9:7 Irrigations + 100 % RDF + MC (182 Kg ha^{-1}) followed by T8:7 Irrigations + 75 % RDF + MC (163 Kg ha^{-1}) and T6:5 Irrigations + 100 % RDF + MC (148 Kg ha^{-1}) while T5:5 Irrigations + 75 % RDF + MC has shown 132 Kg ha^{-1} of total phosphorus uptake on par with the control T10: full irrigations + 100 % RDF (122 Kg ha^{-1}). At flowering stage significantly higher amounts of phosphorus uptake was found in treatments T9 (426 Kg ha^{-1}) and T8 (377 Kg ha^{-1}) while T6 has shown 361 Kg ha^{-1} of phosphorus uptake which was on par with the control T10 (303 Kg ha^{-1}). At harvesting stage significantly higher phosphorus uptake by plants was found in treatments T9 (160 Kg ha^{-1}), T8 (150 Kg ha^{-1}) when compared to control T10 (116 Kg ha^{-1}) (Fig. 4.23)

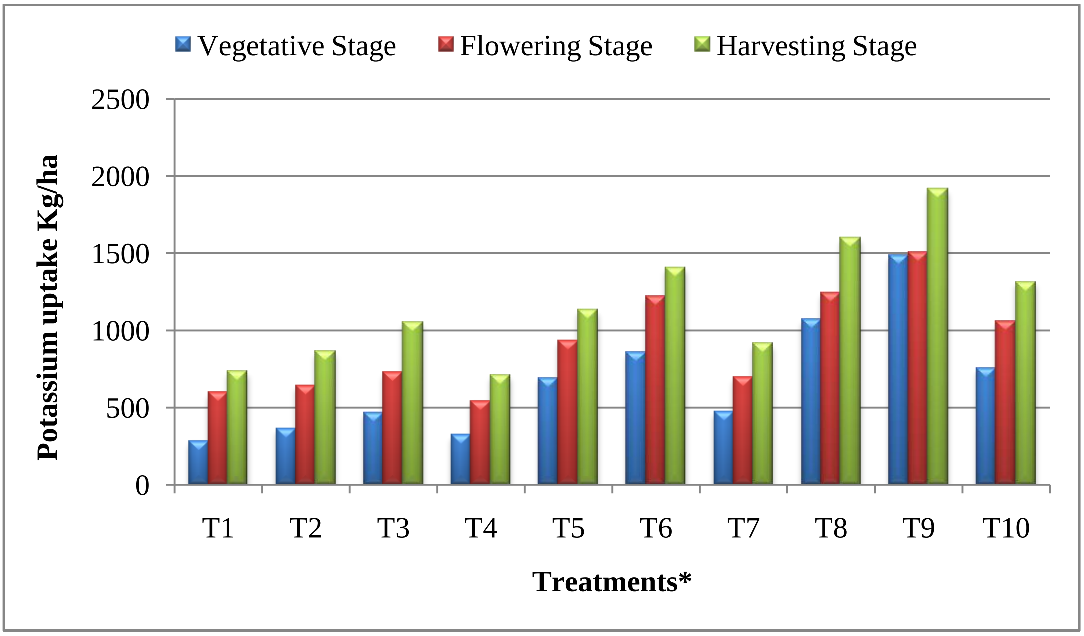


*Treatment details were given in table 3.5

Fig. 4.23. Influence of endophytic microbial consortia on uptake of phosphorus by maize in field under water stress

c) Uptake of potassium (Kg ha^{-1})

At vegetative stage significantly higher uptake of potassium by maize was found (Table 4.35) in treatments T9:7 Irrigations + 100 % RDF + MC (1484 Kg ha^{-1}) followed by T8:7 Irrigations + 75 % RDF + MC (1070 Kg ha^{-1}) while T6:5 Irrigations + 100 % RDF + MC has shown 861 Kg ha^{-1} of total potassium uptake on par with the control T10: full irrigations + 100 % RDF (751 Kg ha^{-1}). At flowering stage significantly higher amounts of potassium uptake was found in treatments T9 (1507 Kg ha^{-1}) and T8 (1245 Kg ha^{-1}) while T6 has shown 1217 Kg ha^{-1} of potassium uptake which was on par with the control T10 (1058 Kg ha^{-1}). At harvesting stage significantly higher potassium uptake by plants was found in treatments T9 (1913 Kg ha^{-1}), T8 (1597 Kg ha^{-1}) when compared to control T10 (1315 Kg ha^{-1}).



*Treatment details were given in table 3.5

Fig. 4.24. Influence of endophytic microbial consortia on uptake of potassium by maize in field under water stress

The present study suggested that application of endophytic microbial consortia has improved the uptake of all macro nutrients including N, P and K at all the growth stages and significantly higher uptake of nutrients was found in T9 and T8 while T6 was found on par with control T10 (Fig. 4.24). The biological nitrogen fixing ability and efficient solubilization of inorganic mineral nutrients of applied microbial consortia might have increased the uptake of nutrients by maize.

Similar results were observed by Krishnaveni (2010) that uptake of both macro and micro-nutrients was enhanced by the application of microbial inoculants. Sahu and Jana, (2000) has suggested that the bacterial strains used in microbial consortia having IAA producing ability influences root biomass thereby increasing the root area available for nutrient uptake and thus enhance uptake of total nutrients by microbial treated plants.

Table 4.35. Influence of endophytic microbial consortia on uptake of macronutrients by maize under water stress imposed field conditions

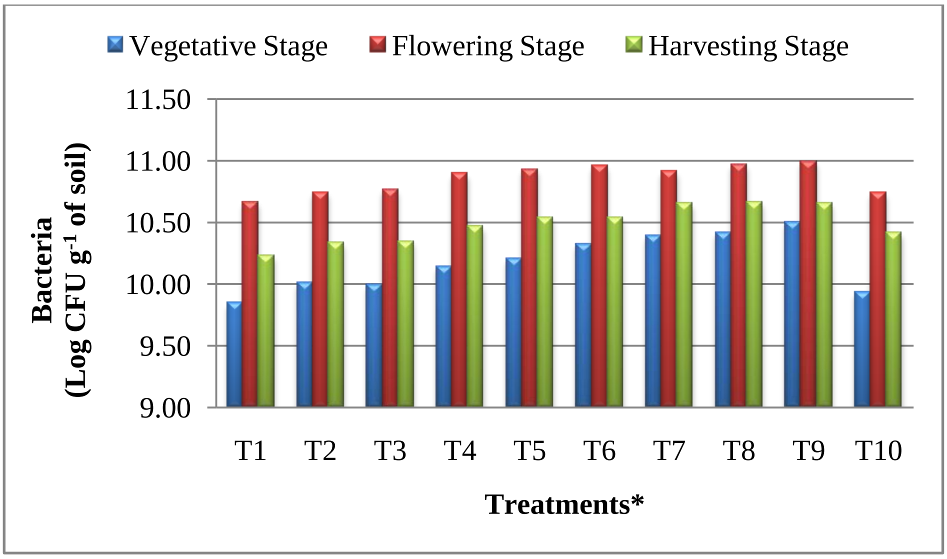
Treatments	Nitrogen uptake (Kg ha^{-1})			Phosphorus uptake (Kg ha^{-1})			Potassium uptake (Kg ha^{-1})		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T_1: 3 Irrigations + 50 % RDF + MC	298	537	509	68	142	54	283	597	733
T_2: 3 Irrigations + 75 % RDF + MC	375	644	530	89	158	77	364	644	862
T_3: 3 Irrigations + 100 % RDF + MC	457	791	683	107	190	96	468	730	1052
T_4: 5 Irrigations + 50 % RDF + MC	333	657	520	71	168	64	321	544	709
T_5: 5 Irrigations + 75 % RDF + MC	518	1031	856	132	266	131	693	934	1130
T_6: 5 Irrigations + 100 % RDF + MC	620	1197	993	148	361	147	861	1217	1408
T_7: 7 Irrigations + 50 % RDF + MC	476	866	655	77	157	74	475	698	913
T_8: 7 Irrigations + 75 % RDF + MC	693	1372	1079	163	377	150	1070	1245	1597
T_9: 7 Irrigations + 100 % RDF + MC	683	1472	1200	182	426	160	1484	1507	1913
T_{10}: Full Irrigations + 100 % RDF (Control)	491	1060	832	122	303	116	751	1058	1315
CD(P=0.05)	64	166	135	28	73	28	136	162	168
S.Em. ±	21	56	45	9	24	9	45	54	56
S.Ed	30	79	64	13	34	13	64	77	79
CV (%)	7	10	10	14	17	15	12	10	8

4.6.6 Influence of endophytic microbial consortia on maize rhizosheric and endospheric microflora under drought stress

a) Total bacteria

At vegetative stage significantly higher population of total bacteria were found in T9:7 Irrigations + 100 % RDF + MC (7.29 Log CFU g^{-1} of soil) followed by T8:7 Irrigations + 75 % RDF + MC (7.21 Log CFU g^{-1} of soil) while the treatments T7:7 Irrigations + 50 % RDF + MC (6.70 Log CFU g^{-1} of soil), T5:5 Irrigations + 75 % RDF + MC (6.45 Log CFU g^{-1} of soil), T6:5 Irrigations + 100 % RDF + MC (6.40 Log CFU g^{-1} of soil) and T4 (6.36 Log CFU g^{-1} of soil) were found to have bacterial population on par with the control T10: Full Irrigations + 100 % RDF (6.23 Log CFU g^{-1} of soil).

At flowering stage increased population of total bacteria was observed significantly higher at T9 (12.75 Log CFU g^{-1} of soil) followed by T8 (10.24Log CFU g^{-1} of soil) while the treatments T5 (9.58 Log CFU g^{-1} of soil) and T6 (9.21 Log CFU g^{-1} of soil) were found on par with the control T10 (9.48 Log CFU g^{-1} of soil). The total population of bacteria were found to get reduced at harvesting stage with significantly higher population in the treatments T5 (9.94 Log CFU g^{-1} of soil) followed by T9 (7.59 Log CFU g^{-1} of soil) when compared to control T10 (5.59 Log CFU g^{-1} of soil) (Table 4.36).



*Treatment details were given in table 3.5

Fig. 4.25. Influence of endophytic microbial consortia on total bacteria of maize rhizosphere in field under water stress

In the present study the inoculated bacterial consortia has improved soil nutrient concentration particularly at vegetative and flowering stage and the pH has maintained near neutral at both the stages. These two soil characters has influenced the bacterial population in rhizosphere recording higher at initial growth stages and flowering stage while reduced bacterial population at harvesting stage as available nutrients were uptaken by plants and soil pH was increased. Our results were in agreement with Ghani *et al.* (2013) which demonstrated highest bacterial growth at neutral pH and corresponding reduction in bacterial growth was observed with increase in pH.

In the treatments induced with drought stress by providing less irrigations (3) decreased bacterial count was recorded when compared to control (Fig. 4.25). As it was hypothesized that bacterial mobility to colonize rhizoshere could be accelerated by mainaining optimum levels of moisture availability (Benizri *et al.,* 2001). In environmentally stressed soils the population of putative bacteria may be as low as 10^4 cells per gram of soil (Timmusk *et al.*, 2011).

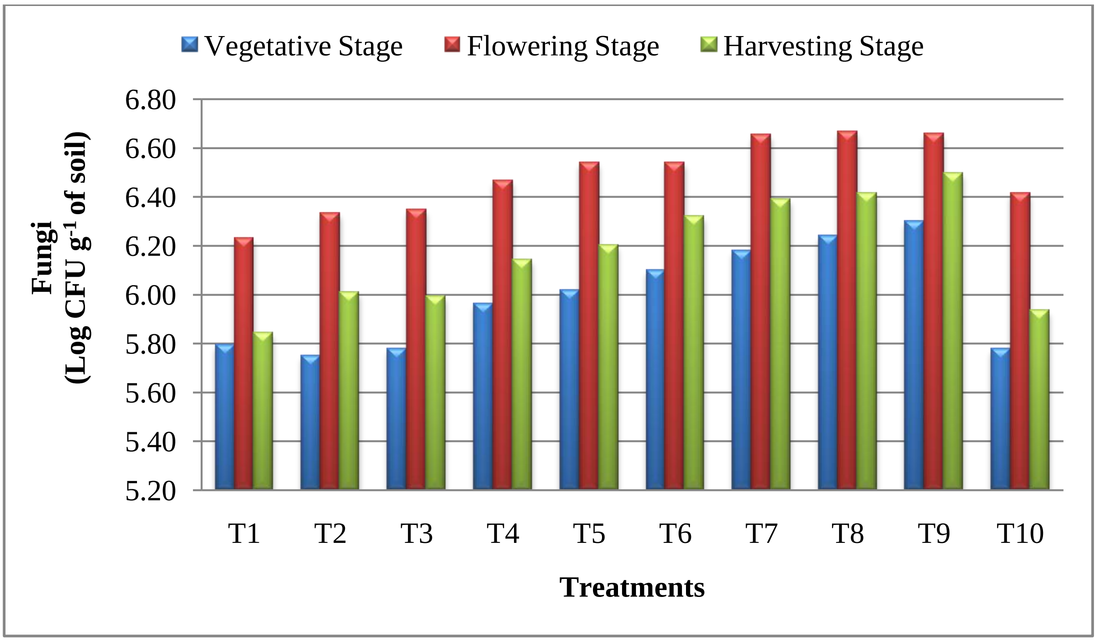
Inoculation of plant growth promoting *Klebsiella* sp. Br1 and *Acinetobacter* sp. S3r2 to maize has improved bacterial population in maize rhizospheric soil significantly with 250–300% over the uninoculated control (Kuan *et al.,* 2016).

b) Total fungi

At vegetative stage no significant difference among the treatments was found regarding total fungal population. However, maximum population of total fungi was observed in T9:7 Irrigations + 100 % RDF + MC (11.39 Log CFU g^{-1} of soil) followed by T6:5 Irrigations + 100 % RDF + MC (10.57 Log CFU g^{-1} of soil), T8:7 Irrigations + 75 % RDF + MC (10.43 Log CFU g^{-1} of soil) and T5:5 Irrigations + 75 % RDF + MC (10.24 Log CFU g^{-1} of soil) while the minimum population of fungi was found in T1:3 Irrigations + 50 % RDF + MC (7.46 Log CFU g^{-1} of soil) and T3:3 Irrigations + 100 % RDF + MC (7.55 Log CFU g^{-1} of soil). The control T10: Full Irrigations + 100 % RDF was found to have 9.57 Log CFU of total fungi per gram of soil.

At flowering stage decreased population of fungi was observed and significantly higher population was found in T9 (7.29 Log CFU g^{-1} of soil) followed by T8 (7.21 Log CFU g^{-1} of soil) when compared to control T10 (6.23 Log CFU g^{-1} of soil). At harvesting stage further reduction of total fungal population was observed with no significant difference among the treatments. Highest fungal population was found in T4 (6.17 Log CFU g^{-1} of soil) followed by T3 (6.07 Log CFU g^{-1} of soil) and T5 (6.01 Log CFU g^{-1} of soil). While lowest population was found in T1 (5.62 Log CFU g^{-1} of soil) and control T10 (5.41 Log CFU g^{-1} of soil) (Table 4.36.

Total fungal population in experimented rhizospheric soil was found more at vegetative stage while trend of decreasing fungal population was observed when crop reached to flowering and then harvesting stage (Fig. 4.26). This decrease in population is due to increased pH in soil as crop proceeds to harvesting stage because the optimum pH for the growth of fungi is acidic (4-6) Two fold higher population of fungi in soil was found at pH 4.0 than at pH 9.0 (Chen *et al.*, 2015).



*Treatment details were given in table 3.5

Fig. 4.26. Influence of endophytic microbial consortia on total fungi of maize rhizosphere in field under water stress

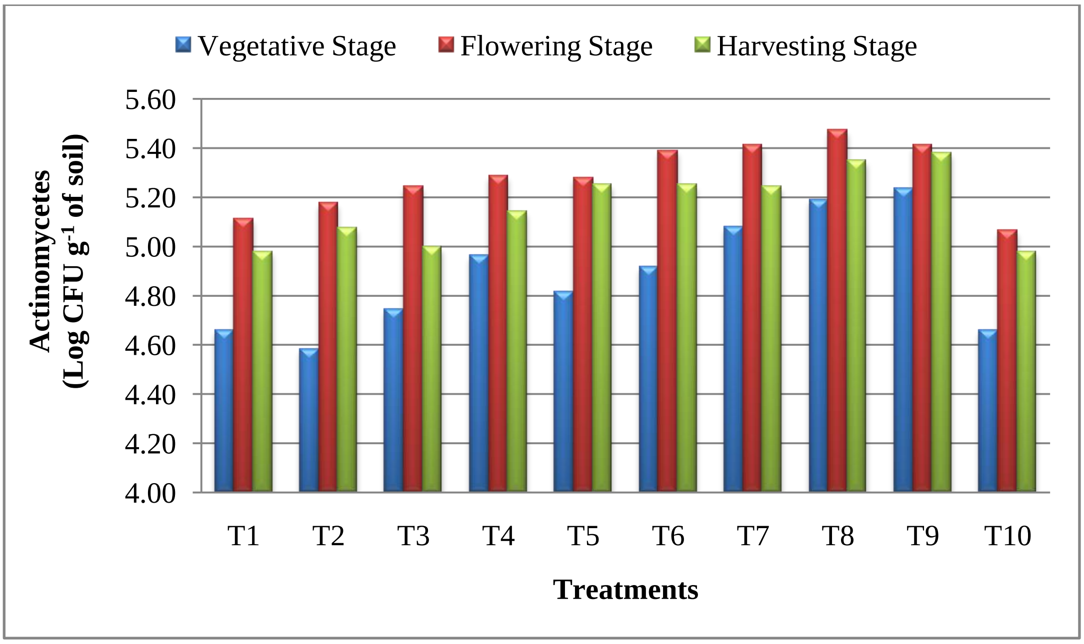
c) Total actinomycetes

At vegetative stage significantly higher count of total actinomycetes was found in T8:7 Irrigations + 75 % RDF + MC (6.43 Log CFU g^{-1} of soil) followed by T9:7 Irrigations + 100 % RDF + MC (6.37 Log CFU g^{-1} of soil), T7:7 Irrigations + 50 % RDF + MC (6.31 Log CFU g^{-1} of soil) and T6:5 Irrigations + 100 % RDF + MC

(6.25 Log CFU g^{-1} of soil) when compared to control T10: Full Irrigations + 100 % RDF (6.04 Log CFU g^{-1} of soil).

An increased population of actinomycetes has been observed at flowering stage. Significantly higher population was found in T9 (10.40 Log CFU g^{-1} of soil) while T6 (9.51 Log CFU g^{-1} of soil) was found to have actinomycetes population on par with the control T10 (9.34 Log CFU g^{-1} of soil). At harvesting stage reduced population of actinomycetes was found at harvesting stage. Significantly higher population was found in T8 (5.72 Log CFU g^{-1} of soil) followed by T9 (5.65 Log CFU g^{-1} of soil), T7 (5.29 Log CFU g^{-1} of soil) when compared to control T10 (4.37 Log CFU g^{-1} of soil) (Table 4.36).

Total actinomycetes population has shown same trend as bacterial population with lower population count at initial growth stage while found to increase at flowering stage and further reduced growth was found at harvesting stage (Fig. 4.27). Actinomycetes are the prominent soil microbes and they grow in close association with the plant roots as more root exudates and available nutrients were available for growth and are one of the important groups of root colonizing organisms (Zhong *et al.*, 2010).



*Treatment details were given in table 3.5

Fig.4.27. Influence of endophytic microbial consortia on total actinomycetes of maize rhizosphere in field under water stress

a) Endophytic bacteria

At vegetative stage endophytic bacteria were found to colonize more of root and stem tissues than leaves. Same trend was followed at flowering stage but the probability of colonization in root, stem and leaf was found equal at harvesting stage while lower population of bacterial colonization was found in kernels.

Endophytic bacterial colonization majorly depends on their motility towards plant roots by the action of chemotaxis because roots exudates rich source of nutrients like organic acids, amino acids and sugars for microbial growth. This is the key activity for establishing plant microbe interaction by associative and symbiotic relation (Yost and Hynes, 2000; Bhattacharjee *et al.,* 2012). Root colonization by microbes is the primary step for plant microbe interation (Bais *et al.,* 2006). Majority of microbes colonize root than shoot (Pereira and Castro 2014). For colonizing above ground parts like leaves and stem microbes should confer selective trait in relation to plant or should mobilize within plant (Hardoim *et al.,* 2008).

At vegetative stage the endophytic bacterial population was found highest in T9:7 Irrigations + 100 % RDF + MC with 5.21 Log CFU g^{-1} of root tissue and 4.83 Log CFU g^{-1} of leaf tissue while T8:7 Irrigations + 75 % RDF + MC was found with 5.05 Log CFU g^{-1} of stem tissue. At flowering stage maximum endophytic bacterial colonization was found in T5:5 Irrigations + 75 % RDF + MC with 5.58 Log CFU g^{-1} of root tissue, T9:7 Irrigations + 100 % RDF + MC with 5.41 Log CFU g^{-1} of stem tissue and 5.09 Log CFU g^{-1} of leaf tissue. At harvesting stage highest endophytic population was found in T9:7 Irrigations + 100 % RDF + MC was found with 5.22 Log CFU g^{-1} of root tissue, T5:5 Irrigations + 75 % RDF + MC with 5.20 Log CFU g^{-1} of stem tissue and 5.00 Log CFU g^{-1} of leaf tissue, T9:7 Irrigations + 100 % RDF + MC with 5.03 Log CFU g^{-1} of kernel (Table 4.37)

In the present study endophytic population was found highest in rood endosphere than the followed by stem, leaf and kernels. Our results were in correlation with the study of Naveed *et al.* (2014b) suggested that the endophytic bacterial strain *Enterobacter* sp. FD17 was reported to colonize efficiently in rhizosphere, root, shoot and leaf tissues maize cultivars with no significant difference in the viable cell number. Maximum population of colonization was encounterd in the rhizosphere and root endosphere when compared to the shoot and leaf endosphere. In addition to the successful colonization by competing with indigenous microbes the endophytic strain FD17 has also found to increase plant growth.

In the current investigation out of all inoculated bacterial consortium based on identical colony morphology we identified that *Klebsiella pneumonea* was found to colonize all plant parts efficiently at all growth stages. *Priestia megaterium* and *Bacillus licheniformis* were found to colonize roots and stem more efficiently while colonizing less in leaves at vegetative stage. Whereas at later growth stages they were found to colonize in leaves and kernels also indicating the movement of bacterial isolates within the plant tissues at different growth stages. *Methylorubrum populi* was found to colonize leaves more efficiently followed by roots and then stem.

Similarly, previous studies reported that diversity of microbial communities were found in various plant tissues like root, stem, leaves, flowers, fruits and seeds at various growth development stages suggesting that different bacterial strains possess distinct capacities to colonize various plant tisuues (Sessitsch *et al.,* 2002; Berg *et al.,* 2005; Okunishi *et al.,* 2005).

In the present study identical colonies of inoculated bacterial consortium were found to isolate from kernels too suggesting that inoculated microbial consortium may act as natural biofertilizer for next generation crop. As bacterial endophytes were found to transfer from other plant tissues to seeds and suggested as natural biofertilizers early from seed germination for new plantlets (Puente *et al.,* 2009; Ruiza *et al.,* 2011). Low number of endophytic bacteria were found in reproductive organs which seems even the less abundance of endophytic population in seeds might be sufficient for vertical transmission by acting as natural biofertilizer and after establishment of bacterial population in next generation plantlet, further increase in cell density takes place and involve in plant growth promotion (Compant *et al.,* 2010).

Table 4.36. Influence of endophytic microbial consortia on microflora of maize rhizosphere under water stress imposed field conditions

Treatments	Bacteria (Log CFU g^{-1} of soil)			Fungi (Log CFU g^{-1} of soil)			Actinomycetes (Log CFU g^{-1} of soil)		
	Vegetative Stage	**Flowering Stage**	**Harvest Stage**	**Vegetative Stage**	**Flowering Stage**	**Harvest Stage**	**Vegetative Stage**	**Flowering Stage**	**Harvest Stage**
T_1: 3 Irrigations + 50 % RDF + MC	9.84	10.66	10.23	5.79	6.23	5.84	4.66	5.11	4.98
T_2: 3 Irrigations + 75 % RDF + MC	10.01	10.74	10.33	5.75	6.33	6.01	4.58	5.18	5.08
T_3: 3 Irrigations + 100 % RDF + MC	9.99	10.77	10.35	5.78	6.35	5.99	4.74	5.24	5.00
T_4: 5 Irrigations + 50 % RDF + MC	10.14	10.89	10.47	5.96	6.47	6.14	4.96	5.29	5.14
T_5: 5 Irrigations + 75 % RDF + MC	10.20	10.93	10.54	6.02	6.54	6.20	4.82	5.28	5.25
T_6: 5 Irrigations + 100 % RDF + MC	10.32	10.96	10.54	6.10	6.54	6.32	4.92	5.39	5.25
T_7: 7 Irrigations + 50 % RDF + MC	10.39	10.91	10.65	6.18	6.65	6.39	5.08	5.41	5.24
T_8: 7 Irrigations + 75 % RDF + MC	10.41	10.97	10.67	6.24	6.67	6.41	5.19	5.47	5.35
T_9: 7 Irrigations + 100 % RDF + MC	10.50	10.99	10.66	6.30	6.66	6.50	5.24	5.41	5.38
T_{10}: Full Irrigations + 100 % RDF (Control)	9.93	10.74	10.41	5.78	6.41	5.93	4.66	5.06	4.98
CD(P=0.05)	0.09	0.05	0.07	0.22	0.07	0.09	0.33	0.14	0.13
S.Em. ±	0.03	0.02	0.02	0.08	0.02	0.03	0.11	0.05	0.04
S.Ed	0.04	0.03	0.04	0.11	0.03	0.04	0.16	0.07	0.06
CV (%)	0.50	0.28	0.40	2.16	0.64	0.82	3.92	1.54	1.42

Table 4.37. Influence of endophytic microbial consortia on microflora of maize endosphere under field conditions

Treatments	Vegetative stage (Log CFU g^{-1} of Tissue)			Flowering stage (Log CFU g^{-1} of Tissue)			Harvesting stage (Log CFU g^{-1} of Tissue)			
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Kernel
T_1: 3 Irrigations + 50 % RDF + MC	4.85	4.70	4.70	5.24	5.15	4.89	5.18	5.10	4.62	4.66
T_2: 3 Irrigations + 75 % RDF + MC	4.99	4.62	4.46	5.33	5.23	4.90	4.97	4.88	4.56	4.72
T_3: 3 Irrigations + 100 % RDF + MC	4.83	4.64	4.63	5.38	5.28	4.75	5.09	4.96	4.68	4.54
T_4: 5 Irrigations + 50 % RDF + MC	5.03	4.89	4.78	5.31	5.22	4.99	5.13	5.08	4.76	4.89
T_5: 5 Irrigations + 75 % RDF + MC	5.04	4.99	4.76	5.58	5.33	4.95	5.25	5.20	5.00	4.92
T_6: 5 Irrigations + 100 % RDF + MC	5.09	4.91	4.67	5.46	5.23	5.03	5.17	4.99	4.84	4.90
T_7: 7 Irrigations + 50 % RDF + MC	5.15	4.97	4.70	5.38	5.22	5.08	5.12	4.92	4.60	4.88
T_8: 7 Irrigations + 75 % RDF + MC	5.15	5.05	4.60	5.54	5.37	5.07	5.22	5.11	4.86	4.96
T_9: 7 Irrigations + 100 % RDF + MC	5.21	4.97	4.83	5.56	5.41	5.09	5.16	5.20	4.91	5.03
T_{10}: Full Irrigations + 100 % RDF (Control)	4.72	4.68	4.66	5.22	5.03	4.73	5.08	4.91	4.66	4.68
CD(P=0.05)	0.21	0.29	N/A	0.12	0.16	0.23	N/A	0.20	N/A	0.26
S.Em. ±	0.07	0.10	0.11	0.04	0.05	0.08	0.06	0.07	0.12	0.09
S.Ed	0.10	0.14	0.15	0.06	0.07	0.11	0.09	0.10	0.17	0.12
CV (%)	2.40	3.49	3.91	1.28	1.71	2.70	2.04	2.31	4.45	3.08

4.6.7 Influence of endophytic microbial consortia on soil enzymes activity of maize rhizosphere under water stress imposed field conditions

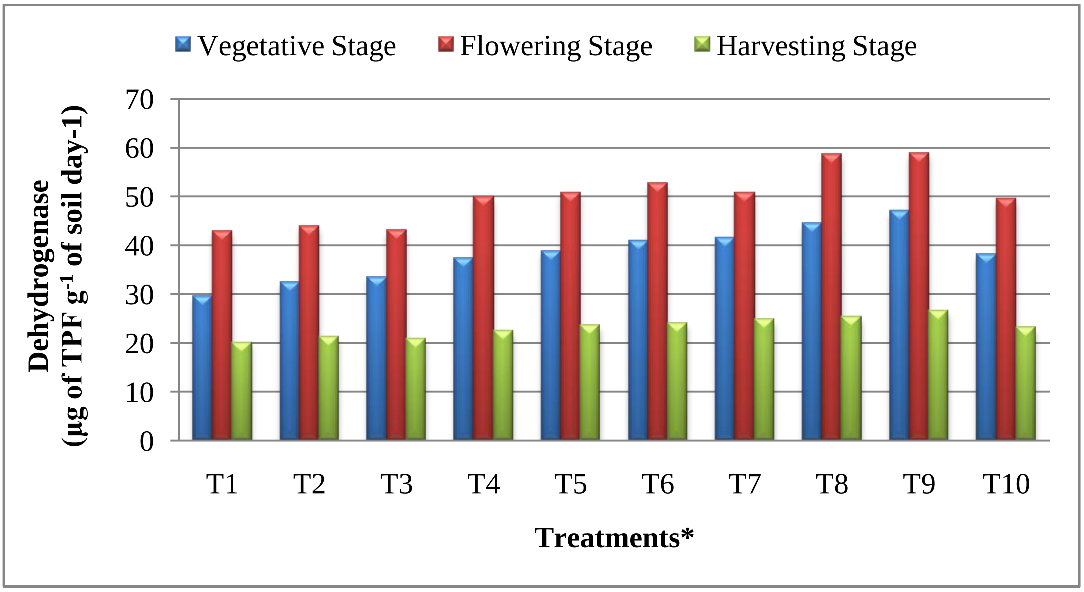
a) Dehydrogenase activity (µg of TPF g^{-1} of soil day^{-1})

At vegetative stage maximum dehydrogenase activity was found in T9:7 Irrigations + 100 % RDF + MC (46.93 µg of TPF g^{-1} of soil day^{-1}) followed by T8:7 Irrigations + 75 % RDF + MC (44.43 µg of TPF g^{-1} of soil day^{-1}) and T7:7 Irrigations + 50 % RDF + MC (41.57 µg of TPF g^{-1} of soil day^{-1}) which are significantly higher than control T10: Full Irrigations + 100 % RDF (38.13 µg of TPF g^{-1} of soil day^{-1}). While T5:5 Irrigations + 75 % RDF + MC (38.81 µg of TPF g^{-1} of soil day^{-1}) and T6:5 Irrigations + 100 % RDF + MC (40.83 µg of TPF g^{-1} of soil day^{-1}) were found to have dehydrogenase activity on par with the control.

At flowering stage maximum and significantly higher dehydrogenase activity was found in T9:7 Irrigations + 100 % RDF + MC (58.90 µg of TPF g^{-1} of soil day^{-1}) followed by T8:7 Irrigations + 75 % RDF + MC (58.63 µg of TPF g^{-1} of soil day^{-1}) while the treatments T7:7 Irrigations + 50 % RDF + MC, T6:5 Irrigations + 100 % RDF + MC, T5:5 Irrigations + 75 % RDF + MC and T4:5 Irrigations + 50 % RDF + MC have shown soil dehydrogenase activity50.80, 52.73, 50.73, 49.90 µg of TPF g^{-1} of soil day^{-1} respectively which are on par with the control T10: Full Irrigations + 100 % RDF (49.56 µg of TPF g^{-1} of soil day^{-1}).

At harvesting stage maximum and significantly higher dehydrogenase activity was found in T9:7 Irrigations + 100 % RDF + MC (26.62 µg of TPF g^{-1} of soil day^{-1}) followed by T8:7 Irrigations + 75 % RDF + MC (24.40 µg of TPF g^{-1} of soil day^{-1}) while the treatments T7:7 Irrigations + 50 % RDF + MC, T6:5 Irrigations + 100 % RDF + MC, T5:5 Irrigations + 75 % RDF + MC and T5:5 Irrigations + 75 % RDF + MC were found to have 24.78, 24.10, 23.18 µg of TPF g^{-1} of soil day^{-1} of soil dehydrogenase activity respectively which are on par with the control T10: Full Irrigations + 100 % RDF (23.18 µg of TPF g^{-1} of soil day^{-1}) (Table 4.38).

Among all enzymes in the soil environment, dehydrogenases are significant and used as an indicator of microbial activity in rhizospheric region (Plate 4.10a). The dehydrogenase activity of the initial field soil (Table 3.4) was found as 23.21 µg of TPF g^{-1} of soil day^{-1}. The soil enzyme activity was obsereved to get increased in vegetative stage and the same trend followed till flowering stage however at harvesting stage soil dehydrogenase activity was found reduced (Fig. 4.28). At all the growth stages significant activity of degydrogenase enzyme was observed in treatments inoculated with microbial consortia though water stress and nutrient stress was given which implies prominent microbial activity in treated plots.



*Treatment details were given in table 3.5

Fig.4.28. Influence of endophytic microbial consortia on dehydrogenase activity of maize rhizosphere soil in field under water stress

The soil enzymes are directly linked with the microbial activity and results obtained in current study clearly showed an increase in soil enzymes in treatments inoculated with microbial consortia. Soil enzyme activity is an important indicator of microbial population intensity and their efficiency in plant growth promotion thus application of these endophytes may enhance soil fertility by improved mineralization, crop productivity and sustainability under stress condition. The results were in accordance with previous investigations (Chouhan *et al.* 2021; Singh *et al.* 2018)

Similarly, Microbial seed endophytes were found to trigger the other soil microbes in soil and induced more secretions of plant metabolites thus enhancing the soil enzymes activity along with increased mineralization activity thereby directly benefiting the plant in the form of productivity and soil health (Mukherjee *et al.*, 2021)

b) Alkaline phosphatase (µg pNP g^{-1} of soil h^{-1})

At vegetative stage significantly higher phosphatase activity was shown by T9:7 Irrigations + 100 % RDF + MC (75.40 µg pNP g^{-1} of soil h^{-1}), T7:7 Irrigations + 50 % RDF + MC (75.00 µg pNP g^{-1} of soil h^{-1}), T8:7 Irrigations + 75 % RDF + MC (74.10 µg pNP g^{-1} of soil h^{-1}) and T6:5 Irrigations + 100 % RDF + MC (70.93 µg pNP g^{-1} of soil h^{-1}) while the treatment T5:5 Irrigations + 75 % RDF + MC (66.83 µg pNP g^{-1} of soil h^{-1}) was found to have phosphatase activity on par with the control T10: Full Irrigations + 100 % RDF (62.50 µg pNP g^{-1} of soil h^{-1}). At flowering stage there was no significant difference was observed among the treatments but maximum phosphatasee activity was found in T8:7 Irrigations + 75 % RDF + MC (94.97 µg pNP g^{-1} of soil h^{-1}) followed by T9:7 Irrigations + 100 % RDF + MC(92.90 µg pNP g^{-1} of soil h^{-1}).

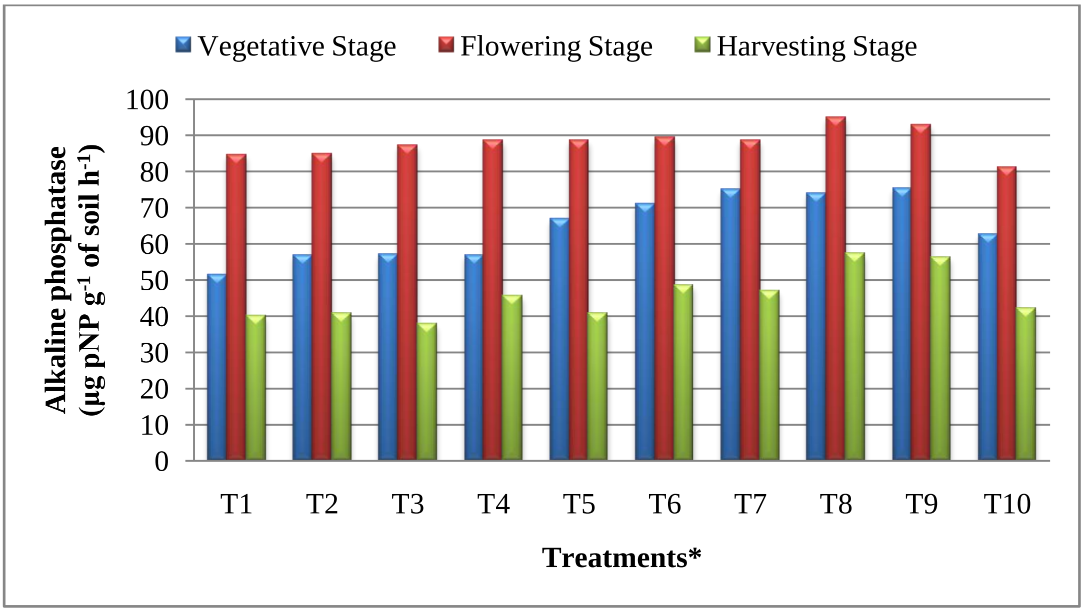
At harvesting stage significantly higher phosphatase activity was shown by T8:7 Irrigations + 75 % RDF + MC (57.20 µg pNP g^{-1} of soil h^{-1}), T9:7 Irrigations + 100 % RDF + MC (56.27 µg pNP g^{-1} of soil h^{-1}) and T6:5 Irrigations + 100 % RDF + MC (48.63 µg pNP g^{-1} of soil h^{-1}) while T7:7 Irrigations + 50 % RDF + MC (40.80 µg pNP g^{-1} of soil h^{-1}) was found to on par with control T10: Full Irrigations + 100 % RDF (42.10 µg pNP g^{-1} of soil h^{-1}) (Table 4.38).

Phosphatase enzymes involves in hydrolyzing recalcitrant form of P into available form of P to the plants. The phosphatase activity in the initial field soil was found as 38.76 µg pNP g^{-1} of soil h^{-1} (Table 3.4). Upon inoculation of developed endophytic microbial consortium the soil enzyme activity has been significantly improved in all the treatments (Plate 4.10b). The phosphatase activity was found to be maximum at flowering stage and decreased activity was observed as the crop proceeds to harvesting stage (Fig. 4.29).

The developed endophytic microbial consortia in the present investigation consist of potential phosphate solubilizing isolates and due to their application the inoculated treatments were found to have increased alkaline phosphatase activity in soil which was assumed due to the exudations of microbial consortia bacteria.

Phosphorus solubilizing microbes were developed with different mechanisms to release phosphates from prevailing sources in soil which include release of alkaline, acid phosphatases and phytase enzymes that involves conversion of organic phosphates to inorganic phosphates and exudates organic acids to solubilize inorganic phosphates (Rinu *et al.* 2012; Adhikari and Pandey, 2019).

The bacterial endophytes GBPI_TWL and GBPI_TWR which were found to have potential of solubilizing phosphorus in three forms including calcium phosphate, aluminium phosphate, and iron phosphate. Field experimentation and quantitative estimation of mechanism involved was revealed the production of soil enzymes alkaline phosphatase, acid phosphatase3 and phytases (Adhikari and Pandey, 2020).



*Treatment details were given in table 3.5

Fig. 4.29. Influence of endophytic microbial consortia on alkaline phosphotase activity of maize rhizosphere soil in field under water stress

Table 4.38. Influence of endophytic microbial consortia on soil enzymes activity of maize rhizosphere under water stress imposed field conditions

Treatments	Dehydrogenase (µg of TPF g^{-1} of soil day^{-1})			Alkaline phosphatase (µg pNP g^{-1} of soil h^{-1})		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T_1: 3 Irrigations + 50 % RDF + MC	29.44	42.93	20.17	51.57	84.57	39.97
T_2: 3 Irrigations + 75 % RDF + MC	32.42	43.97	21.21	56.87	84.83	40.70
T_3: 3 Irrigations + 100 % RDF + MC	33.47	43.10	20.84	57.10	87.27	37.83
T_4: 5 Irrigations + 50 % RDF + MC	37.47	49.90	22.51	56.87	88.53	45.57
T_5: 5 Irrigations + 75 % RDF + MC	38.81	50.73	23.58	66.83	88.60	40.80
T_6: 5 Irrigations + 100 % RDF + MC	40.83	52.73	24.10	70.93	89.37	48.63
T_7: 7 Irrigations + 50 % RDF + MC	41.57	50.80	24.78	75.00	88.57	47.00
T_8: 7 Irrigations + 75 % RDF + MC	44.43	58.63	25.40	74.10	94.97	57.20
T_9: 7 Irrigations + 100 % RDF + MC	46.93	58.90	26.62	75.40	92.90	56.27
T_{10}: Full Irrigations + 100 % RDF (Control)	38.13	49.56	23.17	62.50	81.23	42.10
CD(P=0.05)	2.57	4.82	1.93	5.60	N/A	4.99
S.Em. ±	0.86	1.62	0.65	1.87	3.01	1.67
S.Ed	1.22	2.29	0.92	2.64	4.26	2.36
CV (%)	3.91	5.61	4.85	5.01	5.93	6.34

4.6.8 Influence of endophytic microbial consortia on plant growth parameters of maize under water stress imposed field conditions

a) Plant height (cm):

At vegetative stage plant height was found maximum in T8:7 Irrigations + 75 % RDF + MC (190 cm) followed by T9:7 Irrigations + 100 % RDF + MC (188 cm), T6:5 Irrigations + 100 % RDF + MC (183 cm) and T5:5 Irrigations + 75 % RDF + MC (182 cm) when compared to control T10: Full Irrigations + 100 % RDF (173 cm). And minimum plant height was observed in T1:3 Irrigations + 50 % RDF + MC (147 cm)

At flowering stage maximum plant height was obtained by T9:7 Irrigations + 100 % RDF + MC (290 cm), T8:7 Irrigations + 75 % RDF + MC (289 cm) and T10: Full Irrigations + 100 % RDF (280 cm). And minimum plant height was observed in T1:3 Irrigations + 50 % RDF + MC (255 cm)

At harvesting stage significantly higher plant height was found in T9:7 Irrigations + 100 % RDF + MC (318 cm) while treatments T8:7 Irrigations + 75 % RDF + MC (307 cm), T6:5 Irrigations + 100 % RDF + MC (316 cm) and T5:5 Irrigations + 75 % RDF + MC (314 cm) were observed to have plant height on par with the control T10: Full Irrigations + 100 % RDF (285 cm) (Table 4.39).

At all the growth stages increased maize plant height of was observed in plots treated with microbial consortium though provided with 75% RDF at par with the control (100% RDF). The co-inoculation of *Bacillus megaterium, Azotobacter chroococcum* and *Bacillus mucilaginous* significantly increased maize biomass and plant height equivalent to half of the chemical fertilizer inputs (Wu *et al.,* 2005). Similarly, Rafi *et al.* (2012) reported that coinoculation of *Azospirillum* and PSB significantly increased the shoot height of foxtail millet over the control and the individual inoculants.

Viruel *et al.* (2014) reported that the P-solubilizing bacteria improved seed germination percentage, seedling emergence, shoot length, total dry biomass, and phosphorus (P) concentration of maize plants grown under controlled and field conditions.

b) Biomass (Kg ha^{-1})

At vegetative stage T9:7 Irrigations + 100 % RDF + MC (12194 Kg ha^{-1}), T8:7 Irrigations + 75 % RDF + MC (11777 Kg ha^{-1}) and T6:5 Irrigations + 100 % RDF + MC (11139 Kg ha^{-1}) were found to produce significantly more biomass than control T10: Full Irrigations + 100 % RDF (9666 Kg ha^{-1}) while minimum biomass production was observed in T1:3 Irrigations + 50 % RDF + MC (7277 Kg ha^{-1})

At flowering stage T9:7 Irrigations + 100 % RDF + MC (24054 Kg ha^{-1}) and T8:7 Irrigations + 75 % RDF + MC (22332 Kg ha^{-1}) were found to produce significantly higher amount of biomass than control T10: Full Irrigations + 100 % RDF (20471 Kg ha^{-1}). Whereas treatment T6:5 Irrigations + 100 % RDF + MC was found produce 21749 Kg ha^{-1} of biomass which is on par with the control.

At harvesting stage T9:7 Irrigations + 100 % RDF + MC (28110 Kg ha^{-1}) and T8:7 Irrigations + 75 % RDF + MC (26694 Kg ha^{-1}) were found to produce significantly higher amount of biomass. While T6:5 Irrigations + 100 % RDF + MC (23610 Kg ha^{-1}) and T5:5 Irrigations + 75 % RDF + MC (22138 Kg ha^{-1}) were found produce biomass on par with the control T10: Full Irrigations + 100 % RDF (22416 Kg ha^{-1}) (Table 4.39).

Treatments given with 7 irrigations were found to produce significantly more dry biomass while treatments given with 5 irrigations produced biomass at par with the control (full irrigations. This improved biomass production even under moisture stress conditions was due to the application of stress mitigating endophytic microbial consortium. Similar results were demonstrated by Sandhya *et al.* (2010) that the effect of inoculation of bacterial isolates, *P. entomophila, P. stutzeri, P. putida, P. syringae, P. monteilli* had a more significant positive effect on plant dry biomass under drought stress (by 37.80– 60.36% over uninoculated plants) than under non-stressed conditions (by 22.97–50.75% over uninoculated plants). Under dry conditions, the increased dry biomass and the rhizobacterial population probably increased root and soil microbial respiration which influences composition of soil atmosphere

A significant increase in plant biomass by 30.7% was observed with the inoculation of *Azotobacter* and *Azospirillum* on field-grown maize (Piromyou *et al.*, 2011). Similarly, the co-inoculation of *Bacillus megaterium, Bacillus mucilaginous* and *Azotobacter chroococcum* significantly increased maize plant height and biomass equivalent 50% of the chemical fertilizer inputs (Wu *et al.*, 2005).

4.6.9 Influence of endophytic microbial consortia on yield attributes of maize under water stress imposed field conditions

a) Cob weight (g/cob)

Treatments T9:7 Irrigations + 100 % RDF + MC (263 g), T8:7 Irrigations + 75 % RDF + MC (250 g) and T6:5 Irrigations + 100 % RDF + MC (235 g) were found to produce cobs with significantly more weight (Fig. 4.30a). While T7:7 Irrigations + 50 % RDF + MC (220 g) and T5:5 Irrigations + 75 % RDF + MC (214 g) have recorded cob weight which is on par with control T10: Full Irrigations + 100 % RDF (196 g). Minimum cob weight was observed in T1:3 Irrigations + 50 % RDF + MC (140 g) (Table 4.40).

b) Grain yield (Kg ha^{-1})

Significantly more yields were obtained (Table 4.40) in the treatments T6:5 Irrigations + 100 % RDF + MC (7963 Kg ha^{-1}) followed by T9:7 Irrigations + 100 % RDF + MC with grain yield of 7644 Kg ha^{-1}, T8:7 Irrigations + 75 % RDF + MC (7381 Kg ha^{-1}) and T5:5 Irrigations + 75 % RDF + MC (7139 Kg ha^{-1}) when compared to control T10: Full Irrigations + 100 % RDF (6616 Kg ha^{-1}) while minimum yields were found in T1:3 Irrigations + 50 % RDF + MC (5364 Kg ha^{-1}) (Fig. 4.30b). The higher yield and productivity of is directly linked with increased chlorophyll content by microbial activity thereby achieving high rate of photosynthesis eventually improved plant health.

Jarak *et al.* (2012) reported the ability of different bacterial strains viz., *Azotobacter chrocooccum, Bacillus* sp and *Pseudomonas* sp, to increase nutrient uptake in maize plants thereby enhancing plant growth (*Zea mays* L.) and grain yield. Upregulation of genetic expression of photosynthetic ability in plants by endophytic microbes increases leaf area, chlorophyll content and photosynthates accumulation which is directly linked to the yield, productivity and plant health (Joshi *et al.*, 2019; Harman *et al.,* 2019).

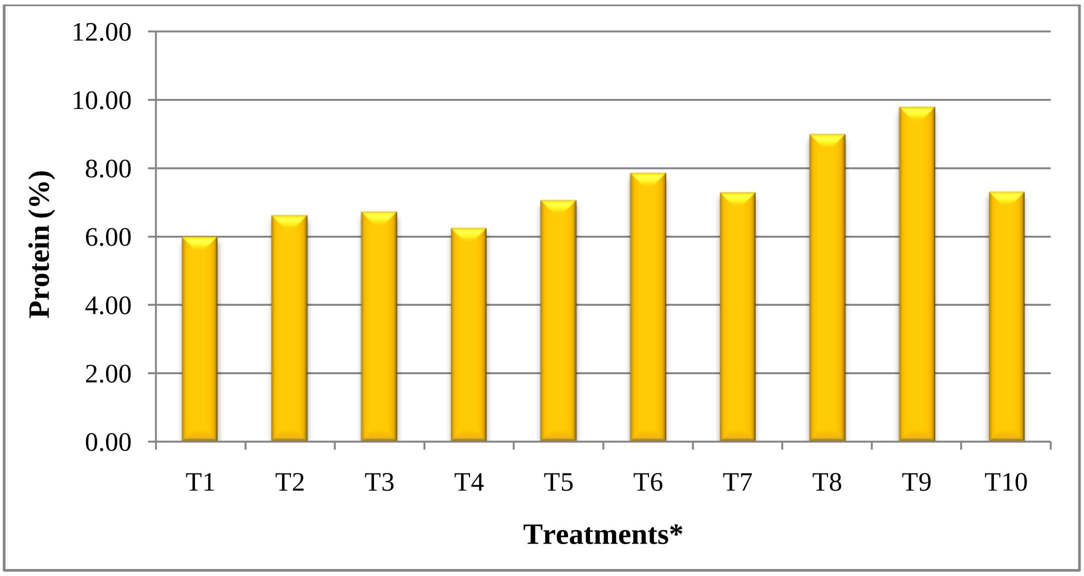
c) Test weight (g/100 seed)

Significantly higher test weight was measured in treatments T9:7 Irrigations + 100 % RDF + MC (49.18 g) and T8:7 Irrigations + 75 % RDF + MC (46.50 g) while treatments T6:5 Irrigations + 100 % RDF + MC (41.76 g) and T5:5 Irrigations + 75 % RDF + MC (41.60 g) were found to get test weight on par with the control T10: Full Irrigations + 100 % RDF (36.00 g) (Fig. 4.30c)

d) Protein (%)

Significantly higher protein content in maize kernels was recorded in treatments T9:7 Irrigations + 100 % RDF + MC (9.78 %) and T8:7 Irrigations + 75 % RDF + MC (8.98 %) while the treatments T7:7 Irrigations + 50 % RDF + MC (7.28 %), T6:5 Irrigations + 100 % RDF + MC (7.85 %) and T5:5 Irrigations + 75 % RDF + MC (7.06 %) were found to have protein content on par with the control T10: Full Irrigations + 100 % RDF (7.29 %) (Fig. 4.31)

Similarly increased protein content by 11.97% and carbohydrate content in seeds by inoculating plant growth promoting 76 rhizobacteria was identified by Stefan *et al*. (2013). Similarly, increased chemical constituents in seed including carbohydrates, proteins and dry matter was recorded due to application of plant growth promoting *Bacillus* sp. (Pandey *et al*., 2019)



*Treatment details were given in table 3.5

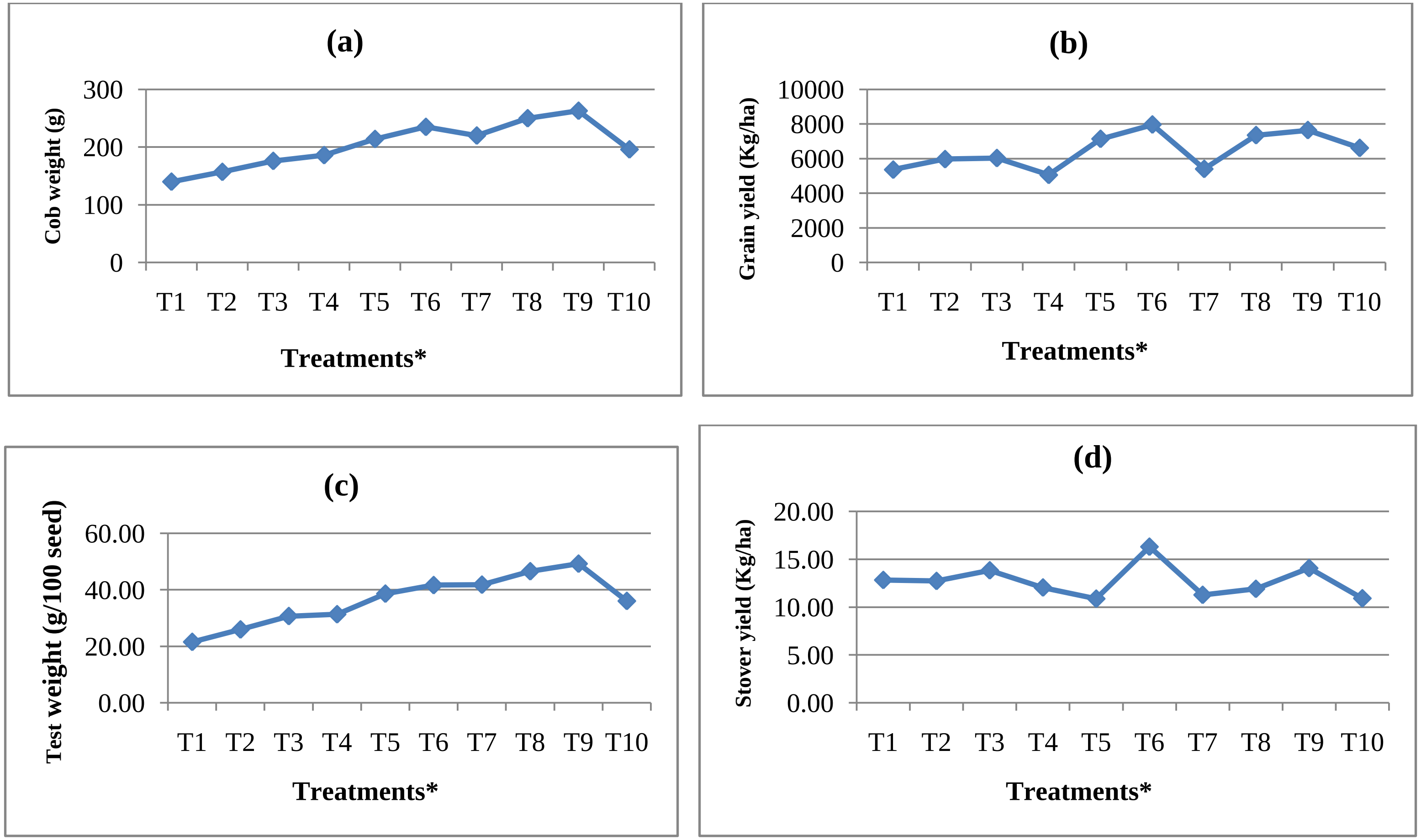
Fig. 4.31. Influence of endophytic microbial consortia on protein content in maize grains grown in field under water stress

Table 4.39. Influence of endophytic microbial consortia on plant growth parameters of maize under water stress imposed field conditions

Treatments	Plant height (cm)			Biomass (Kg ha^{-1})		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T_1: 3 Irrigations + 50 % RDF + MC	147	255	261	7277	13194	15332
T_2: 3 Irrigations + 75 % RDF + MC	159	260	268	8583	13611	17694
T_3: 3 Irrigations + 100 % RDF + MC	167	274	272	9250	15221	20805
T_4: 5 Irrigations + 50 % RDF + MC	160	265	270	7722	13249	16583
T_5: 5 Irrigations + 75 % RDF + MC	182	285	314	10361	18805	22138
T_6: 5 Irrigations + 100 % RDF + MC	183	279	316	11139	21749	23610
T_7: 7 Irrigations + 50 % RDF + MC	166	266	280	8555	15110	18805
T_8: 7 Irrigations + 75 % RDF + MC	190	289	307	11777	22332	26694
T_9: 7 Irrigations + 100 % RDF + MC	188	290	318	12194	24054	28110
T_{10}: Full Irrigations + 100 % RDF (Control)	173	280	285	9666	20471	22416
CD(P=0.05)	19	23	27	845	2125	1929
S.Em. ±	6	7	9	282	710	644
S.Ed	9	11	13	399	1004	911
CV (%)	6	5	5	5	7	5

Table 4.40. Influence of developed endophytic microbial consortia on yield attributes of maize under water stress imposed field conditions

Treatments	Cob weight (g/cob)	Grain yield (Kg/ha)	Test weight (g)	Protein (%)	Starch (%)	Stover yield (Kg/ha)
T_{1}: 3 Irrigations + 50 % RDF + MC	140	5364	21.54	5.99	44.45	12.82
T_{2}: 3 Irrigations + 75 % RDF + MC	157	5969	25.93	6.62	51.84	12.72
T_{3}: 3 Irrigations + 100 % RDF + MC	176	6032	30.66	6.71	57.84	13.83
T_{4}: 5 Irrigations + 50 % RDF + MC	186	5061	31.30	6.24	49.41	12.03
T_{5}: 5 Irrigations + 75 % RDF + MC	214	7139	38.58	7.06	66.33	10.86
T_{6}: 5 Irrigations + 100 % RDF + MC	235	7963	41.60	7.85	73.91	16.31
T_{7}: 7 Irrigations + 50 % RDF + MC	220	5407	41.76	7.28	57.74	11.26
T_{8}: 7 Irrigations + 75 % RDF + MC	250	7351	46.50	8.98	72.65	11.90
T_{9}: 7 Irrigations + 100 % RDF + MC	263	7644	49.18	9.78	73.81	14.06
T_{10}: Full Irrigations + 100 % RDF (Control)	196	6616	36.01	7.29	54.26	10.90
CD(P=0.05)	18	743	7.11	0.71	10.95	3.22
S.Em. ±	6	250	2.37	0.23	3.65	1.07
S.Ed	9	353	3.36	0.33	5.17	1.52
CV (%)	5	7	11.32	5.57	10.52	14.68

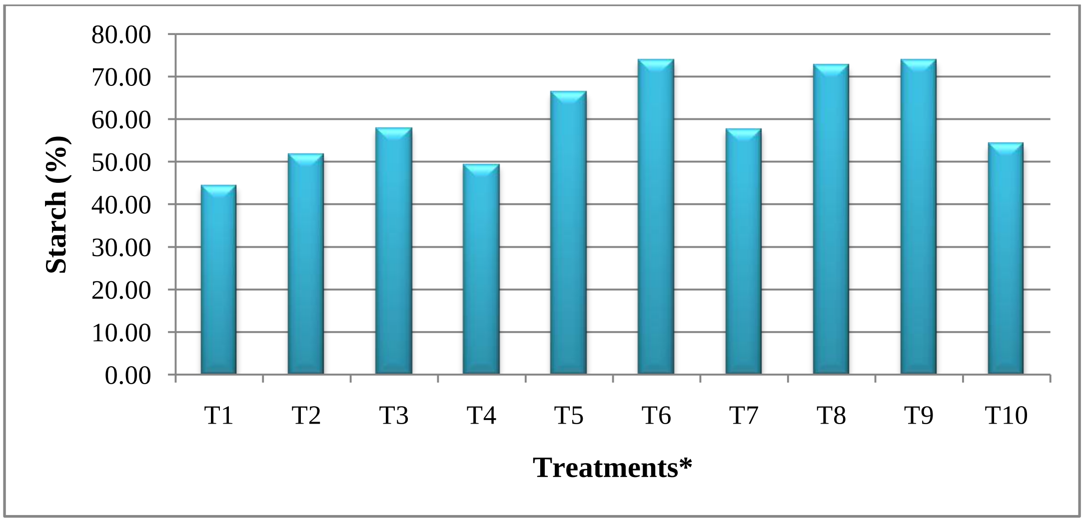


*Treatment details were given in table 3.5

Fig. 4.30. Influence of endophytic microbial consortia on yield attributes maize grown in field under water deficit stress a) Cob Weight b) Grain Yield c) Test Weight d) Stover Yield

e) Starch (%)

Starch content was found significantly higher in treatments T9:7 Irrigations + 100 % RDF + MC (73.81 %), T6:5 Irrigations + 100 % RDF + MC (73.91 %), T8:7 Irrigations + 75 % RDF + MC (72.65 %) and T5:5 Irrigations + 75 % RDF + MC (66.33 %) while T7:7 Irrigations + 50 % RDF + MC has recorded starch content 57.74% which was on par with the control T10: Full Irrigations + 100 % RDF (54.26 %) (Fig. 4.32)



*Treatment details were given in table 3.5

Fig. 4.32. Influence of endophytic microbial consortia on starch content in maize grains grown in field under water stress

Applied endophytic microbial consortium has enhance available nutrients like N, P and K by process of mineralization and increased their uptake by plants, maintained CSI in water deficit stress which aided in increased photosynthesis and accumulation of fixed carbohydrates. Li *et al.* (2017) demonstrated that availability of phosphorus and phosphorus to plants in optimum level contributes to increased photosynthesis which positively linked to improved grain quality with increased essential amino acids, protein and carbohydrate content.

f) Stover yield (Kg ha^{-1})

Maximum stover yield was recorded in T6:5 Irrigations + 100 % RDF + MC (16.31 Kg ha^{-1}) followed by T9:7 Irrigations + 100 % RDF + MC (14.03 Kg ha^{-1}). This increase in stovr yield in inoculated treatments (Fig. 4.30d) was might be due to the action of applied microbial consortia having multiple PGP traits and osmotolerant isolates which helped in colonising the root hair and cortical cells and enhanced the root surface area, elongated root formation under drought stress thereby enhancing nutrient acquisition and regulated plant harmone biosynthetic pathways.

The combined application of bio inoculums (*Serratia* sp.) with 80% recommended dose of N and P enhanced microbial activity which resulted increase of tillering by 28.03% in tillering, grain number per spike by 19.61%, seed test weight (1000 grains) by 10.5% , 9.4% of increased grain yield and 9.2% in straw yield (Sood *et al.,* 2018).

4.6.10. Economics

Influence of developed endophytic microbial consortia on benefit cost ratio of maize under field conditions was calculated by dividing net returns with cost of cultivation. Considerably higher net returns were obtained for T6: 5 Irrigations + 100 % RDF + MC (Rs. 113717/- ha^{-1}) followed by T9:7 Irrigations + 100 % RDF + MC (Rs. 106325/- ha^{-1}) and T8:7 Irrigations + 75 % RDF + MC (Rs. 102539/- ha^{-1}) while the net returns of T5: 5 Irrigations + 75 % RDF + MC (Rs. 99303/- ha^{-1}) were found to be on par with the control T10: Full Irrigations + 100 % RDF (Rs. 84763/- ha^{-1}) (Table 4.41). The benefit cost ratio was found significantly higher in treatments T6 (2.5) followed by T9 (2.3), T8 (2.3) and T5 (2.3) while the B: C ratio of control was found as 1.8.

The results suggested that significant net returns were obtained even under water deficit conditions when compared to control provided with full irrigations because the applied endophytic microbial consortium has improved plant growth and yield by enhancing osmotolerance and mineralization of nutrients under deficit conditions. On the other hand nearly 120 mm of water (2 irrigations) and 25% of RDF can be minimized for crop production without affecting yield and net returns.

The results were comparable with the field trials to evaluate the effect of bioinoculats on yield of maize in rotation with wheat as affected by irrigation regime by Monem *et al.* (2001). An economic partial budget was analyzed and reported that using commercial biofertilizer Cerealin or *Azospirillum brasilense* with half N rate (144 kg N/ha) resulted in a significant increase in yield. Economically commercial bioinoculum Cerealin with half the normal N application had an average higher net benefit, benefit/cost ratios and marginal rate of return values suggesting that possibility of saving 50% of chemical fertilizers.

Similarly it was observed that integrated use of half dose of NP fertilizer with Biopower+ biological potassium fertilizer (BPF) or effective plant growth promoting microorganisms (EM) can give considerable crop yield as of crop yields obtained with full rate of NP fertilizer without microbial inoculums and thereby net returns can be maximized by minimizing production cost with reduced use of chemical fertilizers (Jilani *et al.*, 2007).

Table 4.41. Influence of developed endophytic microbial consortia on benefit cost ratio of maize under field conditions

Treatments	**Total cost of cultivation (Rs ha^{-1})**	**Gross returns (Rs ha^{-1})**	**Net returns (Rs ha^{-1})**	**B:C ratio**
T$_1$:3 Irrigations + 50 % RDF + MC	40400	107,287	66,887	1.7
T$_2$:3 Irrigations + 75 % RDF + MC	42475	119,381	76,906	1.8
T$_3$:3 Irrigations + 100 % RDF + MC	44550	120,633	76,083	1.7
T$_4$:5 Irrigations + 50 % RDF + MC	41400	101,216	59,816	1.4
T$_5$:5 Irrigations + 75 % RDF + MC	43475	142,778	99,303	2.3
T$_6$:5 Irrigations + 100 % RDF + MC	45550	159,267	113,717	2.5
T$_7$:7 Irrigations + 50 % RDF + MC	42400	108,147	65,747	1.6
T$_8$:7 Irrigations + 75 % RDF + MC	44475	147,014	102,539	2.3
T$_9$:7 Irrigations + 100 % RDF + MC	46550	152,875	106,325	2.3
T$_{10}$: Full Irrigations + 100 % RDF (Control)	47550	132,313	84,763	1.8
CD(P=0.05)		14,981	14,981	0.3
S.Em. ±		5,003	5,003	0.1
S.Ed		7,076	7,076	0.2
CV (%)		7	10	10.2

Chapter – V

Summary and Conclusions

Chapter V

SUMMARY AND CONCLUSIONS

Among the abiotic stresses, water stress (drought) has major impact on crop growth and productivity throughout the world and for maize productivity water stress is the major limitation leading to heavy yield losses. On the other hand to maintain proper growth and yield, maize also requires an adequate supply of chemical fertilizers. The excessive use of chemical fertilizers will lead to negative impact on the environment and thus affecting human health. The quality and yield of crop may change due to the association of plant growth promoting endophytic microbes that colonize in plants asymptomatically. Thus, in the present study an approach was developed to minimize use of chemical fertilizers and to improve stress tolerance in maize without affecting ecosystem by using endophytic bacterial inoculants.

- A total of 106 culturable endophytic bacterial morphotypes were isolated and pure cultures were obtained from maize plant tissues including roots, stem, leaf and kernels.
- Among all, 36 water stress tolerant and plant growth promoting isolates were selected based on their osmotolarance at water potential of -0.65 MPa (10% PEG) and other plant growth promoting attributes such as biological nitrogen fixation, phosphate solubilization, potassium solubilization, ACC deaminase activity, exopolysaccharides, HCN, siderophores and IAA production which measured qualitatively.
- The efficiency of water stress mitigation and plant growth promoting attributes of selected isolates were further tested for their growth at varied water potentials of -0.65, -1.57, -2.17 and -2.70 MPa and quantitative production of soluble P_2O_5, soluble K_2O, IAA, siderophores and exopolysaccharides
- Bacterial isolates with less per cent decrease in their growth at higher water potential of -2.17 and -2.70 MPa were considered as osmotolerant or water stress resilient. In the present investigation endophytic bacterial strains VaR3E1 (23.94%), NL3E3 (45.45%), VS1E1 (46.62%), PL3E2 (47.18%) and LL3E1 (54.07%) were found to be efficient osmotolerants with less per cent decrease in the growth at higher water potential of -2.17MPa (30% PEG 6000).

- All the identified efficient bacterial isolates (36) showed distinct phosphate-solubilizing abilities in quantitative study varying in the range of 68 to 573 µg/mL. Maximum release of soluble P_2O_5 was shown by the isolates KL3E1 (573µg/mL) followed by KL3E2 (568µg/mL), LS3E3 (512µg/mL), NC3E2 (468µg/mL) and CC3E3 (461µg/mL) while the isolate VR1E1 was found to release 68 µg/mL of soluble P_2O_5 recording lowest among all solubilizing isolates

- Eighteen isolates were shown potassium solubilization capacity of which maximum was shown by PdS3E1 (39 mg/L) and JC3E2 (38 mg/L) while minimum K solubilization was observed in the isolate KL3E1 (9 mg/L)

- IAA production among bacterial isolates in the present study varied significantly. Minimum production of IAA was shown by the isolate PL3E2 producing 12 µg/mL while maximum production of IAA was shown by the isolates LL3E1 (165µg/mL), RgL3E4 (163µg/mL), PdS3E2 (149µg/mL), PdC3E2 and JC3E2 producing 134 µg/mL of IAA

- Efficient siderophore producing isolates were observed as VaR3E1, JC3E2 and KS3E1 with 93%, 92% and 91% of siderophore forming units respectively

- The exopolysaccharide production was recorded in the range of 6 to 65 mg/mL by 29 isolates. The isolates LS3E3, LS3E1, VaL3E1 and KS3E2 have produced 65,61,61 and 60 mg/mL of EPS respectively

- The presence of ACC deaminase activity in bacteria was found on the basis of ability of bacteria to utilize ACC as a source of nitrogen. On the basis of preliminary qualitative plate assay 2 isolates (RgC3E2, JC3E1) have shown high growth, 16 isolates have shown moderate growth, 14 isolates have shown low growth and 4 strains were found to have no growth

- Endophytic bacterial strain LS3E1 is the only isolate which has shown positive result for HCN production.

- Significant isolates were identified as genera *Gordonia,Microbacterium, Kosakonia, Priestia, Bacillus, Cellulosimicrobium, Pantoea, Pseudomonas, Klebsiella* and *Methylorubrum* by 16S rRNA gene sequencing

- The seed vigor index is significantly higher for *Pantoea dispersa* (5390.8) followed by *Priestia megaterium* (5334.9) and *Bacillius licheniformis* (5265.2). The per cent germination and seed vigor index were recorded less in seedlings treated with *Pseudomonas aeruginosa*

- Bacterial isolates *Bacillus licheniformis* and *Pseudomonas aeruginosa* have shown antagonism effect for the three tested pathogenic fungi *Rhizoctonia solani, Fusarium oxysporium* and *Exserohilum turcicum*. Isolates *Kosakonia radicincitans* and *Kosakonia cowanii* were antagonistic to *Rhizoctonia solani* and *Fusarium oxysporium* while the strains of *Klebsiella pneumoniae* were antagonistic to *Rhizoctonia solani* and *Exserohilum turcicum*. The bacterial isolates *Priestia megaterium, Priestia aryabhattai* and *Methylorubrum populi* were found antagonistic to only *Rhizoctonia solani.*

- Efficient 7 isolates were selected namely *Kosakonia radicincitans* (NL3E3), *Priestia megaterium* (PdS3E1), *Priestia aryabhattai* (PL3E2) *Bacillus licheniformis* (VaR3E1), *Pseudomonas aeruginosa* (LS3E1), *Klebsiella pneumonia* (LS3E3) and *Methylorubrum populi* (LL3E1) for compatiability and endophytic colonization studies

- Microbial consortium *Bacillus megaterium+ Bacillus licheniformis+ Klebsiella pneumoniae+ Methylorubrum populi* was seclected for pot and field experiments.

- Treatments T9, T8, T6 and T5 were found to have higher CSI though given lesser irrigations than the control which might be due to the application of osmotolerant and plant growth promoting microbial consortium

- At flowering stage RWC has shown no significant difference among the treatments given with 5 and 7 irrigations and they are on par with the control given with full irrigations and no microbial inoculums.

- The production of H_2O_2 was higher in treatments given with lower number of irrigations. The H_2O_2 production was increased in flowering stage and then reduced at harvesting stage but not lower than the vegetative stage and lower levels of H_2O_2 was found in microbial treated plants even under drought stress when compared to non inoculated treatments

- At all the growth stages peroxidase content is higher in treatments provided with 75% and 100% RDF under water deficit stress conditions. The increased activity of peroxidase in microbial inoculated treatments under drought stress conditions suggests that microbial inoculation protects plant from stress by scavenging reactive oxygen species like H_2O_2 by increased production of peroxidase leading to improved plant growth under stress.

- At all the growth stages proline content was found highest in treatments (T1, T2 and T3) given with 3 irrigations while the treatments provided with 7 irrigations (T7, T8 and T9) were found to have lesser proline content than the control T10 provided with no water stress and no microbial inoculums. In consortium inoculated treatments provided with 7 irrigations were found to have lesser proline content than the control (un inoculated) indicating that these treatments were less affected by induced water deficit stress.

- Decreased pH of soil was observed which might be due to the release of organic acids through the secretions of microbial inoculants into soil for solubilizing minerals into available forms.

- At all the growth stages EC is higher in treatments given with 3 irrigations than the treatments provided with 5 and 7 irrigations because under moisture limited conditions mineral solutes concentration will be more than the sufficiently moisture available conditions where solutes will get dissolved rapidly and uptaken by plants.

- At vegetative stage T5 was found to have maximum OC while at both flowering and harvesting stage T7 and T8 were observed to have higher OC content. At all growth stages T3 was found to have minimum OC but not less than the control (T10).

- At initial growth stages available nitrogen was found significantly higher in treatments given with 7 irrigations provided 100% or 75% RDF indicating limited application of fertilizers by 25% didn’t affect the nitrogen availability in soil. At vegetative and flowering stage the available P was higher but gradually declined at harvesting stage. The treatments given with water stress (7 and 5 irrigations) and limited nutrients (75% RDF) were found to have significantly higher available P in soil when treated with developed endophytic microbial consortia. The microbial consortia could able to compensate 25% of potassium in treatments given with 75 % RDF even at water stress conditions (7 and 5 irrigations)

- Application of microbial consortium to maize has improved concentration of N, P and K in plants even under drought stress which might be due to the microbial inoculants application which had enhanced the uptake of all plant nutrients. Treatments given with water stress (7 and 5 irrigations) and limited nutrients (50% and 75% RDF) were found to have significantly higher nutrient (N, P, K) content or on par with the control.

- Application of endophytic microbial consortia has improved the uptake of all macro nutrients including N, P and K at all the growth stages and significantly higher uptake of nutrients was found in T9 and T8 while T6 was found on par with control T10

- Enhanced soil dehydrogenase activity and alkaline phosphatase activity was found in treatments provided with 100% and 75% RDF even under water deficit conditions and the enzyme activity was found prominent at flowering stage.

- In the treatments induced with drought stress by providing less irrigations (3) decreased microbial population in rhizosphere and endosphere while maximum count was recorded in T9, T8, T6 and T5 when compared to control T10. Endophytic population was found highest in root endosphere than the followed by stem, leaf and kernels.

- Treatments given with 7 irrigations were found to produce significantly more dry biomass while treatments given with 5 irrigations produced biomass at par with the control (full irrigations)

- Significantly more yields were obtained in the treatments T6:5 Irrigations + 100 % RDF + MC (7963 Kg ha^{-1}) followed by T9:7 Irrigations + 100 % RDF + MC with grain yield of 7644 Kg ha^{-1}, T8:7 Irrigations + 75 % RDF + MC (7381 Kg ha^{-1}) and T5:5 Irrigations + 75 % RDF + MC (7139 Kg ha^{-1}) when compared to control T10: Full Irrigations + 100 % RDF (6616 Kg ha^{-1}).

The following conclusions are drawn from the present investigation

The present study was a preliminary investigation on maize endophytic bacteria for their ability to mitigate water stress and plant growth promotion in pot and field conditions. The results provided empirical evidence for the colonization of maize endophytic bacteria and demonstrated that these microbial consortium have a number of plant growth, soil health and fertility inducing traits. Inoculation of endophytic microbial consortium under limited soil moisture and nutrient availability improved soil enzymatic activity and soil physicochemical properties, plant nutrient status and osmoregulation which positively reflected on plant performance. Microbial consortium inoculated treatments given with 7 irrigations with 100% and 75% RDF were found to produce significantly more dry biomass and grain yield than the un inoculated control which was provided with full irrigations (9) and 100% RDF. Significant net returns were obtained even under water and nutrient limiting conditions and nearly 120 mm of water (2 irrigations) and 25% of RDF can be minimized for crop production without affecting yield and net returns. Thus, the current research advocates the use of endophytic microbial consortium to mitigate stress and to improve soil nutrient status which ultimately enhances the plant health and yield and application of bio-inoculants is considered as eco friendly, economically viable and socially acceptable which completely fulfill the sustainable development goal.

Recommendations for further research

- The mechanism of the interaction between endophytes and host plant need to be elucidated, the mechanism will further comprehensively clarify process of inoculation, role of endophytes in essential nutrient uptake and stress tolerance by host.
- The interactions (negative or positive) between inoculated bacteria with indigenous microflora of host need to be investigated which aid the ease of microbial engineering.
- The modulation of defense-related gene signaling and pathways to counteract water stress by osmotolarant endophytes need to be explored with the help of proteomics, genomics, metabolomics, and nanotechnological tools.
- Studies on the multi-location field evaluation of stress tolerant bacterial endophytes will further strengthen their usefulness.
- Cross-host application of endophytic microbial consortium to study strain-specifc stimulation of gene-mediated pathways in various crops would be useful and path-breaking.
- The development of mathematical models based “customized” inocula would facilitate the stable employment of PGPB in increasing crop production. This would ensure that the great potential of plant growth promoting microbes would find its way to facilitating reproducible field application and sustainable food production under changing climate.

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Note: The pattern of literature cited presented above is in accordance with the guidelines for thesis presentation for Acharya NG Ranga Agricultural University, Guntur.

Appendices

APPENDIX-I

COMPOSITION OF DIFFERENT GROWTH MEDIA/REAGENTS/INDICATORS

S.No	Media	Quantity
1.	**Aleksandrov's Agar Medium** (Sugumaran and Janartham, 2007)	
	Glucose	5.0 g
	Magnesium sulphate	0.5 g
	$FeCl_3$	0.005 g
	Calcium carbonate	0.1 g
	Tricalcium phosphate	2.0 g
	Potassium alumino silicate	2.0 g
	Agar	18.0 g
	Distilled water	1000 ml
	pH (at 25°C)	7.0±0.2
2.	**DF Minimal Medium** (Honma and Shimomura, 1978)	
	Glucose	2.0 g
	Gluconic acid	2.0 g
	Citric acid	2.0 g
	KH_2PO_4	4.0 g
	Na_2HPO_4	6.0 g
	$MgSO_4.7H_2O$	0.2 g
	Micro nutrient solution	10 ml
	Distilled water	990 ml
	pH (at 25°C)	7.0±0.2
3.	**Glucose Phosphate Broth** (Crown and Gen, 1998)	
	Peptone	5.0g
	Beef extract	3.0g
	Glucose	5.0g
	Bromocresol purple solution	15ml
	Distilled water	1000ml
	pH (at 25°C)	7.0±0.2

Cont……

4. **Glucose Peptone Agar** (Crown and Gen, 1998)

Glucose	5.0g
Peptone	10.0g
Agar	18.0g
Distilled water	1000ml
pH (at 25°C)	7.0±0.2

5. **Gram Stain Solutions** (Barthalomew and Mittewar, 1950)

A) Crystal Violet Solution

Crystal violet	10.0g
Ammonium oxalate	4.0g
Ethanol	100ml
Distilled water	1000ml

B) Gram’s Iodine Solution

Iodine	1.0g
Potassium iodide	2.0g
Ethanol	25ml
Distilled water	100ml

C) Counter Stain

2.5 % Safranin in ethanol	10ml
Distilled water	100ml

D) Ethyl Alcohol (Decolouriser)

Ethanol	95ml
Distilled water	5ml

6. **Hofers Alkaline Medium** (Hofer, 1935)

Mannitol	10.0 g
Dipotassium phosphate	0.5 g
Magnesium sulphate	0.2 g
Sodium chloride	0.1 g
Yeast extract	1.0 g
Thymol blue	0.016 g
Agar	15.0 g
Distilled water	1000 ml
pH (at 25°C)	11.0±0.2

Cont……

7.	**King’s B Agar Medium** (Schwyn and Neilands, 1987)	
	Peptone	16.0g
	K_2HPO_4	1.6g
	$MgSO_4$	1.6g
	Glycerol	10.0g
	Agar	18.0g
	Distilled water	1000ml
	pH (at 25°C)	7.5±0.2
8.	**Luria Bertani Broth** (Gordon and Weber, 1951)	
	Casein enzymic hydrolysate	10.0 g
	Yeast extract	5.0 g
	Sodium chloride	10.0 g
	Distilled water	1000 ml
	pH (at 25°C)	7.5±0.2
9.	**MR-VP Broth** (c and Gen, 1998)	
	Buffered peptone	7.0g
	Dextrose	5.0g
	Dipotassium hydrogen phosphate	5.0g
	Distilled water	1000ml
	pH (at 25°C)	6.9±7.1
10.	**Nitrate Broth** (Tille and Forbes, 2014)	
	Peptone	5.0g
	Beef extract	3.0g
	Potassium nitrate	5.0g
	Distilled water	1000ml
	pH (at 25°C)	6.8±7.0
11.	**Nutrient Agar** (Anjum and Chandra, 2015)	
	Peptone	5.0g
	Beef extract	3.0g
	Sodium chloride	5.0g
	Agar	18.0g
	Distilled water	1000ml
	pH (at 25°C)	6.8±7.2

Cont…..

12.	**Nutrient Broth** (Anjum and Chandra, 2015)	
	Peptone	10.0g
	Beef extract	10.0g
	Sodium chloride	5.0g
	Distilled water	1000ml
	pH (at 25°C)	7.3±0.1
13.	**Nutrient Gelatine Agar**(MacFaddin, 2000)	
	Peptone	5.0g
	Beef extract	3.0g
	Gelatine	120.0g
	Distilled water	1000ml
	pH (at 25°C)	6.8±7.0
14.	**Peptone Water**(Juanda, 2005)	
	Peptide digest of animal tissue	10.0g
	Sodium chloride	5.0g
	Distilled water	1000ml
	pH (at 25°C)	7.2±0.2
15.	**Pikovskaya's Agar** (Singh and Ghosh, 2012)	
	Yeast extract	0.5g
	Dextrose	10.0g
	Calcium phosphate	5.0g
	Ammonium sulphate	0.5g
	Potassium chloride	0.2g
	Manganese sulphate	0.0001g
	Magnesium sulphate	0.1g
	Ferrous sulphate	0.0001g
	Agar	18.0g
	Distilled water	1000ml
	pH (at 25°C)	7.0±0.2
16.	**Plate Count Agar**(Vlassak *et al.*, 1992)	
	Casein enzymic hydrolysate	5.0 g
	Yeast extract	2.0 g
	Dextrose	1.0 g

Cont…..

	Agar	18.0 g
	Distilled water	1000 ml
	pH (at 25°C)	5.6±5.8
17.	**Potato Dextrose Agar (**Vlassak *et al.*,1992**)**	
	Potato infusion	200.0g
	Dextrose	20.0g
	Agar	18.0g
	Distilled water	1000ml
	pH (at 25°C)	5.6±5.8
18.	***Pseudomonas* Agar** (Schwyn and Neilands, 1987)	
	Pancreatic digest of casein	10.0 g
	Peptide digest of animal tissue	10.0 g
	Anhydrous dibasic potassium phosphate	1.50g
	Magnesium sulphate	1.50g
	Agar	18.0g
	Distilled water	1000ml
	pH (at 25°C)	7.0±0.2
19.	**Salkowski Reagent** (Gordon and Weber, 1951)	
	35% Perchloric acid	50ml
	0.5M $FeCl_3$	1ml
20.	**Simmons Citrate Agar** (MacFaddin, 2000)	
	Magnesium sulphate	0.2g
	Ammonium dihydrogen phosphate	1.0g
	Dipotassium phosphate	1.0g

Cont…..

	Sodium citrate	2.0g
	Sodium chloride	5.0g
	Bromothymol blue	0.08g
	Agar	18.0g
	Distilled water	1000ml
	pH (at 25°C)	6.8±7.0
21.	**Starch Casein Agar** (MacFaddin, 2000)	
	Starch	10.0g
	Casein	1.0g
	Agar	18.0g
	Distilled water	1000ml
	pH (at 25°C)	7.2±7.4
22.	**Sulphide Indole Motility (SIM) Agar** (Beishir, 1991)	
	HM Peptone	3.0 g
	Peptone	30.0 g
	Peptonized iron	0.20 h
	Sodium thiosulphate	0.025
	Agar	3.0 g
	Distilled water	1000 ml
	pH (at 25°C)	7.3±0.2
23.	**TRIS Minimal Medium** (Honma and Shimomura, 1978)	
	Peptone	5.0 g
	Beef extract	3.0 g
	Glucose	10.0 g
	Zinc phosphate	1.0 g
	Ammonium sulphate	0.5 g
	Potassium chloride	0.2 g
	Yeast extract	0.5 g
	Ferrous sulphate	0.01 g
	Manganese sulphate	0.01 g
	Dipotassium hydrogen phosphate	0.25 g
	Agar	20.0 g
	Distilled water	1000 ml

Cont…..

	pH (at 25°C)	7.0±0.2
24.	**Tryptic Soya Agar** (Vlassak *et al.*,1992)	
	Pancreatic digest of casein	17.0 g
	Papaic digest of soyabean meal	3.0 g
	Dextrose (Glucose)	2.5 g
	Dipotassium hydrogen phosphate	2.5 g
	Agar	15.0 g
	Distilled water	1000 ml
	pH (at 25°C)	7.3±0.2
	pH (at 25°C)	6.8±0.2
25.	**YEM Agar w/ Congo Red)** (Busse and Bottomley, 1989)	
	Yeast extract	1.0g
	Mannitol	10.0g
	Dipotassium hydrogen phosphate	0.5g
	Magnesium sulphate	0.2g
	Sodium chloride	0.1g
	Calcium carbonate	1.0g
	Congo red	0.025g
	Agar	18.0g
	Distilled water	1000ml
	pH (at 25°C)	6.8±7.0
26.	**YEM Broth**(Busse and Bottomley, 1989)	
	Yeast extract	1.0g
	Mannitol	10.0g

Cont…..

	Dipotassium hydrogen phosphate	0.5g
	Magnesium sulphate	0.2g
	Sodium chloride	0.1g
	Calcium carbonate	1.0g
	Distilled water	1000ml
	pH (at 25°C)	6.8±7.0
27.	**Yeast Malt extract Glucose (YMG) Agar (**Ashok *et al*., 2011**)**	
	Glucose	10.0 g
	Yeast extract	3.0 g
	Malt extract	3.0 g
	Peptone	5.0 g
	Agar	18.0 g
	Distilled water	1000 ml
	pH (at 25°C)	7.0±0.2
28.	**Yeast Malt Glucose (YMG) Broth (**Ashok *et al*., 2011**)**	
	Glucose	10.0 g
	Yeast extract	3.0 g
	Malt extract	3.0 g
	Peptone	5.0 g
	Agar	18.0 g
	Distilled water	1000 ml
	pH (at 25°C)	6.2±0.2

APPENDIX-II

BUFFERS AND STOCK SOLUTIONS

DNA Extraction Buffer

2 % (w/v) CTAB (Nalgene)	10 g
100 Mm Tris HCl, pH 8.0	100 ml of 0.5 M Tris HCl (pH8.0)
20 mM EDTA, pH 8.0	20 ml of 0.5 M EDTA (pH 8.0)
1.4 M NaCl	140 ml of 5 M NaCl
PVP (Sigma)	200 mg

All the above ingredients except CTAB were added in respective quantities and final volume was made up to 500ml with double distilled water, the solution was autoclaved. The solution was allowed to attain room temperature and 10g of CTAB was dissolved by intense stirring, stored at room temperature (Heddi *et al.*, 1999).

EDTA (0.5M)

Dissolved 186.1 g of disodium EDTA .2 H_2O in 800 ml of H_2O and stirred using magnetic stirrer and adjusted pH to 8.0 with NaOH

Ethidium Bromide

Stock 20 mg/ml can be prepared by dissolving 1 gm of ethidium bromide in 50 ml of water.

RNase: (20 mg/ml)

20 mg of RNase (Sigma) was dissolved in 500 µl of double distilled water + 500 µl of 50% Glycerol (Qualigens) and the solution was heated at 95˚C for 10 min and stored at -20˚C.

TAE Buffer (pH 8.0)

For 10X stock solution

400 mM Tris base

200 mM Glacial acetic acid

10 mM EDTA

Dissolve in appropriate amount of sterile water.

TBE (electrophoresis buffer- pH 8.4):

To prepare a 10X solution 54g Trizma base, 22.5g of Boric acid along with 20ml of EDTA (0.5M, pH 8.0) IN 500 ml of water. For running the gel, 10X is made to 1X solution.

TE buffer (pH 8.0)

10 mM Tris HCl

1 mM EDTA

6X Gel loading buffer

0.25 % (w/v) bromophenol blue

0.25 % (w/v) xylene cyanol

40 % (w/v) sucrose in water

10 X PCR buffer

500 mM KCl and 100 mM Tris-Cl. pH 8.0

APPENDIX-III

16S rRNA GENE SEQUENCES OF ENDOPHYTIC BACTERIAL ISOLATES

1. AR3E2 (*Gordonia hongkongensis* strain GAD1811)

>Forward Seq data

GTGTGGGTTCCTTTTCACGGGGATCCGTGCCGTAGCTAACGCATTAAGTAC
CCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAA
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GTTCCCGGGCCTTGTACACACCGCCCGTCACGTCATGAAAGTCGGTAACA
CCCGAAGCCGGTGGCCTAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGG
ATCGGCGATTGGGACGAAGTCGTAACAA GGGTAACCG

>Reverse Seq

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CCACACCTAGTACCCACCGTTTACGGCGTGGACTACCAGGGTATCTAATCC
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CTCACGCTTCGTCCCTGGTGAAAGAGGTTTACAACCCGAAGGCCGTCATC
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ACCAACCACCATGCGACAGTTGGTCATATCCGGTATTAGACCCAGTTTCCCAGGCTTATCCCAGAGTGCAGGGCAGATCACCCACGTGTTACTCACCCGTTCGCCACTCGAGTACCCAGCAAGCTGGGCCTTTCCGTTCGACTTGCATGTGTTAAGCACGCCGCCAGCGTTCGTCCTGAGC

>Reverse complement

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> Consensus data

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2. VR1E1 ***(Microbacterium hydrothermale strain GAD1812*)**

>Forward Seq data
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>Reverse Seq Data
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>Reverse complement
TTCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAA
CGGTGAAGCCAAGCTTGCTTGGTGGATCAGTGGCGAACGGGTGAGTAACA
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>Consensus data
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3. NL3E3 (*Kosakonia radicincitans strain GAD1813)*

>Forward Seq data
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>Reverse Seq Data
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ACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCT
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ACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCT GAGCC

>Reverse complement
GGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGT
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TAAGT

>Consensus
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4. NC3E2 (*Kosakonia cowanii* strain GAD1814)

>Forward Seq data

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>Reverse Seq Data

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>Reverse complement

GCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTA
ACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
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AAG

>Consensus data

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GGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTT
TGTGATTCATGACTGGGGTGAAGTCGTAA

5. PdS3E1 (*Priestia megaterium* strain GAD181_5)

>Forward Seq data
GAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTG
GGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGC
ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC
CAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCCTTCGGG
GGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGAT
GTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCAT
TTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGG
GGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCT
ACAATGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCAAGCCAATCCC
ATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAG
CTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCC
GGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAA
GTCGGTGGAGTAACCGTAAGGAGCTAGCCGCCTAAGGTGGGACAGATGAT
TGGGGTGAAGTCGT

>Reverse Seq Data
TGCGTTAGCTGCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTC
ATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACG
CTTTCGCGCCTCAGCGTCAGTTACAGACCAAAAAGCCGCCTTCGCCACTG
GTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCGCTT
TTCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCACGGTTGAG
CCGTGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCGCGCTTTACGCC
CAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCA
CGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACGAGCAGTTA
CTCTCATACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTT

CATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATT
CCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTG
GCCGATCACCCTCTCAGGTCGGCTATGCATCGTTGCCTTGGTGAGCCGTTA
CCTCACCAACTAGCTAATGCACCGCGGGCCCATCTGTAAGTGATAGCCGA
AACCATCTTTCAATCATCTCCCATGAAGGAGAAGATCCTATCCGGTATTAG
CTTCGGTTTCCCGAAGTTATCCCAGTCTTACAGGCAGGTTGCCCACGTGTT
ACTCACCCGTCCGCCGCTAACGTCATAGAAGCAAGCTTCTAATCAGTTCGC
TCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCAT CCTGAG

>Reverse complement
CTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACT
GATTAGAAGCTTGCTTCTATGACGTTAGCGGCGGACGGGTGAGTAACACG
TGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAAT
ACCGGATAGGATCTTCTCCTTCATGGGAGATGATTGAAAGATGGTTTCGG
CTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAAC
GGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCAC
ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGA
ATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATG
AAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTATGAGA
GTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTA
CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTA
TTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCC
ACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAA
GAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGA
GGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGC
GCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC
GTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCA
GCT AACGCA

>Consensus data
CTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACT
GATTAGAAGCTTGCTTCTATGACGTTAGCGGCGGACGGGTGAGTAACACG
TGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAAT
ACCGGATAGGATCTTCTCCTTCATGGGAGATGATTGAAAGATGGTTTCGG
CTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAAC

GGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCAC
ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGA
ATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATG
AAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTATGAGA
GTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTA
CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTA
TTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCC
ACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAA
GAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGA
GGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGC
GCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC
GTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCA
GCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAAC
TCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAAT
TCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTC
TAGAGATAGAGCGTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGG
TTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC
AACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGTGACTGC
CGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCT
TATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAA
GACCGCGAGGTCAAGCCAATCCCATAAAACCATTCTCAGTTCGGATTGTA
GGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAG
CATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACAC
CACGAGAGTTTGTAACACCCGAAGTCGGTGGAGTAACCGTAAGGAGCTAG
CCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGT

6. PdS3E2 (*Priestia aryabhattai* strain GAD181_6)

>Forward Seq data
TTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGC
CTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCC
CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCT
TACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCCTTC
GGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA
GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCA

GCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAG
GTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACG
TGCTACAATGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCAAGCCAAT
CCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGA
AGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTC
CCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCG
AAGTCGGTGGAGTAACCGTAAGGAGCTAGCCGCCTAAGGTGGGACAGAT
GATTGGGGTGAAGTCGTAACAAGGTAACC GAA

>Reverse Seq Data
TAATGCGTTAGCTGCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCA
CTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCC
ACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAAAAAGCCGCCTTCGCCA
CTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCG
CTTTTCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCACGGTTG
AGCCGTGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCGCGCTTTAC
GCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTG
GCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACGAGCA
GTTACTCTCGTACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAG
CCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAA
GATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAG
TGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCGTTGCCTTGGTGAGCC
GTTACCTCACCAACTAGCTAATGCACCGCGGGCCCATCTGTAAGTGATAG
CCGAAACCATCTTTCAATCATCTCCCATGAAGGAGAAGATCCTATCCGGT
ATTAGCTTCGGTTTCCCGAAGTTATCCCAGTCTTACAGGCAGGTTGCCCAC
GTGTTACTCACCCGTCCGCCGCTAACGTCATAGAAGCAAGCTTCTAATCAG
TTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTT CATCCTGAGCA

>Reverse complement
TGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAA
CTGATTAGAAGCTTGCTTCTATGACGTTAGCGGCGGACGGGTGAGTAACA
CGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTA
ATACCGGATAGGATCTTCTCCTTCATGGGAGATGATTGAAAGATGGTTTCG
GCTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAA
CGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCA

CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGG
AATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGAT
GAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACGAG
AGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACT
ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATT
ATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCC
CACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGA
AGAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGG
AGGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGG
CGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC
GTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCA
G CTAACGCATTA

>Consensus data
TGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAA
CTGATTAGAAGCTTGCTTCTATGACGTTAGCGGCGGACGGGTGAGTAACA
CGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTA
ATACCGGATAGGATCTTCTCCTTCATGGGAGATGATTGAAAGATGGTTTCG
GCTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAA
CGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCA
CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGG
AATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGAT
GAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACGAG
AGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACT
ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATT
ATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCC
CACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGA
AGAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGG
AGGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGG
CGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC
GTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCA
GCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAAC
TCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAAT
TCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTC
TAGAGATAGAGCGTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGG

TTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC
AACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGTGACTGC
CGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCT
TATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAA
GACCGCGAGGTCAAGCCAATCCCATAAAACCATTCTCAGTTCGGATTGTA
GGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAG
CATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACAC
CACGAGAGTTTGTAACACCCGAAGTCGGTGGAGTAACCGTAAGGAGCTAG
CCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAACCG
AA

7. RgL3E4 (*Klebsiella pneumoniae* strain GAD181_7)

>Forward Seq data
GTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTG
GGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGC
ACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTAC
CTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGA
ACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTT
GGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCC
GGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGG
GATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTA
CAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCA
TAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGT
CGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCG
GGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAG
TAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTG
GGGTGAAGTCGTAACA

>Reverse Seq Data
ACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAATCGAC
ATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACG
CTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCACCGG
TATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCCC
CCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCAGTTCCCAGGTTGAGC
CCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGCGCTTTACGCCC

AGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCAC
GGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATCGCCAAGGTTATTA
ACCTTAACGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCGAAGGCCTTC
TTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTC
CCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGG
CTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTAC
CCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCATGAGGCCCG
AAGGTCCCCCACTTTGGTCTTGCGACATTATGCGGTATTAGCTACCGTTTC
CAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTC
CGCCGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCAT
GTGTTAGGCCTGCCGCCAGCGTTCAATCTGA

>Reverse complement
TCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGC
ACAGAGAGCTTGCTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCT
GGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATA
CCGCATAATGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCAT
CAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTA
GGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTG
AGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA
ATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCG
GGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGTTAAGGTTAATAACCTT
GGCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCA
GCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAA
AGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAAC
CTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTA
GAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGT
GGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGT
CGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGT

>Consensus data
TCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGC
ACAGAGAGCTTGCTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCT
GGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATA

CCGCATAATGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCAT
CAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTA
GGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTG
AGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA
ATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCG
GGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGTTAAGGTTAATAACCTT
GGCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCA
GCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAA
AGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAAC
CTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTA
GAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGT
GGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGT
CGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTA
AATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTG
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GAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATT
GGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCG
TGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTT
GTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACT
GGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGC
TACACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGC
AAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAACTCG
ACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTG
AATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGG
TTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTG
TGATTCATGACTGGGGTGAAGTCGTAACA

8. JC3E2 (*Priestia megaterium* strain GAD181_8)

>Forward Seq data

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CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACC
TTACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCCTT

CGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTG
AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCC
AGCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAA
GGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACAC
GTGCTACAATGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCAAGCCA
ATCCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACAT
GAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGT
TCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACC
CGAAGTCGGTGGAGTAACCGTAAGGAGCTAGCCGCCTAAGGTGGGACAG
ATGATTGGGGTGAAGTCGTAACAA

>Reverse Seq Data

TTAATGCGTTAGCTGCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGC
ACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCC
CACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAAAAAGCCGCCTTCGCC
ACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCC
GCTTTTCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCACGGTT
GAGCCGTGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCGCGCTTTA
CGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCT
GGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACAAGC
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AGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCA
GTGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCGTTGCCTTGGTGAGC
CGTTACCTCACCAACTAGCTAATGCACCGCGGGCCCATCTGTAAGTGATA
GCCGAAACCATCTTTCAATCATCTCCCATGAAGGAGAAGATCCTATCCGG
TATTAGCTTCGGTTTCCCGAAGTTATCCCAGTCTTACAGGCAGGTTGCCCA
CGTGTTACTCACCCGTCCGCCGCTAACGTCATAGAAGCAAGCTTCTAATCA
GTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCTGA

>Reverse complement

TCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACTG
ATTAGAAGCTTGCTTCTATGACGTTAGCGGCGGACGGGTGAGTAACACGT
GGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAATA

CCGGATAGGATCTTCTCCTTCATGGGAGATGATTGAAAGATGGTTTCGGCT
ATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGG
CTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACAC
TGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAAT
CTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAA
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GGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCAC
GGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAG
GAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCG
CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT
AAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCT
AACGCATTAA

>Consensus data

TCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACTG
ATTAGAAGCTTGCTTCTATGACGTTAGCGGCGGACGGGTGAGTAACACGT
GGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAATA
CCGGATAGGATCTTCTCCTTCATGGGAGATGATTGAAAGATGGTTTCGGCT
ATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGG
CTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACAC
TGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAAT
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AACTGCTTGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACG
TGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATT
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GGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAG
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AACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCA
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TGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGA
CCGCGAGGTCAAGCCAATCCCATAAAACCATTCTCAGTTCGGATTGTAGG
CTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCA
TGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCA
CGAGAGTTTGTAACACCCGAAGTCGGTGGAGTAACCGTAAGGAGCTAGCC
GCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAA

9. PL3E2 (*Priestia aryabhattai* strain GAD181_9)

>Forward Seq data

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GCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAA
CCTTACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCC
TTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCG
TGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGC
CAGCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGA
AGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACA
CGTGCTACAATGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCAAGCC
AATCCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACA
TGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACG
TTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACC
CGAAGTCGGTGGAGTAACCGTAAGGAGCTAGCCGCCTAAGGTGGGACAG
ATGATTGGGGTGAAGTCGTAAC

>Reverse Seq Data

TAATGCGTTAGCTGCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCA
CTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCC

ACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAAAAAGCCGCCTTCGCCA
CTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCG
CTTTTCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCACGGTTG
AGCCGTGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCGCTTTAC
GCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTG
GCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACGAGCA
GTTACTCTCGTACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAG
CCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAA
GATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAG
TGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCGTTGCCTTGGTGAGCC
GTTACCTCACCAACTAGCTAATGCACCGCGGGCCCATCTGTAAGTGATAG
CCGAAACCATCTTTCAATCATCTCCCATGAAGGAGAAGATCCTATCCGGT
ATTAGCTTCGGTTTCCCGAAGTTATCCCAGTCTTACAGGCAGGTTGCCCAC
GTGTTACTCACCCGTCCGCCGCTAACGTCATAGAAGCAAGCTTCTAATCAG
TTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCTGAGCCA

>Reverse complement

TGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGA
ACTGATTAGAAGCTTGCTTCTATGACGTTAGCGGCGGACGGGTGAGTAAC
ACGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCT
AATACCGGATAGGATCTTCTCCTTCATGGGAGATGATTGAAAGATGGTTTC
GGCTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTA
ACGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCC
ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGG
GAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGA
TGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACGA
GAGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAAC
TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAAT
TATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGC
CCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAG
AAGAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTG
GAGGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAG
GCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACG

CCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGC
AGCTAACGCATTA

>Consensus data

TGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGA
ACTGATTAGAAGCTTGCTTCTATGACGTTAGCGGCGGACGGGTGAGTAAC
ACGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCT
AATACCGGATAGGATCTTCTCCTTCATGGGAGATGATTGAAAGATGGTTTC
GGCTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTA
ACGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCC
ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGG
GAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGA
TGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACGA
GAGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAAC
TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAAT
TATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGC
CCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAG
AAGAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTG
GAGGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAG
GCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACG
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AGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAA
CTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA
TTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACT
CTAGAGATAGAGCGTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATG
GTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGTGACT
GCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCC
CCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTG
CAAGACCGCGAGGTCAAGCCAATCCCATAAAACCATTCTCAGTTCGGATT
GTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGAT
CAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA
CACCACGAGAGTTTGTAACACCCGAAGTCGGTGGAGTAACCGTAAGGAGC
TAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAAC

10. CC3E3 (*Cellulosimicrobium funkei* strain GAD181_10)

>Forward Seq data

GGGCTCATTTCCACGAGTTCCGTGCCGCAGCAAACGCATTAAGTGCCCCG
CCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGC
CCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAAC
CTTACCAAGGCTTGACATGCACGGGAAGCCACCAGAGATGGTGGTCTCTT
TGGACACTCGTGCACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA
GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTCCCATGTTGCCA
GCGGGTTATGCCGGGGACTCATGGGAGACTGCCGGGGTCAACTCGGAGGA
AGGTGGGGATGACGTCAAATCATCATGCCCCTTATGTCTTGGGCTTCACGC
ATGCTACAATGGCCGGTACAAAGGGCTGCGATGCCGTAAGGTGGAGCGA
ATCCCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCA
TGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATAC
GTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCACGAAAGTCGGTAACA
CCCGAAGCCCATGGCCCAACCGTTCGCGGGGGGAGTGGTCGAAGGTGGG
ACTGGCGATTGGGACTAAGTCGTAACAAGGTAA

>Reverse Seq Data

GGGACTTAATGCGTTTGCTGCGGCACGGAACTCGTGGAATGAGCCCCACA
CCTAGTGCCCAACGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTC
GCTCCCCATGCTTTCGCTCCTCAGCGTCAGTTGCGGCCCAGAGACCTGCCT
TCGCCATCGGTGTTCCTCCTGATATCTGCGCATTCCACCGCTACACCAGGA
ATTCCAGTCTCCCCTACCGCACTCTAGTCTGCCCGTACCCGATGCAAGCTC
GAGGTTGAGCCTCGAGTTTTCACACCAGACGCGACAAACCGCCTACGAGC
TCTTTACGCCCAATAATTCCGGACAACGCTTGCGCCCTACGTATTACCGCG
GCTGCTGGCACGTAGTTAGCCGGCGCTTCTTCTGCAGGTACCGTCACTTGC
GCTTCTTCCCTGCTGAAAGAGGTTTACAACCCGAAGGCCTTCATCCCTCAC
GCGGCGTCGCTGCATCAGGCTTTCGCCCATTGTGCAATATTCCCCACTGCT
GCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGGTCGCC
CTCTCAGGCCGGCTACCCGTCGTCGCCTTGGTAGGCCATCACCCCACCAAC
AAGCTGATAGGCCGCGAGCCCATCCCTGACCGAAAAACTTTCCAACCACC
CCCATGCGAGAGCGGCTCATATCCGGTATTAGCCCCGGTTTCTCGGAGTTA
TCTCGAAGTCAAGGGCAGGTTACTCACGTGTTACTCACCCGTTCGCCACTA

ATCCACCCAGCAAGCTGGGCATCATCGTTCGACTTGCATGTGTTAAGCAC
GCCGCCAGCGTTCGTCCTGAGCC

>Reverse complement

GGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGAT
GATGCCCAGCTTGCTGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTG
AGTAACCTGCCCTTGACTTCGAGATAACTCCGAGAAACCGGGGCTAATAC
CGGATATGAGCCGCTCTCGCATGGGGGTGGTTGGAAAGTTTTTCGGTCAG
GGATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTGATGGCCTACCAAG
GCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTG
AGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA
ATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGAAGGCCTTC
GGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGCAAGTGACGGTACCTGC
AGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
GCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTT
GTCGCGTCTGGTGTGAAAACTCGAGGCTCAACCTCGAGCTTGCATCGGGT
ACGGGCAGACTAGAGTGCGGTAGGGGAGACTGGAATTCCTGGTGTAGCG
GTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCTC
TGGGCCGCAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGAT
TAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGC
TCATTCCACGAGTTCCGTGCCGCAGCAAACGCATTAAGTCCC

>Consensus data

GGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGAT
GATGCCCAGCTTGCTGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTG
AGTAACCTGCCCTTGACTTCGGGATAACTCCGGGAAACCGGGGCTAATAC
CGGATATGAGCCGCTCTCGCATGGGGGTGGTTGGAAAGTTTTTCGGTCAG
GGATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTGATGGCCTACCAAG
GCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTG
AGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA
ATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGAAGGCCTTC
GGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGCAAGTGACGGTACCTGC
AGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
GCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTT

GTCGCGTCTGGTGTGAAAACTCGAGGCTCAACCTCGAGCTTGCATCGGGT
ACGGGCAGACTAGAGTGCGGTAGGGGAGACTGGAATTCCTGGTGTAGCG
GTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCTC
TGGGCCGCAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGAT
TAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGC
TCATTCCACGAGTTCCGTGCCGCAGCAAACGCATTAAGTGCCCCGCCTGG
GGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCA
CAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACC
AAGGCTTGACATGCACGGGAAGCCACCAGAGATGGTGGTCTCTTTGGACA
CTCGTGCACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCTCGTCCCATGTTGCCAGCGGGTT
ATGCCGGGGACTCATGGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGG
GGATGACGTCAAATCATCATGCCCCTTATGTCTTGGGCTTCACGCATGCTA
CAATGGCCGGTACAAAGGGCTGCGATGCCGTAAGGTGGAGCGAATCCCA
AAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGT
CGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCC
GGGCCTTGTACACACCGCCCGTCAAGTCACGAAAGTCGGTAACACCCGAA
GCCCATGGCCCAACCGTTCGCGGGGGGAGTGGTCGAAGGTGGGACTGGCG
ATT GGGACTAAGTCGTAACAAGGTAA

11. VaR3E1 (*Bacillus licheniformis* strain GAD181_11)

>Forward Seq data

TGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCAAACGCATTAAGCACTCC
GCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGG
CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC
CTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTCCCCTT
CGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA
GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCA
GCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAG
GTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACG
TGCTACAATGGGCAGAACAAAGGGCAGCGAAGCCGCGAGGCTAAGCCAA
TCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTG
AAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTT
CCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCC

GAAGTCGGTGAGGTAACCTTTTGGAGCCAGCCGCCGAAGGTGGGACAGAT
GATTGGGGTGAAGTCGTAACAAGGTAACC

>Reverse Seq Data
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ACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCC
CACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAGAGAGTCGCCTTCGCC
ACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCC
ACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCCCGGTT
GAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCGCGCTTTA
CGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCT
GGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACCGCCC
TATTCGAACGGTACTTGTTCTTCCCTAACAACAGAGTTTTACGATCCGAAA
ACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGA
AGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCA
GTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGC
CGTTACCTCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGGTA
GCTAA

>Reverse complement
TTAGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGG
TAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGG
CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTA
GGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGT
GATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTAC
CGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCT
AACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGG
AATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAA
AGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTG
CAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGAT
GTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCT
GAGGCGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
ACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTG
CTGCAGCAAACGCATTAA

>Consensus data

TTAGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGG
TAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGG
CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTA
GGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGT
GATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTAC
CGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCT
AACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGG
AATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAA
AGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTG
CAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGAT
GTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCT
GAGGCGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
ACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTG
CTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACT
GAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGG
TTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAC
AACCCTAGAGATAGGGCTTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCA
TGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGAC
TGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCC
CCTTATGACCTGGGCTACACACGTGCTACAATGGCAGAACAAAGGGCAG
CGAAGCCGCGAGGCTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATC
GCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGAT
CAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA
CACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTGGAGC
CAGCCGCCGAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTA
ACC

12. KL3E1 (*Kosakonia cowanii* strain GAD181_12)

>Forward Seq data

GCCCTTGGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGG
GGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCA
CAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACC

TGGTCTTGACATCCACAGAACTTGGCAGAGATGCATTGGTGCCTTCGGGA
ACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTT
GGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTA
GGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGG
GATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTA
CAATGGCGCATACAAAGAGAAGCAATCTCGCGAGAGCTAGCGGACCTCAT
AAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTC
GGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGG
GCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGT
AGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGG
GGTGAAGTCGTAAC

>Reverse Seq Data

TTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTC
GACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCC
ACGCTTTCGCACCTGAGCGTCAGTCTTCGTCCAGGGGGCCGCCTTCGCCAC
CGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTAC
CCCCCTCTACGAGACTCAAGCCTGCCAGTTTCGGATGCAGTTCCCAGGTTG
AGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGCGCTTTAC
GCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTG
GCACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATCGACGCGGT
TATTAACCGCATCGCCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGG
CCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAG
TGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGC
CGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCAAGA
GGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTAC
CGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCA
CCCGTCCGCCACTCGTCAGCGAAGCAGCAAGCTGCTTCCTGTTACCGTTCG
ACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTGAGC

>Reverse complement

GCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTA
ACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT

GTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTA
ATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGC
CATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCAC
CTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAA
CTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCA
CAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTT
CGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATGCGGTTAATAACC
GCGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAG
CAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGT
AAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCA
ACCTGGGAACTGCATCCGAAACTGGCAGGCTTGAGTCTCGTAGAGGGGGG
TAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCG
GTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGC
GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGAT
GTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTT
AA

>Consensus data

GCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTA
ACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
GTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTA
ATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGC
CATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCAC
CTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAA
CTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCA
CAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTT
CGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATGCGGTTAATAACC
GCGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAG
CAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGT
AAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCA
ACCTGGGAACTGCATCCGAAACTGGCAGGCTTGAGTCTCGTAGAGGGGGG
TAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCG
GTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGC
GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGAT

GTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTT
AAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATT
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CGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTGGCAGAGATGCAT
TGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTC
GTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTT
TGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAAC
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CTACACACGTGCTACAATGGCGCATACAAAGAGAAGCAATCTCGCGAGAG
CTAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTC
GACTCCATGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGT
GAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGG
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13. KS3E1 (*Pantoea dispersa* strain GAD181_13)

>Forward Seq data

CCCTTGGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGG
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GGCCTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAA
CTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTCG
GCCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGG
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AATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCAT
AAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTC
GGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGG
GCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGT
AGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGG
GGTGAAGTCGTAACA

>Reverse Seq Data

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GACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCC

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CGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTAC

CCCCCTCTACGAGACTCAAGCCTGCCAGTTTCAAATGCAGTTCCCAGGTTA

AGCCCGGGGATTTCACATCTGACTTAACAGACCGCCTGCGTGCGCTTTACG

CCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGG

CACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATCGGCGAGGTT

ATTAACCTCACCGCCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGC

CTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAAT

ATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGT

GTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCC

ATTACCCCACCTACTAGCTAATCCCATCTGGGCACATCCGATGGTGTGAGG

CCCGAAGGTCCCCCACTTTGGTCTCGCGACGTTATGCGGTATTAGCTACCG

TTTCCAGTAGTTATCCCCCTCCATCGGGCAGTTTCCCAGACATTACTCACC

CGTCCGCCACTCGCCACCCAAAGAGCAAGCTCTTCTGTGCTGCCGTTCGAC

TTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTGA

>Reverse complement

TTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTC

GACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCC

ACGCTTTCGCACCTGAGCGTCAGTCTTCGTCCAGGGGGCCGCCTTCGCCAC

CGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTAC

CCCCCTCTACGAGACTCAAGCCTGCCAGTTTCAAATGCAGTTCCCAGGTTA

AGCCCGGGGATTTCACATCTGACTTAACAGACCGCCTGCGTGCGCTTTACG

CCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGG

CACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATCGGCGAGGTT

ATTAACCTCACCGCCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGC

CTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAAT

ATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGT

GTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCC

ATTACCCCACCTACTAGCTAATCCCATCTGGGCACATCCGATGGTGTGAGG

CCCGAAGGTCCCCCACTTTGGTCTCGCGACGTTATGCGGTATTAGCTACCG

TTTCCAGTAGTTATCCCCCTCCATCGGGCAGTTTCCCAGACATTACTCACC

CGTCCGCCACTCGCCACCCAAAGAGCAAGCTCTTCTGTGCTGCCGTTCGAC
TTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTGA

>Consensus data

TCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGCAGC
ACAGAAGAGCTTGCTCTTTGGGTGGCGAGTGGCGGACGGGTGAGTAATGT
CTGGGAAACTGCCCGATGGAGGGGGATAACTACTGGAAACGGTAGCTAAT
ACCGCATAACGTCGCGAGACCAAAGTGGGGGACCTTCGGGCCTCACACCA
TCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAATGGCTCACCT
AGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACT
GAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC
AATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTC
GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGGTGAGGTTAATAACCT
CGCCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGC
AGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTA
AAGCGCACGCAGGCGGTCTGTTAAGTCAGATGTGAAATCCCCGGGCTTAA
CCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCTCGTAGAGGGGGGT
AGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGG
TGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCG
TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG
TCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTA
AGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTG
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GGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCG
TGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTT
GTTGCCAGCGGTTCGGCCGGGAACTCAAAGGAGACTGCCGGTGATAAACC
GGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGC
TACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAG
CAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTC
GACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGT
GAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGG
GTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTT

GTGATTCATGACTGGGGTGAAGTCGTAACA

14. LS3E1 (*Pseudomonas aeruginosa*)

>Forward Seq data

CCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGA
GTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAA
GCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGG
CCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTC
AGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGG
TTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGG
TGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT
GACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAA
TGGTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAA
ACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGA
ATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCC
TTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCCAGAAGTAGC
TAGTCTAACCGCAAGGGGGACGGTTACCACGGAGTGATTCATGACTGGGG
TGAAGTCGTAACAAGGTTAACCGACGACCCCCTTGCGGTTAGACTAGCTA
CTTCTGGAGCAACCCACTCC

>Reverse Seq Data

TTATCGCGTTAGCTGCGCCACTAAGATCTCAAGGATCCCAACGGCTAGTC
GACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCC
ACGCTTTCGCACCTCAGTGTCAGTATCAGTCCAGGTGGTCGCCTTCGCCAC
TGGTGTTCCTTCCTATATCTACGCATTTCACCGCTACACAGGAAATTCCAC
CACCCTCTACCGTACTCTAGCTCAGTAGTTTTGGATGCAGTTCCCAGGTTG
AGCCCGGGGATTTCACATCCAACTTGCTGAACCACCTACGCGCGCTTTACG
CCCAGTAATTCCGATTAACGCTTGCACCCTTCGTATTACCGCGGCTGCTGG
CACGAAGTTAGCCGGTGCTTATTCTGTTGGTAACGTCAAAACAGCAAGGT
ATTAACTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGAC
CTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAAT
ATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGT
GTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTAGGCCT
TTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGTGAGGT

CCGAAGATCCCCCACTTTCTCCCTCAGGACGTATGCGGTATTAGCGCCCGT
TTCCGGACGTTATCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCC
GTCCGCCGCTGAATCCAGGAGCAAGCTCCCTTCATCCGCTCGACTTGCATG
TGTTAGGCCTGCCGCCAGCGTTCATCTGAGC

>Reverse complement

GCTCAGATGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGATG
AAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATGCCTAGG
AATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGC
ATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGA
TGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCG
ACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGAC
ACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGG
GCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATT
GTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGT
TTTGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCG
CGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCG
CGCGTAGGTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGG
GAACTGCATCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAAT
TTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCG
AAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGG
AGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGAC
TAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAA

>Consensus data

GCTCAGATGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGATG
AAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATGCCTAGG
AATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGC
ATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGA
TGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCG
ACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGAC
ACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGG
GCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATT
GTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGT

TTTGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCG
CGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCG
CGCGTAGGTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGG
GAACTGCATCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAAT
TTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCG
AAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGG
AGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGAC
TAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTC
GACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGG
GGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAG
AACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGC
CTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGT
GAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACC
AGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGG
AAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACAC
ACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCT
AATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGC
GTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATAC
GTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTC
CAGAAGTAGCTAGTCTAACCGCAAGGGGGACGGTTACCACGGAGTGATTC
ATGACTGGGGTGAAGTCGTAACAAGGTTAACCGACGACCCCCTTGCGGTT
AGACTAGCTACTTCTGGAGCAACCCACTCC

15. LS3E3 (*Klebsiella pneumoniae* strain DSM 30104)

>Forward Seq data

CCCTTGGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGG
GAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCAC
AAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCT
GGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAA
CTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTCG
GCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGG
ATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTAC
AATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCAT

AAAGTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTC
GGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGG
GCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGT
AGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGG
GGTGAAGTCGTAACAAGGT

>Reverse Seq Data

TTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAATC
GACATCGTTTACAGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCC
ACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCAC
CGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTAC
CCCCCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCAGTTCCCAGGTTG
AGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGCGCTTTAC
GCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTG
GCACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATCGACGAGGT
TATTAACCTCACCGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCGAAGG
CCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAG
TGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGC
CGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCATGAG
GCCCGAAGGTCCCCCACTTTGGTCTTGCGACGTTATGCGGTATTAGCTACC
GTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCAC
CCGTCCGCCGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACT
TGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTGAGCCACGA

>Reverse complement

TCGTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAG
CGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGCGGCGGACGGGTGAGT
AATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAG
CTAATACCGCATAACGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCA
TGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCT
CACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTG
GAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATT
GCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGC

CTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGGTGAGGTTAATA
ACCTCGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGC
CAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGG
CGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGC
TCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGG
GGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATA
CCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAA
AGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAAC
GATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACG
CGTTAA

>Consensus data

TCGTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAG
CGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGCGGCGGACGGGTGAGT
AATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAG
CTAATACCGCATAACGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCA
TGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCT
CACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTG
GAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATT
GCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGC
CTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGGTGAGGTTAATA
ACCTCGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGC
CAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGG
CGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGC
TCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGG
GGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATA
CCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAA
AGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAAC
GATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACG
CGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATG
AATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGC
AACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAGAT
GGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCA
GCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAT

CCTTTGTTGCCAGCGGTTCGGCCGGGAACTCAAAGGAGACTGCCAGTGAT
AAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACC
AGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCG
AGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCA
ACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTA
CGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGA
GTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCA
CTTTGTGATTCATGACTGGGGTGAAGTCGTAACAAGGT

16. LL3E1 (*Methylorubrum populi* GAD18_16)

>Forward Seq data

GGGTTGGGCCTGCTTGCAGGTCAGTGGCGCCGCTAACGCATTAAGCATTC
CGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGG
GCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCTAAGCAACGCGCAGAA
CCTTACCATCCCTTGACATGGCATGTTACCTCGAGAGATCGAGGATCCTCT
TCGGAGGCGTGCACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG
AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCACGTCCTTAGTTGCC
ATCATTCAGTTGGGCACTCTAGGGAGACTGCCGGTGATAAGCCGCGAGGA
AGGTGTGGATGACGTCAAGTCCTCATGGCCCTTACGGGATGGGCTACACA
CGTGCTACAATGGCGGTGACAGTGGGACGCGAAACCGCGAGGTTGAGCA
AATCCCCAAAAGCCGTCTCAGTTCGGATTGCACTCTGCAACTCGGGTGCAT
GAAGGCGGAATCGCTAGTAATCGTGGATCAGCACGCCACGGTGAATACGT
TCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTCTTACCC
GACGGCGCTGCGCCAACCGCAAGGGGGCAGGCGACCACGGTAGGGTCAG
CGACTGGGGTGAAGTCGT

>Reverse Seq Data

TGGCGTTAGCGGCGCCACTGACCTGCAAGCAGGCCAACGGCTGGCATTCA
TCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGC
TTTCGCGCCTCAGCGTCAGAACCGGACCAGACAGCCGCCTTCGCCACTGG
TGTTCTTGCGAATATCTACGAATTTCACCTCTACACTCGCAGTTCCGCTGT
CCTCTTCCGGTCTCAAGCCAACCAGTATCGAAGGCAATTCTGTGGTTGAGC
CACAGGCTTTCACCCCCGACTTAATCGGCCGCCTACGCGCCCTTTACGCCC
AGTGATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCAC

GAAGTTAGCCGGGGCTTATTCTTCCGGTACCGTCATTATCGTCCCGGACAA
AAGAGCTTTACAACCCTAAGGCCTTCATCACTCACGCGGCATGGCTGGAT
CAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGT
CTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTA
CTGATCGTCGCCTTGGTAGGCCGTTACCCCACCAACAAGCTAATCAGACG
CGGGCCGATCCTTCGGCAGTAAACCTTTCCCCATAAGGGCGTATCCGGTAT
TAGCTCAAGTTTCCCTGAGTTATTCCGAACCGAAGGGCACGTTCCCACGTG
TTACTCACCCGTCTGCCACTGACTTCCGAAGAAGCCCGTTCGACTTGCATG
TGTTAAGCCTGCCGCCAGCGTTCGCTCTGAGCCAGGATCAAACTCTA

>Reverse complement

TAGAGTTTGATCCTGGCTCAGAGCGAACGCTGGCGGCAGGCTTAACACAT
GCAAGTCGAACGGGCTTCTTCGGAAGTCAGTGGCAGACGGGTGAGTAACA
CGTGGGAACGTGCCCTTCGGTTCGGAATAACTCAGGGAAACTTGAGCTAA
TACCGGATACGCCCTTATGGGGAAAGGTTTACTGCCGAAGGATCGGCCCG
CGTCTGATTAGCTTGTTGGTGGGGTAACGGCCTACCAAGGCGACGATCAG
TAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCA
GACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGC
CTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCT
CTTTTGTCCGGGACGATAATGACGGTACCGGAAGAATAAGCCCCGGCTAA
CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGCTCGGAA
TCACTGGGCGTAAAGGGCGCGTAGGCGGCCGATTAAGTCGGGGGTGAAA
GCCTGTGGCTCAACCACAGAATTGCCTTCGATACTGGTTGGCTTGAGACCG
GAAGAGGACAGCGGAACTGCGAGTGTAGAGGTGAAATTCGTAGATATTC
GCAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCCGGTTCTGACGCTGA
GGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCAC
GCCGTAAACGATGAATGCCAGCCGTTGGCCTGCTTGCAGGTCAGTGGCGC
CGCTAACGCCA

> Consensus data

TAGAGTTTGATCCTGGCTCAGAGCGAACGCTGGCGGCAGGCTTAACACAT
GCAAGTCGAACGGGCTTCTTCGGAAGTCAGTGGCAGACGGGTGAGTAACA
CGTGGGAACGTGCCCTTCGGTTCGGAATAACTCAGGGAAACTTGAGCTAA
TACCGGATACGCCCTTATGGGGAAAGGTTTACTGCCGAAGGATCGGCCCG

CGTCTGATTAGCTTGTTGGTGGGGTAACGGCCTACCAAGGCGACGATCAG
TAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCA
GACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGC
CTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCT
CTTTTGTCCGGGACGATAATGACGGTACCGGAAGAATAAGCCCCGGCTAA
CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGCTCGGAA
TCACTGGGCGTAAAGGGCGCGTAGGCGGCCGATTAAGTCGGGGGTGAAA
GCCTGTGGCTCAACCACAGAATTGCCTTCGATACTGGTTGGCTTGAGACCG
GAAGAGGACAGCGGAACTGCGAGTGTAGAGGTGAAATTCGTAGATATTC
GCAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCCGGTTCTGACGCTGA
GGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCAC
GCCGTAAACGATGAATGCCAGCCGTTGGCCTGCTTGCAGGTCAGTGGCGC
CGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAA
CTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA
TTCTAAGCAACGCGCAGAACCTTACCATCCCTTGACATGGCATGTTACCTC
GAGAGATCGAGGATCCTCTTCGGAGGCGTGCACACAGGTGCTGCATGGCT
GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA
ACCCACGTCCTTAGTTGCCATCATTCAGTTGGGCACTCTAGGGAGACTGCC
GGTGATAAGCCGCGAGGAAGGTGTGGATGACGTCAAGTCCTCATGGCCCT
TACGGGATGGGCTACACACGTGCTACAATGGCGGTGACAGTGGGACGCGA
AACCGCGAGGTTGAGCAAATCCCCAAAAGCCGTCTCAGTTCGGATTGCAC
TCTGCAACTCGGGTGCATGAAGGCGGAATCGCTAGTAATCGTGGATCAGC
ACGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACC
ATGGGAGTTGGTCTTACCCGACGGCGCTGCGCCAACCGCAAGGGGGCAGG
CGACCACGGTAGGGTCAGCGACTGGGGTGAAGTCGT

17. RI1T1R (*Pseudomonas hibiscicola*)

>Forward Seq data

GTTGGGTGCAATTTGGCACGCAGTATCGAAGCTAACGCGTTAAGTTCGCC
GCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGG
CCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAAC
CTTACCTGGCCTTGACATGTCGAGAACTTTCCAGAGATGGATTGGTGCCTT
CGGGAACTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGA
GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAG

CACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGA
AGG

>Reverse Seq Data

CGGCGAACTTAACGCGTTAGCTTCGATACTGCGTGCCAAATTGCACCCAA
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CTTCGCCATGGATGTTCCTCCTGATCTCTACGCATTTCACTGCTACACCAG
GAATTCCGCTACCCTCTACCACATTCTAGTCGCCCAGTATCCACTGCAGTT
CCCAGGTTGAGCCCAGGGCTTTCACAACGGACTTAAACGACCACCTACGC
ACGCTTTACGCCCAGTAATTCCGAGTAACGCTTGCACCCTTCGTATTACCG
CGGCTGCTGGCACGAAGTTAGCCGGTGCTTATTCTTTGGGTACCGTCATCC
CAACCAGGTATTAGCCGGCTGGATTTCTTTCCCAACAAAAGGGCTTTACA
ACCCGAAGGCCTTCTTCACCCACGCGGTATGGCTGGATCAGGCTTGCGCC
CATTGTCCAATATTCCCCACTGCTGCCTCCCGTACGAGTCTGGACCGTGTC
TCAGTTCCAGTGTGGCTGATCATCCTCTCGGACCAGCTACGGATCGTCGCC
TTGGTGGGCCTTTACCCCGCCAGCTAGCTAATCCGACATCGGCTCATT

>Reverse complement

AATGAGCCGATGTCGGATTAGCTAGCTGGCGGGGTAAAGGCCCACCAAGG
CGACGATCCGTAGCTGGTCCGAGAGGATGATCAGCCACACTGGAACTGAG
ACACGGTCCAGACTCGTACGGGAGGCAGCAGTGGGGAATATTGGACAAT
GGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGG
TTGTAAAGCCCTTTTGTTGGGAAAGAAATCCAGCCGGCTAATACCTGGTTG
GGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCC
GCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGC
GTGCGTAGGTGGTCGTTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTG
GGAACTGCAGTGGATACTGGGCGACTAGAATGTGGTAGAGGGTAGCGGA
ATTCCTGGTGTAGCAGTGAAATGCGTAGAGATCAGGAGGAACATCCATGG
CGAAGGCAGCTACCTGGACCAACATTGACACTGAGGCACGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGA
ACTGGATGTTGGGTGCAATTTGGCACGCAGTATCGAAGCTAACGCGTTAA
GTTCGCCG

> Consensus data

AATGAGCCGATGTCGGATTAGCTAGCTGGCGGGGTAAAGGCCCACCAAGG
CGACGATCCGTAGCTGGTCCGAGAGGATGATCAGCCACACTGGAACTGAG
ACACGGTCCAGACTCGTACGGGAGGCAGCAGTGGGGAATATTGGACAAT
GGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGG
TTGTAAAGCCCTTTTGTTGGGAAAGAAATCCAGCCGGCTAATACCTGGTTG
GGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCC
GCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGC
GTGCGTAGGTGGTCGTTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTG
GGAACTGCAGTGGATACTGGGCGACTAGAATGTGGTAGAGGGTAGCGGA
ATTCCTGGTGTAGCAGTGAAATGCGTAGAGATCAGGAGGAACATCCATGG
CGAAGGCAGCTACCTGGACCAACATTGACACTGAGGCACGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGA
ACTGGATGTTGGGTGCAATTTGGCACGCAGTATCGAAGCTAACGCGTTAA
GTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGA
CGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCG
AAGAACCTTACCTGGCCTTGACATGTCGAGAACTTTCCAGAGATGGATTG
GTGCCTTCGGGAACTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGT
GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTA
GTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAAC
CGGAGGAAGG

18. RI7T2S (*Arthrobacter pascens*)

>Forward Seq data

GGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTG
CCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGA
AGAACCTTACCAAGGCTTGACATGGACCGGACCGCCGCAGAAATGTGGTT
TCCCCTTTGGGGCCGGTTTACAGGTGGTGCATGGTTGTCGTCAGCTCGTGT
CGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTT
GCCAGCGGTTCGGCCGGGGACTCATAGGAGACTGCCGGGGTCAACTCGGA
GGAAGGTGGGGACGACGTCAAATCATCATGCCCCTTATGTCTTGGGCTTC
ACGCATGCTACAATGGCCGGTACAAAGGGTTGCGATACTGTGAGGTGGAG
CTAATCCCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACC
CCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCAGTGAA

TACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCACGAGAGTTGGTA
ACACCCGAAGCCCGTGGCCTAACCCCATGTGGGAGGGAGCTCT

>Reverse Seq Data

GGGGCACTTAATGCGTTAGCTACGGCGCGGAAAACGTGGAATGTCCCCCA
CACCTAGTGCCCAACGTTTACGGCATGGACTACCAGGGTATCTAATCCTGT
TCGCTCCCCATGCTTTCGCTCCTCAGCGTCAGTTAATGCCCAGAGACCTGC
CTTCGCCATCGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAG
GAATTCCAGTCTCCCCTACATCACTCTAGTCTGCCCGTACCCACCGCAGAT
CCGGAGTTGAGCCCCGGACTTTCACGGCAGACGCGACAAACCGCCTACGA
GCTCTTTACGCCCAATAATTCCGGATAACGCTTGCGCCCTACGTATTACCG
CGGCTGCTGGCACGTAGTTAGCCGGCGCTTCTTCTGCAGGTACCGTCACTT
TCGCTTCTTCCCTACTGAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTC
ACGCGGCGTCGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTG
CTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGGTCA
CCCTCTCAGGCCGGCTACCCGTCGTCGCCTTGGTAGGCCATTACCCCACCG
ACAAGCTGATAGGCCGCGAGTCCGTCC

>Reverse complement

GGACGGACTCGCGGCCTATCAGCTTGTCGGTGGGGTAATGGCCTACCAAG
GCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTG
AGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA
ATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCG
GGTTGTAAACCTCTTTCAGTAGGGAAGAAGCGAAAGTGACGGTACCTGCA
GAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGG
CGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTG
TCGCGTCTGCCGTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTA
CGGGCAGACTAGAGTGATGTAGGGGAGACTGGAATTCCTGGTGTAGCGGT
GAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCTCTG
GGCATTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTA
GATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGGAC
ATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCC

> Consensus data

GGACTCGCGGCCTATCAGCTTGTCGGTGGGGTAATGGCCTACCAAGGCGA
CGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGAC
ACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGG
GCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTT
GTAAACCTCTTTCAGTAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAG
AAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAA
GCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCG
TCTGCCGTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGC
AGACTAGAGTGATGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAAT
GCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCTCTGGGCATT
AACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACC
CTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGGACATTCCA
CGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACG
GCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGC
GGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTG
ACATGGACCGGACCGCCGCAGAAATGTGGTTTCCCCTTTGGGGCCGGTTT
ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAA
GTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCGGTTCGGCCGG
GGACTCATAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGAC
GTCAAATCATCATGCCCCTTATGTCTTGGGCTTCACGCATGCTACAATGGC
CGGTACAAAGGGTTGCGATACTGTGAGGTGGAGCTAATCCCAAAAAGCCG
GTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCG
CTAGTAATCGCAGATCAGCAACGCTGCAGTGAATACGTTCCCGGGCCTTG
TACACACCGCCCGTCAAGTCACGAGAGTTGGTAACACCCGAAGCCGGTGG
CCTAACCCCTTGTGGGAGGGAGCTCT

19. RI4T3R (*Bacillus subtilis*)

>Forward Seq data

GTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTC
CGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGG
GCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAA
CCTTACCAGGTCTTGACATCCTCTGACAATCCTAGAGATAGGACGTCCCCT
TCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTG
AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCC

AGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAA
GGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACAC
GTGCTACAATGGACAGAACAAAGGGCAGCGAAACCGCGAGGTTAAGCCA
ATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGT
GAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGT
TCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTA

>Reverse Seq Data

CTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAG
CACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCC
CCACGCTTTCGCTCCTCAGCGTCAGTTACAGACCAGAGAGTCGCCTTCGCC
ACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCC
ACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCCCGGTT
GAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGAGCCCTTTA
CGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCT
GGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACCGCCC
TATTCGAACGGTACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAA
ACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGA
AGATTCCCTACTGCTGCCT

>Reverse complement

AGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAA
CGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGG
AAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAG
AAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCA
AGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAA
GTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGG
GGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAA
ATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCT
GTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATA
CCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTC
CGCCCCTTAGTGCTGCAGCTAACGCATTAAG

> Consensus data

AGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAA
CGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGG
AAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAG
AAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCA
AGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAA
GTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGG
GGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAA
ATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCT
GTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATA
CCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTC
CGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTAC
GGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTT
GACATCCTCTGACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGT
GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA
AGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGG
GCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGAC
GTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGG
ACAGAACAAAGGGCAGCGAAACCGCGAGGTTAAGCCAATCCCACAAATC
TGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATC
GCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTG
TACACACCGCCCGTCACACCACGAGAGTTTGTA

20. RI5T12R (*Bacillus licheniformis*)

>Forward Seq data

GCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCAAACGCATTAA
GCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGA
CGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCG
AAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCT
TCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGT
GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTA
GTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCG
GAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCT
ACACACGTGCTACAATGGGCAGAACAAAGGGCAGCGAAGCCGCGAGGCT

AAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGA
CTGCGTGAAGCTGGAATCGCTATTAATCGCGGATCAGCATGCCGCGGTGA

>Reverse Seq Data

GCTTAATGCGTTTGCTGCAGCACTAAAGGGCGGAAACCCTCTAACACTTA
GCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTC
CCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAGAGAGTCGCCTTCGC
CACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTC
CACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCCCGGT
TGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCGCGCTTT
ACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTG
CTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACCGC
CCTATTCGAACGGTACTTGTTCTTCCCTAACAACAGAGTTTTACGATCCGA
AAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCG
GAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCC
CAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGA
GCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGGT
AGCTAAAAGCCACCTTTTATAATTGAACCATGCGGTTCAATCAAGCATCC
GGTATTAGCCCCGGTTTCCCGGAGTTATCTCAGTCTTACAGGCAGGTTACG
CACGTGTTACTCAC

CCGTC

>Reverse complement

GACGGGTGAGTAACACGTGCGTAACCTGCCTGTAAGACTGAGATAACTCC
GGGAAACCGGGGCTAATACCGGATGCTTGATTGAACCGCATGGTTCAATT
ATAAAAGGTGGCTTTTAGCTACCACTTACAGATGGACCCGCGGCGCATTA
GCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCT
GAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACG
GGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGC
AACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAG
GGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACC
AGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGG
CAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCAGGCGGTTTCTT
AAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACT

GGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTG
AAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGT
CTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCGAACAGGATTAGA
TACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTT
TCCGCCCTTTAGTGCTGCAGCAAACGCATTAAGC

> Consensus data

GACGGGTGAGTAACACGTGCGTAACCTGCCTGTAAGACTGAGATAACTCC
GGGAAACCGGGGCTAATACCGGATGCTTGATTGAACCGCATGGTTCAATT
ATAAAAGGTGGCTTTTAGCTACCACTTACAGATGGACCCGCGGCGCATTA
GCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCT
GAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACG
GGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGC
AACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAG
GGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACC
AGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGG
CAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTT
AAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACT
GGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTG
AAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGT
CTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCGAACAGGATTAGA
TACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTT
TCCGCCCTTTAGTGCTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGT
ACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC
GGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTC
TTGACATCCTCTGACAACCCTAGAGATAGGGCTTCCCCTTCGGGGGCAGA
GTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT
AAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTG
GGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGA
CGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGG
GCAGAACAAAGGGCAGCGAAGCCGCGAGGCTAAGCCAATCCCACAAATC
TGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATC
GCTATTAATCGCGGATCAGCATGCCGCGGTGA

21. RI6T10S (*Klebsiella pneumonia*)

>Forward Seq data

TTGGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAAT
CGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAA
GAACCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTG
CCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTG
TGAAATGTTGGGTTTAGTCCCGCTACG

>Reverse Seq Data

GTCGATTTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTC
CAAATCGACATCGTTTACAGCGTGGACTACCAGGGTATCTAATCCTGTTTG
CTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTT
CGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAA
TTCTACCCCCCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCAGTTCCC
AGGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGCG
CTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGG
CTGCTGGCACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATCGAC
GAGGTTATTAACCTCACCGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCC
GAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATT
GTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAG
TTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGG
TGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGC
ATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACGTTATGCGGTATTAG
CTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTA
CTCACCCGTCCGCCGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCT
CGACTTGCATGTGTT

>Reverse complement

AACACATGCAAGTCGAGCGGTAGCACAGAGAGCTTGCTCTCGGGTGACGA
GCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGAT
AACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTG
GGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGCTA
GTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGA
GGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG

GCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGC

CGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGG

AAGGCGGTGAGGTTAATAACCTCGTCGATTGACGTTACCCGCAGAAGAAG

CACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCG

TTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCG

GATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGG

CTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCG

TAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGA

CTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG

GTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGT

GGCTTCCGGAGCTAACGCGTTAAATCGAC

> Consensus data

AACACATGCAAGTCGAGCGGTAGCACAGAGAGCTTGCTCTCGGGTGACGA

GCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGAT

AACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTG

GGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGCTA

GTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGA

GGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG

GCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGC

CGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGG

AAGGCGGTGAGGTTAATAACCTCGTCGATTGACGTTACCCGCAGAAGAAG

CACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCG

TTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCG

GATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGG

CTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCG

TAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGA

CTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG

GTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGT

GGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCA

AGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA

TGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCA

CAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTG

CTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTTAGTCCCGCTACG

22. RI8T7L (*Methylobacterium radiotolerance*)

>Forward Seq data

GCTGTTGGGGTGCTTGCACCGGCAGTAGGCGCAGCTAACGCTTTGAGCATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCATCCTTTGACATGGCGTGTTACCCAGAGAGATCTGGGGTCCCCTTCGGGGGCGCGCACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCACGTCCTTAGTTGCCATCATTCAGTTGGGCACTCTAGGGAGACTGCCGGTGATAAGCCGCGAGGAAGGTGTGGATGACGTCAAGTCCTCATGGCCCTTACGGGATGGGCTACACACGTGCTACAATGGCGGTGACAGTGGGATGCGAAGGAGCGATCTGGAGCAAATCCCCAAAAGCCGTCTCGGTTCGGATTGCACTCTGCAACTCGAGTGCATGAAGGCGGAATCGCTACTAATCGTGGATCAGCATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCATCACACCATGGGA

>Reverse Seq Data

TCCGACTCCAGGCGGGAATGCTTCAATGCGTTAGCTGCGCTACTGCGGTGCAAGCACGCCAACAGCTGGCATTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTAATGGTCCAGTTGGCCGCCTTCGCCACCGGTGTTCTTGCGAATATCTACGAATTTCACCTCTACACTCGCAGTTCCACCAACCTCTACCATACTCAAGCGTCCCAGTATCGAAGGCCATTCTGTGGTTGAGCCACAGGCTTTCACCCCCGACTTAAAACGCCGCCTACGCGCCCTTTACGCCCAGTGATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCCGGGGCTTATTCCTCCGGTACCGTCATTATCGTCCCGGATAAAAGAGCTTTACAACCCTAAGGCCTTCATCACTCACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTACTGATCGTCGCCTTGGTAGGCCGTTACCCCACCAACTAGCTAATCAGACGCGGGCCGATCTTCCGGCAGTAAACCTTTCCCCAGAAGGGCGTATCCGGGATTAGCCCTAGTTTCC

>Reverse complement

GGAAACTAGGGCTAATCCCGGATACGCCCTTCTGGGGAAAGGTTTACTGC
CGGAAGATCGGCCCGCGTCTGATTAGCTAGTTGGTGGGGTAACGGCCTAC
CAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGG
ACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCT
TAGGGTTGTAAAGCTCTTTTATCCGGGACGATAATGACGGTACCGGAGGA
ATAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGC
TAGCGTTGCTCGGAATCACTGGGCGTAAAGGGCGCGTAGGCGGCGTTTTA
AGTCGGGGGTGAAAGCCTGTGGCTCAACCACAGAATGGCCTTCGATACTG
GGACGCTTGAGTATGGTAGAGGTTGGTGGAACTGCGAGTGTAGAGGTGAA
ATTCGTAGATATTCGCAAGAACACCGGTGGCGAAGGCGGCCAACTGGACC
ATTACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCCGTAAACGATGAATGCCAGCTGTTGGCGTGCTTG
CACCGCAGTAGCGCAGCTAACGCATTGAAGCATTCCCGCCTGGAGTCGGA

> Consensus data

GGAAACTAGGGCTAATCCCGGATACGCCCTTCTGGGGAAAGGTTTACTGC
CGGAAGATCGGCCCGCGTCTGATTAGCTAGTTGGTGGGGTAACGGCCTAC
CAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGG
ACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCT
TAGGGTTGTAAAGCTCTTTTATCCGGGACGATAATGACGGTACCGGAGGA
ATAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGC
TAGCGTTGCTCGGAATCACTGGGCGTAAAGGGCGCGTAGGCGGCGTTTTA
AGTCGGGGGTGAAAGCCTGTGGCTCAACCACAGAATGGCCTTCGATACTG
GGACGCTTGAGTATGGTAGAGGTTGGTGGAACTGCGAGTGTAGAGGTGAA
ATTCGTAGATATTCGCAAGAACACCGGTGGCGAAGGCGGCCAACTGGACC
ATTACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCCGTAAACGATGAATGCCAGCTGTTGGCGTGCTTG
CACCGGCAGTAGGCGCAGCTAACGCTTTGAGCATTCCGCCTGGGGAGTAC
GGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCATCCTTTG
ACATGGCGTGTTACCCAGAGAGATCTGGGGTCCCCTTCGGGGGCGCGCAC

ACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAA
GTCCCGCAACGAGCGCAACCCACGTCCTTAGTTGCCATCATTCAGTTGGGC
ACTCTAGGGAGACTGCCGGTGATAAGCCGCGAGGAAGGTGTGGATGACGT
CAAGTCCTCATGGCCCTTACGGGATGGGCTACACACGTGCTACAATGGCG
GTGACAGTGGGATGCGAAGGAGCGATCTGGAGCAAATCCCCAAAAGCCG
TCTCGGTTCGGATTGCACTCTGCAACTCGAGTGCATGAAGGCGGAATCGC
TACTAATCGTGGATCAGCATGCCACGGTGAATACGTTCCCGGGCCTTGTAC
ACACCGCCCATCACACCATGGGA

23. RI10T12L(*Methylorubrum populi*)

>Forward Seq data

CAAGCCGGTTGGGCCTGCTTGCAGGTCAGTGGCGCCGCTAACGCATTAAG
CATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGAC
GGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCTAAGCAACGCGC
AGAACCTTACCATCCCTTGACATGGCATGTTACCTCCAGAGATCGGGGAT
CCTCTTCGGAGGCGTGCACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT
CGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCACGTCCTTAGT
TGCCATCATTCAGTTGGGCACTCTAGGGAGACTGCCGGTGATAAGCCGCG
AGGAAGGTGTGGATGACGTCAAGTCCTCATGGCCCTTACGGGATGGGCTA
CACACGTGCTACAATGGCGGTGACAGTGGGACGCGAAACCGCGAGGTTG
AGCAAATCCCCAAAAGCCGTCTCAGTTCGGATTGCACTCTGCAACTCGGG
TGCATGAAGGCGGAATCGCTAGTAATCGTGGATCAGCACGCCACGGTGAA
TACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTCT
TACCCGACGGCGCTGCGCCAACCGCAAGGAGGCAGGCGACCACGGTAGG
GTCAGCGACGAGGGTGAAGT

>Reverse Seq Data

GAATGCTTAATGCGTTAGCGGCGCCACTGACCTGCAAGCAGGCCAACGGC
TGGCATTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGC
TCCCCACGCTTTCGCGCCTCAGCGTCAGAACCGGACCAGACAGCCGCCTT
CGCCACTGGTGTTCTTGCGAATATCTACGAATTTCACCTCTACACTCGCAG
TTCCGCTGTCCTCTTCCGGTCTCAAGCCAACCAGTATCGAAGGCAATTCTG
TGGTTGAGCCACAGGCTTTCACCCCCGACTTAATCGGCCGCCTACGCGCCC
TTTACGCCCAGTGATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGCGGC

TGCTGGCACGAAGTTAGCCGGGGCTTATTCTTCCGGTACCGTCATTATCGT
CCCGGACAAAAGAGCTTTACAACCCTAAGGCCTTCATCACTCACGCGGCA
TGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCC
CGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCA
GACCAGCTACTGATCGTCGCCTTGGTAGGCCGTTACCCCACCAACAAGCT
AATCAGACGCGGGCCGATCCTTCGGCAGTAAACCTTTCCCCATAAGGGCG
TATCCGGTATTAGCTCAAGTTTCCCTGAGTTATTCCGAACCGAAGGGCACG
TTCACACGTGTTACTCACGCGTCTGCCACTG

>Reverse complement

CAGTGGCAGACGCGTGAGTAACACGTGTGAACGTGCCCTTCGGTTCGGAA
TAACTCAGGGAAACTTGAGCTAATACCGGATACGCCCTTATGGGGAAAGG
TTTACTGCCGAAGGATCGGCCCGCGTCTGATTAGCTTGTTGGTGGGGTAAC
GGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCAC
ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATG
AAGGCCTTAGGGTTGTAAAGCTCTTTTGTCCGGGACGATAATGACGGTAC
CGGAAGAATAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGA
AGGGGGCTAGCGTTGCTCGGAATCACTGGGCGTAAAGGGCGCGTAGGCG
GCCGATTAAGTCGGGGGTGAAAGCCTGTGGCTCAACCACAGAATTGCCTT
CGATACTGGTTGGCTTGAGACCGGAAGAGGACAGCGGAACTGCGAGTGTA
GAGGTGAAATTCGTAGATATTCGCAAGAACACCAGTGGCGAAGGCGGCTG
TCTGGTCCGGTTCTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGA
TTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCCAGCCGTTGGC
CTGCTTGCAGGTCAGTGGCGCCGCTAACGCATTAAGCATTC

> Consensus data

CAGTGGCAGACGCGTGAGTAACACGTGTGAACGTGCCCTTCGGTTCGGAA
TAACTCAGGGAAACTTGAGCTAATACCGGATACGCCCTTATGGGGAAAGG
TTTACTGCCGAAGGATCGGCCCGCGTCTGATTAGCTTGTTGGTGGGGTAAC
GGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCAC
ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATG
AAGGCCTTAGGGTTGTAAAGCTCTTTTGTCCGGGACGATAATGACGGTAC

CGGAAGAATAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGA
AGGGGGCTAGCGTTGCTCGGAATCACTGGGCGTAAAGGGCGCGTAGGCG
GCCGATTAAGTCGGGGGTGAAAGCCTGTGGCTCAACCACAGAATTGCCTT
CGATACTGGTTGGCTTGAGACCGGAAGAGGACAGCGGAACTGCGAGTGTA
GAGGTGAAATTCGTAGATATTCGCAAGAACACCAGTGGCGAAGGCGGCTG
TCTGGTCCGGTTCTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGA
TTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCCAGCCGTTGGC
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AGCGGTGGAGCATGTGGTTTAATTCTAAGCAACGCGCAGAACCTTACCAT
CCCTTGACATGGCATGTTACCTCCAGAGATCGGGGATCCTCTTCGGAGGC
GTGCACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCACGTCCTTAGTTGCCATCATTCA
GTTGGGCACTCTAGGGAGACTGCCGGTGATAAGCCGCGAGGAAGGTGTGG
ATGACGTCAAGTCCTCATGGCCCTTACGGGATGGGCTACACACGTGCTAC
AATGGCGGTGACAGTGGGACGCGAAACCGCGAGGTTGAGCAAATCCCCA
AAAGCCGTCTCAGTTCGGATTGCACTCTGCAACTCGGGTGCATGAAGGCG
GAATCGCTAGTAATCGTGGATCAGCACGCCACGGTGAATACGTTCCCGGG
CCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTCTTACCCGACGGC
GCTGCGCCAACCGCAAGGAGGCAGGCGACCACGGTAGGGTCAGCGACGA
GGGTGAAGT

24. RI2T9L (*Ochrobactrum grignonense*)

>Forward Seq data

GCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATTCC
GCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGG
CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAAC
CTTACCAGCCCTTGACATACCGGTCGCGGACACAGAGATGTGTCTTTCAGT
TCGGCTGGACCGGATACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGT
GAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCTTTAGTTGC
CATCATTTAGTTGGGCACTCTAAAGGGACTGCCAGTGATAAGCTGGAGGA
AGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGCTACACA
CGTGCTACAATGGTGGTGACAGTGGGCAGCAAGCACGCGAGTGTGAGCTA
ATCTCCAAAAGCCATCTCAGTTCGGATTGCACTCTGCAACTCGAGTGCATG

AAGTTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTT
CCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTTCTGCCCG
AAGGCACTGTGCTAACCGTAAGGAGGCAGGTGACCACGGTAGGGTCAGC
GACTGGGGTGAAGTCGTAACAAGGTAACCG

>Reverse Seq Data

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ATTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCC
CACGCTTTCGCACCTCAGCGTCAGTAATGGTCCAGTGAGCCGCCTTCGCCA
CTGGTGTTCCTCCGAATATCTACGAATTTCACCTCTACACTCGGAATTCCA
CTCACCTCTACCATACTCAAGACTTCCAGTATCAAAGGCAGTTCCAGAGTT
GAGCTCTGGGATTTCACCCCTGACTTAAAAGTCCGCCTACGTGCGCTTTAC
GCCCAGTAAATCCGAACAACGCTAGCCCCCTTCGTATTACCGCGGCTGCT
GGCACGAAGTTAGCCGGGGCTTCTTCTCCGGTTACCGTCATTATCTTCACC
GGTGAAAGAGCTTTACAACCCTAGGGCCTTCATCACTCACGCGGCATGGC
TGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTA
GGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACC
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AACGCGGGCTCATCCTTTGCCGATAAATCTTTCCCCCGAAGGGCACATAC
GGTATTAGCACAAGTTTCCCTGAGTTATTCCGTAGCAAAAGGTAGATTCCC
ACGCGTTACTCACCCGTCTGCCGCTCGCCTTGCGGGGCGCTCGACTTGCAT
GTGTTAAGCCTGCCGCCAGCGTTCGTTCTGAGCCAGGAGT

>Reverse complement

ACTCCTGGCTCAGAACGAACGCTGGCGGCAGGCTTAACACATGCAAGTCG
AGCGCCCCGCAAGGCGAGCGGCAGACGGGTGAGTAACGCGTGGGAATCT
ACCTTTTGCTACGGAATAACTCAGGGAAACTTGTGCTAATACCGTATGTGC
CCTTCGGGGGAAAGATTTATCGGCAAAGGATGAGCCCGCGTTGGATTAGC
TAGTTGGTGGGGTAAAGGCCCACCAAGGCGACGATCCATAGCTGGTCTGA
GAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGG
AGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCAT
GCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTCACCGGTGA
AGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCA
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GAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACACCAGT
GGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAA
TGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAAC

> Consensus data

ACTCCTGGCTCAGAACGAACGCTGGCGGCAGGCTTAACACATGCAAGTCG
AGCGCCCCGCAAGGCGAGCGGCAGACGGGTGAGTAACGCGTGGGAATCT
ACCTTTTGCTACGGAATAACTCAGGGAAACTTGTGCTAATACCGTATGTGC
CCTTCGGGGGAAAGATTTATCGGCAAAGGATGAGCCCGCGTTGGATTAGC
TAGTTGGTGGGGTAAAGGCCCACCAAGGCGACGATCCATAGCTGGTCTGA
GAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGG
AGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCAT
GCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTCACCGGTGA
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GCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAA
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GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCA
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GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCTTTA
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GAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGCT
ACACACGTGCTACAATGGTGGTGACAGTGGGCAGCAAGCACGCGAGTGTG
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GCATGAAGTTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAAT

ACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTTCT
GCCCGAAGGCACTGTGCTAACCGTAAGGAGGCAGGTGACCACGGTAGGG
TCAGCGACTGGGGTGAAGTCGTAACAAGGTAACCG

25. RI3T11R (*Priestia megaterium*)

>Forward Seq data

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ACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTTCCC
CTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTT
GCCAGCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAG
GAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACA
CACGTGCTACAATGGATGGAACAAAGGGCAGCGAAGCCGCGAGGTGAAG
CCAATCCCATAAAACCATTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTG
CATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATA
CGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAAC
ACCCGAAGTCGGTGGGGTAACCTTTTGGAGCCAGCCGCCTAAGGTGGGAC
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>Reverse Seq Data

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CCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCG
CCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATT
CCGCTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCCCG
GTTGAGCCGGGGGCTTTCACATCAGACTTAAGGAACCGCCTGCGCGCGCT
TTACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCT
GCTGGCACGTAGTTAGCCGTGGCTTTCTGGTCAGGTACCGTCAAGGTACC
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AAGGCCTTCATCGCTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGC
GGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTC
CCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTG

AGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGCCCATCTGTAAGTG
ACAGCCGAAACCGTCTTTCAGCTTTCCCTCATGTGAGGGAAAGGATTATCC
GGTATTAGCTCCGGTTTCCCGAAGTTATCCCAGTCTTACAGGCAGGTTGCC
CACGTGTTACTCACCCGTCCGCCGCTGATCTTCAAAAGCAAGCTAATGAA
GATCCGCTCGACTTGCATGTATTAGGCACG

>Reverse complement

CGTGCCTAATACATGCAAGTCGAGCGGATCTTCATTAGCTTGCTTTTGAAG
ATCAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTG
GGATAACTTCGGGAAACCGGAGCTAATACCGGATAATCCTTTCCCTCACA
TGAGGGAAAGCTGAAAGACGGTTTCGGCTGTCACTTACAGATGGGCCCGC
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AGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCA
GACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTC
TGACGGAGCAACGCCGCGTGAGCGATGAAGGCCTTCGGGTCGTAAAGCTC
TGTTGTCAGGGAAGAACAAGTACCGGAGTAACTGCCGGTACCTTGACGGT
ACCTGACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATAC
GTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGC
GGTTCCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCAT
TGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGCGGAATTCCACGTG
TAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGG
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GGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTT
AGAGGGTTTCCGCCCTTTAGTGCTGCAGCAAACGCATTAAGC

> Consensus data

CGTGCCTAATACATGCAAGTCGAGCGGATCTTCATTAGCTTGCTTTTGAAG
ATCAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTG
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TGAGGGAAAGCTGAAAGACGGTTTCGGCTGTCACTTACAGATGGGCCCGC
GGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGT
AGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCA
GACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTC
TGACGGAGCAACGCCGCGTGAGCGATGAAGGCCTTCGGGTCGTAAAGCTC

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ACCTGACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATAC
GTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGC
GGTTCCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCAT
TGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGCGGAATTCCACGTG
TAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGG
CTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCGAACA
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AGAGGGTTTCCGCCCTTTAGTGCTGCAGCAAACGCATTAAGCACTCCGCCT
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CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTA
CCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTTCCCCTTCGG
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TGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCA
TTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTG
GGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGC
TACAATGGATGGAACAAAGGGCAGCGAAGCCGCGAGGTGAAGCCAATCC
CATAAAACCATTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAA
GCCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCC
CGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGA
AGTCGGTGGGGTAACCTTTTGGAGCCAGCCGCCTAAGGTGGGACAGATGA
TTGGGGTGAAGTCGTAACAAGGTAACC