Studies on Growth Promoting Microorganisms in Rhizosphere of Wild *Musa* Species of Nagaland

by

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DEPARTMENT OF BOTANY

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September 10, 2024

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I, **Ms. Mum Tatung** bearing Ph. D. Registration No. **Ph.D./BOT/00279 dated August 23, 2019** hereby declare that the subject matter of my Ph. D. thesis entitled "**Studies on Growth Promoting Microorganisms in Rhizosphere of Wild *Musa* Species of Nagaland** " is a record of work done by me, and that the contents of this thesis did not form the basis for award of any previous degree to me or to anybody else known to the best of my knowledge. This thesis has not been submitted by me for any other research degree in any other university/institute.

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Abbreviations

PGPM	**Plant growth promoting microorganisms**
PSM	**Phosphate solubilising microorganisms**
Da	**Dalton**
CTAB	**Cetyltrimethylammonium bromide**
NaOCl	**Sodium hypochlorite**
EB	**Ethidium bromide**
LSD	**Least Significance Test**
AuNPs	**Gold nanoparticles**
Tc	**Toxin complex**
PSF	**Phosphate solubilising fungi**
pH	**Potential of hydrogen**
SEM	**Standard error of the mean**
C	**Control**
RPM	**Revolutions Per Minute**
Trp	**Tryptophan**
Kb	**Kilobase**
IAM	**Indole-3-acetamide**
PGPR	**Plant growth promoting rhizobacteria**
NPK	**Nitrogen, Phosphorus and Potassium**
Ca3(PO4)2	**Calcium phosphate**
IPyA	**Indole-3-pyruvate**
TSO	**Tryptophan side-chain oxidase**
TAM	**Tryptamine**
IAN	**Indole-3-acetonitrile**
w/v	**Weight by volume**
BNF	**Biological nitrogen fixation**
HPO_4^{2-}	**Hydrogen phosphate**
HNO_3	**Nitric acid**
$H_2PO_4^-$	**Dihydrogen phosphate**
DDT	**Dichlorodiphenyltrichloroethane**

Fe	**Iron**
Zn	**Zinc**
K	**Potassium**
Mg	**Magnesium**
N	**Nitrogen**
GA	**Gibberellic acid**
P	**Phosphorus**
Cl−	**Chlorine ion**
MDA	**Malondialdehyde**
Mg2+	**Magnesium Ions**
K+	**Potassium ions**
Fe3+,	**Iron ions**
Al3+.	**Aluminum ions**
Mg2+	**Magnesium ions**
Ca2+	**Calcium ions**
Na+	**Sodium ions**
CAT	**Catalases**
PGPF	**Plant growth promoting fungi**
PSB	**Phosphate solubilizing bacteria**
O. D	**Optical density**
18s rRNA	**18S ribosomal RNA**
ABA	**Abscisic acid**
AMF	**Arbuscular mycorrhizal fungi**
MIC	**Minimum inhibitory concentration**
ITS1	**internal transcribed spacer**
ITS4	**internal transcribed spacer**
PSI	**Phosphate solubilizing index**
PSU	**Percent siderophore unit**
DPPH	**1,1-diphenyl-2-picrylhydrazyl**
IAA	**Indole 3-acetic acid**
POD	**peroxidase activity**
AAO	**Ascorbic acid oxidase**

CAS	**Chrome AzuralSulphonate**
CAGR	**Compound annual growth rate**
MIC	**Minimum inhibitory concentration**
PDA	**Potato dextrose agar**
RBA	**Rose Bengal Agar**
µl	**Microliter**
Cm	**Centimeter**
Gm	**Gram**
rRNA	**Ribosomal nucleic acid**
BLAST	**Basic Local Alignment Search Tool**
DNA	**Deoxyribose Nucleic Acid**
ºC	**Degree Celsius**
PVK	**Pikovskaya's**
NB	**Nutrient broth**
NA	**Nutrient agar**
NH4	**Ammonia**
NCBI	**National Centre for Biotechnology Information**
ACC deaminase	**1-aminocyclopropane-1-carboxylic acid deaminase**
DAPG	**2,4-diacetylphloroglucinol**
SOD	**Superoxide dismutase**
POD	**Peroxidase**
MEGA	**Molecular evolutionary genetics analysis**
EPGPR	**Extracellular PGPR**
IPGPR	**Intracellular PGPR**
PCR	**Polymerase chain reaction**
Hrs	**Hours**
g/ml	**Gram per millilitre**
HCL	**Hydrochloric acid**
ml	**Millilitre**
nm	**Nanometre**
M	**Molar**
NBRIP	**National Botanical Research Institute's phosphate**

$MgSO_4 \cdot 7H_2O$	**Magnesium sulfate**
KCl	**Potassium chloride**
$(NH_4)_2SO_4$	**Ammonium sulfate**
$MgCl_2 \cdot 6H_2O$	**Magnesium chloride**
IROMPs	**Iron regulated outer membrane proteins**
NaOCl	**Sodium hypochlorite**
PSI	**Pressure per square inch**
min	**Minutes**
µg	**Microgram**
Sp.	**Species**
BC	**Before Christ**
USD	**US dollar**
FAO	**Food and Agricultural Organization**
H_2O_2	**Hydrogen peroxide**
Cr	**Chromium**
LsLea	**Late embryogenesis abundant protein**
MBCAs	**Microbial biocontrol agents**
HCN	**Hydrogen cyanide**
PHL	**2,4-diacetylphloroglucinol**
PLT	**Pyoluteorin**
PRN	**Pyrrolnitrin**
PCA	**Phenazine-1-carboxylic acid**
PCN	**Phenazine-1-carboxamide**
DMHDA	**Phenazine,N, N-dimethyl hexadecylamine**
BCA	**Biocontrol agents**
EPA	**Environmental Protection Agency**
ISR	**Induced systemic resistance**
BTH	**Benzothiadiazole**
VOCs	**Volatile Organic Compounds**
mVOCs	**Microbial volatile organic compounds**
SAR	**Systemic acquired resistance**
SA	**Salicylic acid**

PR proteins	**Pathogenesis-related proteins**
JA	**Jasmonic acid**
ET	**Ethylene**
NPR1 gene	**Nonexpressor of pathogenesis-related genes 1**
Sec	**Seconds**
MYB72 and MYC2	**Transcription factors**
gL^{-1}	**Gram per litre**
DMA	**Dimethylarsinic acid**
v/v	**Volume/volume**
^oC	**Degree Celsius**
PHPB	**Plant health-promoting bacteria**
Ca	**Calcium**
La	**Lanthanum**
Pb	**Lead**
Cu	**Copper**
Cd	**Cadmium**
Cr	**Chromium**
Hg	**Mercury**
Co	**Cobalt**
Th	**Thorium**
U	**Uranium**
Pu	**Plutonium**
As	**Arsenic**
Ni	**Nickel**
K_2HPO_4	**Dipotassium phosphate**
Kg/ha	**Kilogram per hectare**
LA	**lactic acid**
MR-negative	**Methyl red negative**
CO_2	**Carbon dioxide**
SSM	**Solid surface motility**
TCP	**Tricalcium phosphate**
IROMPs	**Iron regulated outer membrane proteins**

ROS	**Reactive oxygen species**
ppb	**Parts per billion**
MDA	**Malondialdehyde**
$FeCl_3$, $6H_2O$	**Ferric chloride**
mM	**Millimolar**
HCl	**Hydrochloric acid**
HDTMA	**Hexadecyltrimethylammonium**
ddH_2O	**Double distilled water**
$CdCl_2$, H_2O	**Cadmium chloride**
$CuSO_4$	**Copper sulfate**
$NISO_4.H_2O$	**Nickel sulfate**
$NaAsO_2$	**Sodium arsenite**
K_2 $(SbO)_2C_8H_4O_{40}$, $3H_2O$	**Antimony Potassium Tartrate Trihydrate**
ZnSO4.7H_2O	**Zinc sulfate**
K_2CrO_4	**Potassium chromate**
PSB	**Phosphate solubilizing bacteria**
EPS	**Extracellular polymeric substance**
DOC	**Dissolved organic carbon**

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Chapter-1

Introduction

Rhizosphere

The term "Rhizosphere" was initially coined by Hiltner (1904), to describe the area located few centimetres from the root system, where soil microbes and root exudates interact (Das et al., 2013). The term "Rhizoplane" refers to the region containing the root surface (**Figure 1.1**), while, thefurthestregion from the root system is termed "Bulk soil". The rhizosphere stands out for hosting highest microbial population, surpassing the bulk soil by 10-1000 (Hiltner, 1904). It is a nutrient rich-zone compared to non-rooted bulk soil, owingto the accumulation of diverse plant exudates that serve as an abundant source of energy and nutrients for microbes (Gray and Smith, 2005).

Root exudates are comprised of low molecular weight soluble organic substances, which may contain sugars, amino acids, organic acids, enzymes, and other substances such as nucleotide, flavanone, fatty acids, proteins, sterols, lipids, aliphatics, aromatics, and carbohydrates (Hu et al., 2018; Vives-Peris et al., 2018). These substances act as

signalling molecules between the plant and microbes. However, root exudates are highly susceptible to influences from various abiotic and biotic factors (Vives-Peris et al., 2018). Root exudates can exert both negative and positive effects on the organism residing in the rhizosphere. Microorganisms metabolize these deposits, releasing biologically active compounds such as phytohormones (auxins, cytokinins, gibberellins, and ABA), antifungal compounds, enzymes, and compatible solutes. These compounds contribute to improved plant growth and influence the organisms within this habitat in various ways (Weise et al., 2013).

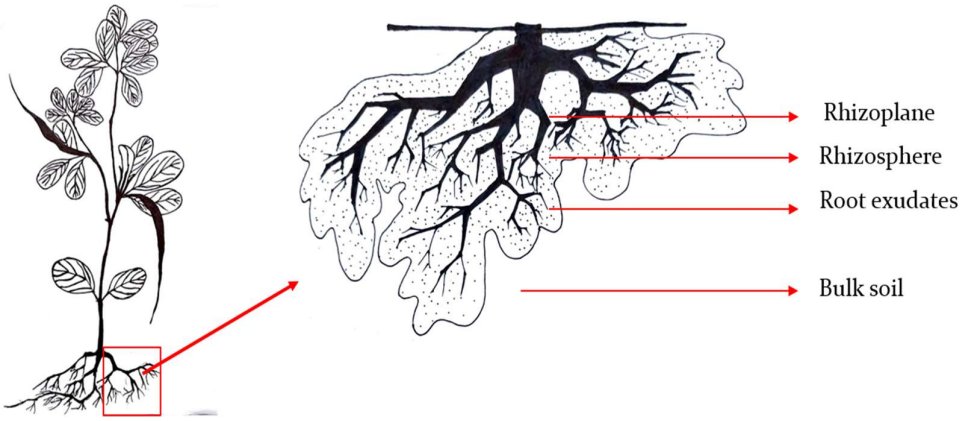

Figure 1.1: Plant root system. Rhizoplane, Rhizosphere, Root exudates and Bulk soil

Plant Growth Promoting Microorganism (PGPM)

Soil hosts some of the planet's most diverse microbiomes, crucial for both recycling nutrients and storing carbon (Bahram et al., 2018). According to Nannipieri et al. (2003), microbial diversity is a general term used to include genetic diversity, within microbial species; diversity of bacterial and fungal species in microbial communities; and ecological diversity (Praeg et al., 2020; Coleman et al., 2018). Microbial activity in the rhizosphere is pivotal for plant health, aiding in nutrient uptake, enhancing soil fertility

and providing protection against pathogen (Berendsen et al., 2012; Kumar et al., 2012b). Microbial populations exist in diverse ecological niches, including extreme environments present in the lithosphere and hydrosphere, where their metabolic abilities are vital for geochemical nutrient cycling (Daniel, 2005; Jha et al., 2011; Kumar et al., 2011). Fungi, algae, protists, bacteria are some of the groups of microbes that resides in the rhizospheric region and among these bacteria is the highest in population following fungi. Fungal and bacterial organisms are considered to be one of the most important groups of microbes in the soil (Li et al., 2021). They dominate the terrestrial soil habitats in terms of biodiversity, biomass and their influence over essential soil process (Bahram et al., 2018). They support sustainable agriculture development and protect the environment (Das et al., 2013). Bacterial community which helps in the plant growth and resides in the rhizosphere are called the plant growth promoting rhizobacteria (Kloepper et al., 1978), whereas fungal community that are found in the rhizosphere, or inside the plant and promote growth are called plant growth promoting fungi (Elsherbiny et al., 2023). Plants release various chemicals into the rhizosphere, facilitating the colonization of microorganisms (Kundan et al., 2015). The composition of rhizobacterial and rhizofungal communities can differ between plants due to variations in root exudates compositions. Factors such as soil *pH*, moisture, temperature, nutrients level, alongside root exudates composition, influence microbial population (Mishra et al., 2015). Among the well-studied root associated fungi are arbuscular mycorrhizal fungi (AMF), which forms symbiotic relationship with approximately 80% of the land plants species, including agricultural crops. AMF provides numerous benefits to the plants including enhanced uptake of mineral nutrients and water in exchange of carbon source from plants (Mitter et al., 2021). The PGPM has emerged as a promising solution, as it enhances plant growth (Msimbira and Smith, 2020). They employ various mechanisms, including

phosphate solubilisation (Sharma et al., 2013; Doillom et al., 2020), siderophore production (Ghosh et al., 2017; Chowdappa et al., 2020), and indole-3-acetic acid (IAA) production (Gusmiaty et al., 2019; Tarroum et al., 2022), alongside other beneficial traits such as stress tolerance and biocontrol activity (Jentschke and Godbold, 2000; Torres-Cruza et al*.,* 2018) (**Figure 1.2**). Hence, are considered to be great biofertilizer candidates. Especially due to the increase in the awareness about the harmful effect of agrochemicals people are moving towards the use of organic biofertilizers for implementing sustainable agriculture. Many countries have already started putting strict regulations in the use of such harmful chemicals and encourage the use of microbes for enhancing the plants growth (Odoh et al., 2020; Sarkar et al., 2022). Although it is not feasible to completely eliminate the use of synthetic agrochemicals overnight, the use of biofertilizers can reduce their usage to a great extent (Tatung and Deb, 2023). According to the estimates, the global biofertilizers market is projected to have a size of USD 2,314.30 million in 2023 and is anticipated to expand to USD 4,096.84 million by 2028, exhibiting a compound annual growth rate (CAGR) of 12.10% during the forecast period of 2023-2028 (www.mordorintelligence.com). Hence, it is required to study more bacterial and fungal strains to develop biofertilizers, pesticide, fungicides, etc. in order to reduce the reliance on harmful agrochemicals.

Few examples of PGPF are *Penicillium* sp., *Pythium* sp., *Trichoderma* sp. (Murali et al., 2012), *T. harzianum* TaK12 and *T. aureoviride* TaN16 (Sarkar et al., 2022), *Daldinia eschscholtzii*, *Sarocladium oryzae*, *Rhizoctonia oryzae, Penicillium allahabadense*, and *Aspergillus foetidus* (Syamsia et al., 2021). *Acrophialophora jodhpurensis* (Daroodi et al., 2022), *A. niger* (Galeano et al., 2021) and mostly reported PGPR genera are *Agrobacterium, Arthrobacter, Azobacterer, Azospirillum, Bacillus,*

Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcus and *Pseudomonas* etc (Bhattacharyya and Jha, 2012).

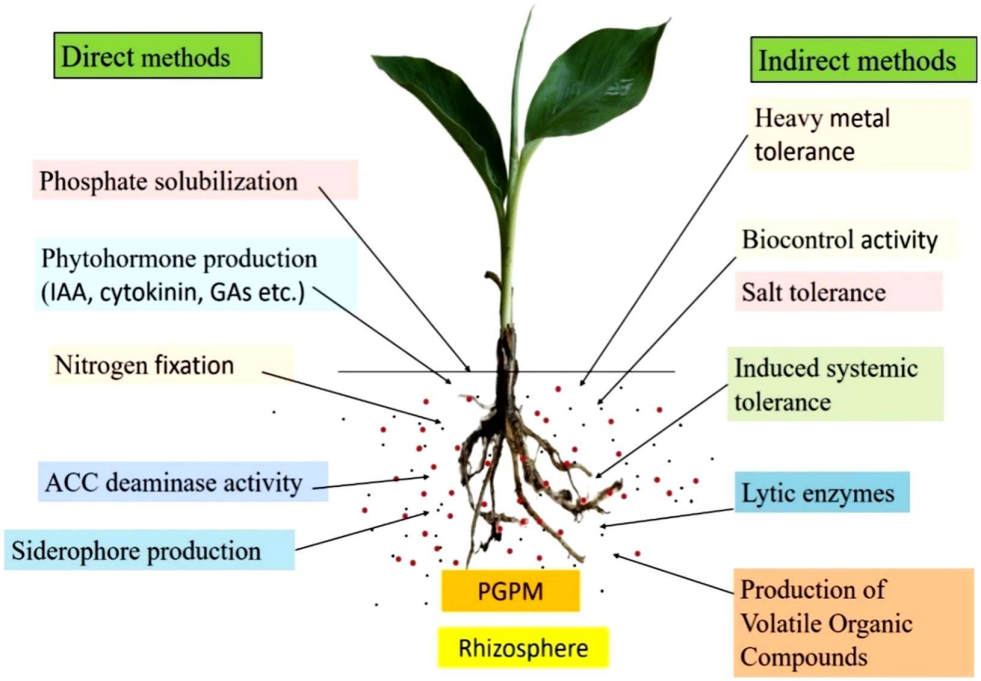

Figure 1.2: Enhancement of plant growth and development by plant growth microorganism (PGPM) through direct and indirect ways.

Plant Growth Promoting Bacteria

Plant-associated bacteria can be classified into beneficial, deleterious, and neutral groups based on their effects on plant growth (Dobbelaere et al., 2003). According to Whipps (2001), there are three basic categories of interactions (neutral, negative, or positive) generally exists between the rhizobacteria and growing plants. A variety of bacteria interact with plant roots while scattered throughout the soil and frequently connected to soil particles. It is remarkable to note that the idea of beneficial bacteria can be traced back to as early as 372- 287 BC. Theophrastus, during that time, proposed the combination of various soils as a way of enhancing the quality of the soils. However, the

idea was not very clear back then. It was much later when Bottomley reported the use of soil microorganisms for promoting plant growth as documented by Ruzzi et al. (2015). However, Kloepper et al. (1978), were the first to utilize the term PGPR to refer to soilbacteria that colonize plant roots after being inoculated onto the seed and promote plant growth. When growing alongside the host plants, PGPR is an essential component of the rhizosphere biota that can promote the host's development. Due to their great flexibility in a variety of conditions, rapid growth rate and biochemical plasticity to metabolize a wide range of natural and xenobiotic molecule, PGPR appeared as effective rhizobacteria in establishing themselves in soil ecosystem. (Kloepper et al., 1989). As stated by Cook (2002), PGPR is a key element of agricultural practices with intrinsic genetic potential. Numerous genera of bacteria have been identified as PGPR, with *Bacillus* sp. and *Pseudomonas* spp. being the most common (Podile and Kishore, 2006). Additionally, Grey and Smith (2005) showed that different PGPR associations had varying levels of bacterial closeness to the root and relationship intimacy. In general, PGPR can be separated into extracellular PGPR, existing in the rhizosphere, on the rhizoplane, or in the spaces between the cells of the root cortex, and intracellular PGPR, which exist inside root cells, generally in specialized nodular structure (Figueido et al., 2011). Some examples of EPGPR are *Agrobacterium* sp., *Arthrobacter* sp., *Azobacterer* sp., *Azospirillum* sp., *Bacillus* sp., *Burkholderia* sp., *Caulobacter* sp., *Chromobacterium* sp., *Erwinia* sp., *Flavobacterium* sp., *Micrococcus* sp. and *Pseudomonas* sp. etc. (Bhattacharyya and Jha, 2012). Similarly, some examples of IPGPR are *Allorhizobium* sp., *Azorizobium* sp., *Bradyrhizobium* sp., *Mesorhizobium* sp. etc. (Bhattacharyya and Jha, 2012). Functionally PGPR involve several direct and indirect

mechanisms like plant-microbes symbiosis, develops colonization space competition, enhance nutrients absorption by plant and decrease plant pathogen activities (Lugtenberg et al., 2002). The use of bacterial characteristics that directly promote plant development is referred to as one of the direct methods. Auxin, ACC deaminase, cytokinin, nitrogen fixation, phosphate solubilization, and iron sequestration by bacterial siderophores are a few of the processes that fall under this category. Indirect mechanism refers to bacterial traits that inhibit the functions of one or more plant pathogenic organisms both fungi and bacteria (**Figure 1.2, Table 1.1**). The population of the rhizobacteria is influenced by the soil pH, moisture, temperature, and soil nutrients (Mishra et al., 2015). Studies on the effects of PGPR inoculation on plants have demonstrated that PGPR aids plant growth (**Table 1.2**). A better solution to the issue of the negative impacts of chemical fertilizers, according to Gupta et al. (2015), is to use PGPR as biofertilizer. Rodriguez and Fraga (1999), have suggested that there are several benefits in utilizing PGPR as a bio-fertilizer over chemical fertilizers as the former does not accumulate in the food chain and the target species seldom ever develop resistance, in contrast to chemical fertilizers. However, Gange et al. (2018), have made the case that the inoculated bacteria occasionally might not get along with those that are already present in the rhizosphere hence a detailed study is required before the application of the PGPR to the targeted area.

Table 1.1: Reports on rhizobacterial strains with their PGPR traits

Name of the PGPR	Host Plant	PGPR Traits	References
Pseudomonas aeruginosa	*Musa* spp.	IAA production, siderophore production, inorganic phosphate solubilisation, fungal antibiosis (DAPG production)	Ayyadurai et al., 2005

Methylobacterium sp. 2A	*Solanum tuberosum* L. cv. Desirée	Salinity stress tolerance, biocontrol activity against *P. infestans, Botrytis cinerea* and *Fusarium graminearum*, IAA production, phosphate solubilisation, Dinitrogen fixation	Grossi et al., 2020
Kosakonia arachidis, Pseudomonas putida, and *Pseudomonas monteilii*	*Musa itinerans*	Phosphate solubilisation, siderophore production, and salinity tolerance	Tatung and Deb, 2023
Bacillus megaterium	*Retama monosperma* (L.) Boiss.	IAA production	Dahmani et al., 2020
B. velezensis, P. peoriae, B. altitudinis	-	Biocontrol agents against *Pythium ultimum, Rhizoctonia solani, Fusarium oxysporum, Xanthomonas axonopodis, Pseudomonas syringae*	Liu et al., 2017
Rhodobacter sphaeroides, Lactobacillus plantarum, and *Saccharomyces cerevisiae*	*Cucumis sativus* L. cv. Black Pearls	Indole acetic acid and or organic acids production.	Kang et al., 2014
Pseudomonas putida	*Cicer arietinum* L.	Increased N, P, K content, increased soil pH, increased SOD and POD enzymatic activity, increased oxalic acid and citric acid concentration in root exudates, increase plantbiomass	Israr et al., 2016
Acinetobacter sp.BR-12, *Klebsiella* sp. BR-15	*Oryza sativa* L. cv. BR29	Phosphate solubilisation by releasing organic acids	Islam et al., 2006
Pseudmonas aeruginosa	-	Phosphate solubilisation by releasing organic acids, broad-spectrum antifungal activity against *Alternaria brassicae, Alternaria brassicicola, Alternaria alternate. Collectotrichum gleosporoides, Fusarium oxysporum, Fusarium solani, Penicillium expansum, Phytopthoracapisci, Rhizoctonia solani, Verticillium theobromae,* IAA production, possess biosurfactant activity	Bakthavatchalu et al., 2012
*Enterobacter lignolyticus*TG1, *Burkholderia* sp. TT6, *Bacillus pseudomycoides* SN29, *Pseudomonas aeruginosa* KH45	*Camellia sinensis* (L.) O. Kuntze	Phosphate solubilisation, IAA production, siderophore production, and ammonia production	Dutta et al., 2015

Azotobacter sp., *Azospirullum* sp., *Pseudomonas* sp.	*Triticum aestivum* L.	Antagonistic activity against *Rhizoctonia solani*	Singh et al., 2015
Pseudomonas, Rhizobium, Enterobacter, Chronobacter, Kosakonia, Beijerinckia and *Pantoea. Kosakonia pseudosacchari*	-	Siderophore production	Arora, 2015
Klebsiella pneumonia	*Piper nigrum* L.	IAA production	Jasim et al., 2014
Variovorax boronicumulans	-	Biodegradation of acrylamide, production of siderophore, Ammonia, hydrogen cyanide, and the phytohormone salicylic acid	Liu et al., 2013
Burkholderia cepacian	-	Biocontrol activity against *Fusarium* sp., production of siderophore	Vessey, 2003

Table1.2: Reports on bacterial strains as plant growth promoting microorganism tested on different crop plants

PGPR	Test Plants	Effect on the plant's growth	References
Kosakonia arachidis, Pseudomonas putida, and *P. monteilii*	*Cicer arietinum* L.	Significant enhanced the growth parameter such as shoot and root length, root and shoot fresh weight and dry weight	Tatung and Deb, 2023
Bacillus cereus, Bacillus safensis, Bacillus pumilus, Klebsiella variicola, Lelliottia amnigena, Pseudomonas koreensis, and *Serratia marcescens*	*Brassica juncea* and *Helianthus annuus*	In *Brassica juncea*, enhancement in root length and weight were recorded by 53 and 93% respectively against control, while, fresh weight of shoot was improved by 72% on soil adjunct with PGPR and 67% with PGPR in heavy metal contaminated condition. Similar trend was also registered on dry weight basis. Whereas, PGPR consortia treated *Helianthus annuus* registered increased shoot and root lengths both fresh and dry weight basis.	Tatung and Deb, 2024
Pseudomonas putida	*Brassica nigra* L.	Increased chlorophyll content, shoot length, and in the number of leaves as compared to control. Increased	Bharucha et al., 2013

		number of adventitious roots was observed which provides more surface area to the roots for better utilization of nutrients	
Pseudomonas fluorescence	*Spinacia oleracea* L.	Increased shoot and root length	Yamini et al., 2021
B. cereus and *Bacillus subtilis*	*Capsicum annuum* L.	Plants inoculated with B. subtilis ITC-N67 showed an increase in stem diameter and root volume, whereas inoculation with B. cereus ITC-BL18 increased the number of flower buds, fresh biomass of roots and total fresh biomass	Peña-Yam et al., 2016
Bacillus amyloliquefaciens and *Serratia marcescens*	*Zingiber officinale* Rosc.	Both in green house and field test *B. amyloliquefaciens* and *S. marcescens* registered markedly higher sprouting and lower disease incidence and greater rhizome yield, while control registered the lowest sprouting, maximum soft rot incidence and lowest rhizome yield.	Dinesh et al., 2015
Burkholderia cenocepacia	*Zea mays* L.	The addition of both *B. cenocepacia* CR318 and inorganic phosphate [$Ca_3(PO_4)_2$] combined significantly improved several growth parameters of corn plants relative to the addition of *B. cenocepacia* CR318 alone, $Ca_3(PO_4)_2$ alone, or no treatment. led to an 80 % increase in chlorophyll content, 68 % increase in whole-plant wet weight, 59 % increase in whole-plant dry weight, 74 % increase in root wet weight, and 98 % increase in root dry weight compared to $Ca_3(PO_4)_2$ alone after 6 weeks of growth	You et al., 2020
Pseudomonas stutzeri, Bacillus subtilis, Stenotrophomonas maltophilia, and *Bacillus amyloliquefaciens*	*Cucumis sativus* L.	Increased levels of germination, seedling vigour, growth, and N content in root and shoot tissue compared to non-treated control plants	Islam et al., 2016
Pseudomonas alcaliphila and *Pseudomonas hunanensis*	*Phaseolus vulgaris* L.	Bacterial inoculation significantly enhanced the root growth parameters of *Phaseolus vulgaris*	Alali et al., 2021
Bacillus amyloliquefaciens	*Lycopersicon Esculentum*	Significantly suppressed *R. solanacearum* and bacterial wilt in tomatoes when the culture medium was supplemented with	Chou et al., 2022

	L.	1% (w/v) soybean meal.	
Bacillus subtilis	*Capsicum annuum* L.	Inoculation of *B. subtilis* reduced the incidence of *Fusarium* wilt in pepper significantly, induced systemic resistance. Treated plants grew 27.24e54.53% taller than controls. It also enhanced the yield of pepper by shortening the time to 50 percent flowering to 17.26 days, increasing the average fruit weight 36.92%, and increasing the average yield per plant 49.68%.	Yu et al., 2011a

Plant Growth Promoting Fungi

Fungi play a crucial role in ecosystems and offer numerous benefits in various fields, including food production, medicine, agriculture, and healthcare advancements (Imran et al., 2021). Plant growth promoting fungi (PGPF), which are beneficial fungi (mycorrhizal, rhizospheric, and endophytic) contribute to growth and development of plants. Their ability to enhance plant growth makes them suitable for organic based agriculture and presents a new innovative approach with reduced reliance on traditional inorganic fertilizers (Sarkar et al., 2022). Fungi extend the root system of the plants, aiding in nutrient and water uptake and improving the physical properties of the soil by modifying its structure. For example, fungal hyphae can create macroaggregate by entangling soil particles with each other (Pirttilä et al., 2021). Among the well-studied root associated fungi are arbuscular mycorrhizal fungi (AMF), which forms symbiotic relationship with approximately 80% of the land plants species, including agricultural crops. AMF provides numerous benefits to the plants including enhanced uptake of mineral nutrients and water in exchange of carbon source from plants (Mitter et al., 2021). The PGPF and plant root association has shown to modulate plant growth, mineral nutrient uptake, increased biomass, and yield of crop plants (Deshmukh et al., 2006). PGPF suppress plant pathogens in the rhizosphere through production of hydrolytic

enzymes and plant hormones, and mineral solubilisation (N, P, and Fe). Other mechanisms include mycoparasitism, siderophore production, competition for saprophytic colonization, and the induction of systemic resistance (Lewis and Papavizas, 1991). Various studies have highlighted the potential benefits of different fungi (**Table 1.3**). For example, *Phoma* sp. when inoculated increased the fresh biomass and number of cucumbers (Byrappa et al., 2005), while *Aspergillus niger* and *Aspergillus caespitosus* enhanced content of protein, carbohydrate, total phenolic, anti-oxidant activity and diosgenin in *Trigonella foenum-graecum* (Thakor et al., 2023). The fungi *Aspergillus terreus* demonstrated antifungal effects against *Aspergillus fumigatus*, a human pathogen (Jalili et al., 2020). Also, in the study conducted by Sarkar et al. (2022), *Trichoderma harzianum* TaK12 and *Trichoderma aureoviride* TaN16 two phosphate solubilising and IAA producing PGPF were found to enhance maximum shoot and root length of rice plant; while, *Penicillium olsonii* improved the height, leaf area, dry weight, and total chlorophyll content of the rice seedlings (Tarroum et al., 2022). In a study conducted by Imran et al. (2021), the application of a consortium consisting of plant growth promoting fungi (PGPF) such as *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. resulted in significantly increased wheat yield. These studies demonstrate the strong potential of PGPF as biofertilizers. Overall, the use of these fungi in sustainable agriculture practices offers promising solutions to reduce the reliance on chemical fertilizers and improve crop productivity. Few examples of PGPF are *Penicillium* sp., *Pythium* sp., *Trichoderma* sp. (Murali et al., 2012), *Trichoderma harzianum* TaK12 and *Trichoderma aureoviride* TaN16 (Sarkar et al., 2022), *Daldinia eschscholtzii*, *Sarocladium oryzae*, *Rhizoctonia oryzae, Penicillium allahabadense*, and *Aspergillus foetidus* (Syamsia et al., 2021). *Acrophialophora jodhpurensis* (Daroodi et al., 2022), *Aspergillus niger* (Galeano et al., 2021).

Table 1.3: Reports on fungal strains as Plant growth promoting microorganism tested on different crop plants

PGPF	Test Plant	Effect on the Plant's Growth	References
Penicillium commune	*Vigna mungo* L.	Increase in the growth parameters such as shoot and root length, fresh and dry weight of the shoot and root length	Banerjee and Dutta, 2019
Talaromyces sp.	*Capsicum annuum* L.	antagonism against *Colletotrichum capsici* and presented significant enhancement in the seed and plant growth parameters	Naziya et al., 2019
Aspergillus flavus	*Solanum lycopersicum* L.	Antagonisms against *Alternaria phragmospora* and increase in the plant growth parameters such plant fresh weight and plant length.	Abdel-Motaal et al., 2020
Penicillium sp.	*Cenchrus americanus* L., *Solanum melongena* L. and *Solanum lycopersicum* L. seedlings	Enhanced seed germination and seedling vigour	Mahadevamurthy et al., 2016
Aspergillus niger	*Triticum aestivum* L.	Increased the assessed biometric parameters, reduction in the population of pathogenic fungi *Gibberella*, *Fusarium*, *Monographella*, *Bipolaris*, and *Volutella*	Wang et al., 2018
Phoma sp.	*Cucumis sativus* L.	Increase in the plant biomass and length after 6 and 10 weeks of planting	Byrappa et al., 2005
*Penicillium*sp., *Pythium* sp., *Trichoderma* sp.	*Cenchrus americanus* L.	Enhancement of seed germination and vigour, diseases protection against downy mildew of pearl millet	Murali et al., 2012
Aspergillus niger and *Aspergillus caespitosus*	*Trigonella foenumgraecum* L. seeds	Increased protein content, carbohydrate content, total phenolic content, anti-oxidant activity, diosgenin content.	Thakor et al., 2023
Trichoderma harzianum TaK12 and *Trichoderma aureoviride* TaN16	*Oryzae sativa* L.	Increased shoot and root length of rice plant	Sarkar et al., 2022
Penicillium olsonii	*Nicotiana tabacum* L.	Enhanced the plant salt tolerance by increasing the levels of total chlorophyll, proline, CAT, and SOD activities. In addition, the treated	Tarroum et al., 2022

		plants accumulated less Na+ in their roots but more K+ in their leaves. The A3CFF was also found to induce the expression of five salt stress related genes (NtSOS1, NtNHX1, NtHKT1, NtSOD, and NtCAT1)	
Acrophialophora jodhpurensis	*Solanum lycopersicum* L.	Significant increase in plant growth parameters and reduction in the progress of the diseases caused by *A. alternata*	Daroodi et al., 2022
Aspergillus sp, *Penicillium* sp, *Rhizopus* sp	*Triticum aestivum* L.	Significant increase in the plant growth and yield	Imran et al., 2021
Trichoderma viride	*Vigna radiate* L., *Vigna mungo* L.and *Sesamum indicum* L.	Increase in the fresh and dry weight, seed germination, vigour index and inhibition of *Fusarium oxysporum* (82%), and *Aspergillus niger* (94%).	Kumar et al., 2017
Penicillium chrysogenum	*Zea mays* L.	Increased shoot length, dry and fresh biomass, total chlorophyll and proline content	Galeano et al., 2023
Aspergillus niger	*Phaseolus vulgaris* L.	Increased in shoot length, root fresh weight, root and shoot dry weight	Galeano et al., 2021
Trichoderma harzianum	*Solanum lycopersicum* L.	Increased chlorophyll content, shoot length, fresh and dry weight of shoot and roots, and reduced *F. oxysporum* wilt disease	Bader et al., 2019
Aspergillus flavus, Aspergillus niger, Mucor circinelloides and *Pencillium oxalicum*	*Solanum lycopersicum* L.	Increased growth of healthy and infected plants against *Fusarium oxysporum*	Attia et al., 2022
Penicillium menonorum	*Cucumis sativus* L.	Increased in dry biomass of cucumber roots (57%) and shoots (52%), Chlorophyll, starch, protein, and P contents were increased by 16%, 45%, 22%, and 14%, respectively	Babu et al., 2015
Ampelomyces sp. and *Penicillium* sp.	*Solanum lycopersicum* L.	*Ampelomyces* sp. Increase in plant growth under drought condition while *Penicillium* sp. increased plant growth and root biomass under salinity stress (300 mM)	Morsy et al., 2020
Gibberella intermedia	Waito-C *Oryza sativa*germinals	Increase in the shoot growth by the production of gibberellins	Khalmuratova et al., 2021
Trichoderma longibrachiatum	*Triticum aestivum* L.	Significantincrease in the relative water content (leaves and roots), chlorophyll content, and root activity, accelerated	Zhang et al., 2016

		accumulation of proline content in leaves, antioxidant enzymes-superoxide dismutase, peroxidase, and catalase in wheat seedlings (under salt stress); the relative expression of SOD, POD, and CAT genes in these wheat seedlings were significantly up-regulated	
Fusarium sp.	Salt-sensitive *Oryza sativa* variety IR-64	High assimilation and chlorophyll stability index	Ramaiah et al., 2020
Bipolaris sp.	*Glycine max* (L.) Merr.	Significantly increased shoot and root length, shoot and root fresh and dry weight and chlorophyll content under NaCl stress (200 mM).	Khan et al., 2022
Stemphylium lycopersici	*Zea mays* L.	Increase Ca2+, K+, Mg2+, N, and P contents under salt stress, antioxidant enzyme. Decreased MDA content, Na+ ion content, Cl− ion, Na+/K+, and Na+/Ca2+	Ali et al., 2022b
Paraglomus occultum	*Lycopersicon esculentum* L.	Higher root and shoot length, shoot dry weight (28%), yield (20%) as well as increased in potassium (2%), calcium (13%), Mg (24%) and Fe (37%) content.	Alam et al., 2023
Rhizophagus intraradices	*Solanum tuberosum* L.	Increase minituber number (116%), minituber weight (181%), shoot dry weight (248%), root dry weight (120%), chlorophyll content (57%), ascorbic acid (8%) content, K (27%), Zn (24%) and Fe (17%) content.	Barogh et al., 2023
Rhizophagus irregularis	*Lycopersicon Esculentum* L.	Substantial improvement in the growth and quality of the crop and highest values of total dry weight, survival rate, N content and P content.	Roussis et al., 2022
Penicillium pinophilum	*Lycopersicon esculentum* L.	Improved growth indices and boosted fruit weights as well as significantly reduced disease incidence caused by *Verticillium dahliae*.	Ibiang et al., 2021
Trichoderma longibrachiatum, *T. asperellum*, and *T. atroviride*	*Glycine max* (L.) Merr.	Exhibited a high percentage of antagonistic activity against *Rhizoctonia solani*, the causal pathogen of root rot disease of soybean plants.	Sallam et al., 2021
Penicillium oxalicum and	*Solanum melongena* L.	Increase in seedling height, leaf size, and root length, dry	Li et al., 2021b

Aspergillus brunneoviolaceus		and fresh weights of seedlings as well as early flowering.	
Penicillium chrysogenum	*Arachis hypogaea* L.	Inhibited the virulent plant pathogens *Ralstonia solanacearum*, that causes bacterial wilt in groundnut	Chowdappa et al., 2020
Trichoderma koningii	*Cynara cardunculus* L.	Significantly higher values of plant height, diameter, chlorophyll content and leaf dry weight.	Suebrasri et al., 2020
Lecanicillium psalliotae	*Elettaria cardamomum* L.	Significantly increased shoot and root length, shoot and root biomass, terminal leaf length and width, number of secondary roots and leaves and leaf chlorophyll content compared to untreated plants.	Kumar et al., 2018b
Xylaria regalis	*Capsicum frutescens* L.	Significant increase in shoot and root length, dry matter production of shoot and root, chlorophyll, nitrogen, and phosphorus contents.	Adnan et al., 2018
Trichoderma virens	*Zea mays* L.	Induced systemic resistance against the foliar pathogen *Cochliobolus heterostrophus* which cause corn leaf blight.	Mukherjee et al., 2018
Trichoderma harzianum	*Pistacia vera* L.	Exhibited the highest growth inhibition percentages against disease causing *Aspergillus flavus, Rhizoctonia solani* and *Sclerotinia sclerotiorum.*	Dolatabad et al., 2017
Aspergillus niger and *Aspergillus parasiticus*	*Vigna radiate* L.	Increased growth of plants in terms of root length, shoot length, number of leaves and fresh weight as well as dry weight as compared to un-inoculated control.	Patel et al., 2017

Sustainable Agricultural Practices

As the world population continues to surge, agriculture has expanded to unprecedented levels. A major problem today is meeting the growing demand for crops while using less synthetic chemical fertilizers and pesticides. However, the excessive use of synthetic pesticides and fertilizers has led to pollution of land and water, thereby

adversely affecting all forms of life on the planet. Also, continues usage of these chemicals has led to a decrease in productivity and yield of the land with each passing year. After the historic green revolution movement which began between 1943 and the late 1970s in Mexico, the amount of cereal production in developing countries had increased in huge quantity (Ameen et al., 2017). Although populations had more than doubled, the production of cereal crops tripled during this period, with only a 30%increase in land area cultivated (Pingali et al., 2012). However, the negative impacts it has left on many aspects of human society cannot be avoided, few examples being overexploitation of water sources and excessive use of pesticides. These chemicals are being accumulated in the agricultural soil and will remain there for a long time. Long-term exposure to pesticides such as organochlorines, creosote, and sulfalate has been correlated with higher cancer rates and organochlorines DDT, chlordane, and lindane as tumour promoters in animals (Ameen et al., 2017). A viable alternative to chemical fertilizers is the use of biological fertilizers which can effectively increase productivity as well as prevent our ecosystem from further degradation. The market of the biofertilizers is expected to reach 3.8$ billion by 2025 from 2$ billion in 2019 (Riaz et al., 2020). Research on PGPM as biofertilizers, biopesticides and biocontrol agents has demonstrated its potential as viable substitute for synthetic pesticides and fertilizers. PGPM in nature aid plants to grow by using different mechanisms like protecting them from phytopathogens and by producing chemical compounds along with other various ways. The potential of PGPM to function as biofertilizers while maintaining environmental integrity holds great promise for the advancement of sustainable agriculture. PGPM can be a great alternative to such chemicals; since, it can increase crop production without harming the environment, using microbes as biofertilizers has attracted a lot of attention in recent years. Bioinoculants containing rhizobacteria are now

being utilized globally to enhance plant growth and development in the face of different stressors such as heavy metal exposures (Ma et al., 2011a; Ma et al., 2011b; Wani and Khan, 2010), pesticide degradation/tolerance (Ahemed and Khan, 2012), biological control of phytopathogens and insects along with the normal plant growth promoting properties such as, phytohormone (Asghar et al., 2002; Tank and Saraf, 2010), Siderophore (Tian et al., 2009; Jahanianet al., 2012), nitrogen fixation (Glick et al., 2012), phosphate solubilisation (Glick, 2012; Zheng et al., 2018), production of antibiotics and act as biological control of pest and diseases in addition to ameliorating stress conditions (Whipps et al., 2001; Beneduziet al., 2012), competitive exclusion of pathogens or removal of phytotoxic substances (Bashan and de- Bashan, 2010).

Mechanism of PGPM on Plant Growth

PGPM can enhance the plants growth through both direct and indirect methods. Direct methods involve activity such as Phosphate solubilisation and production of indole 3-acetic acid (IAA). On the other hand, indirect methods encompass the production of siderophore, induced systemic resistance, and the ability to confer biotic and abiotic stress such as tolerance to heavy metal and salinity (**Figure 2**).

Direct Mechanisms

PGPR offer various direct benefits to plants such as solubilization of inorganic potassium and phosphates, nitrogen fixation, phytohormone production and siderophore production. This enrichment is achieved by elevating individual ion fluxes on the root surface in the presence of PGPR (Gouda et al., 2018). The microbes synthesize biologically active compounds, including phytohormones (auxins, cytokinins, gibberellins, and ABA), antifungal compounds, enzymes, and compatible solute.

Phosphate solubilisation: Although soil usually contain high amount of the total phosphorus, its availability to the plants is very low and often a limiting factor of the

plant's growth (Mikanova and Novakova, 2002). Phosphorus (P) is one of the essential elements that are necessary for plant development and growth; it makes up about 0.2% of a plant dry weight (Azziz et al., 2012; Tak et al., 2012). The effectiveness of applied P fertilizers is typically limited to around 30 % due to its fixation in soils. In acidic soils, P is fixed in the form of iron/aluminium phosphate; on the other hand, in neutral to alkaline soils, P fixation occurs in the form of calcium phosphate. This fixation process hinders the availability of P for plants, leading to reduced efficiency of P fertilizers (Sharma et al., 2013). It is a major growth-limiting nutrient after nitrogen, even though abundantly available in soils in both organic and inorganic forms (Khan et al., 2009). This low availability of phosphorus to plants is because the majority of soil P is found in insoluble forms, while the plants absorb it only in two soluble forms- the monobasic ($H_2PO_4^-$) and the diabasic (HPO_4^{2-}) ions (Bhattacharyya and Jha, 2012). Micro-organisms such as PGPR and PGPM can mineralize organic phosphorus in soil by solubilising complex-structured phosphates *viz*., tricalcium phosphate, rock phosphate, aluminium phosphate, etc. which turns organic phosphorous to inorganic form ultimately aiding the phosphate availability to plants (Goswami et al., 2016). Phosphate anions may be immobilized through precipitation with cations such as Ca2+, Mg^{2+}, Fe^{3+}, and Al^{3+}. The release of insoluble and fixed forms of phosphorus is an important aspect of increasing soil phosphorus availability (Islam et al., 2006). Phosphorus plays a key role in root development, root traits anatomy modifications and root hair density with a significant contribution in increasing yield of crops. Consequently, P deficiency can cause significant reductions of crop yield. For this reason, P applications remain one of the main agricultural practices to meet plant needs. Traditionally to overcome the P deficiency in soils, there are frequent applications of phosphatic fertilizers in agricultural fields. There is found to be an association between bacterial diversity and soil P content

in the soil, higher the phosphate fertilization more the total bacterial diversity (Liang et al., 2023). Several authors attribute the solubilisation of inorganic insoluble phosphate by microorganisms to the production of organic acids such as formic acid, acidic, propionic, lactic, glycolic, fumaric and succinic acid and chelating oxo-acids from sugar (Nautiyal, 1999; Dutta et al., 2015). These organic acids released by microorganisms act as good chelators of divalent cations of Ca^{2+} coupled with the release of phosphates from insoluble complexes or may also form soluble complexes with metal ions co-complex with insoluble phosphorus, thereby releasing the phosphorus moiety (Bhattacharya, 2019). Although microbial inoculation is in use for improving soil fertility during the last century, however, a meagre work has been reported on phosphorus solubilisation compared to nitrogen fixation. Pande et al. (2017), studied the effect of *Alcaligenes aquatilis* and *Burkholderia cepacian,* phosphate solubilising PGPR on maize and found significant increase in the growth and development of the plant. Similar outcome was found by Sarker et al. (2014), *Pseudomonas* sp. significantly increased the growth and nutrient uptake by wheat. *Pseudomonas aeruginosa*, *Enterobacter lignolyticus*, *Burkholderia*, *Bacillus pseudomycoides, Pseudomonas aeruginosa, Acinetobacter* sp., *Klebsiella* sp.*, Pseudomonas putida, Rhodobacter sphaeroides, Lactobacillus plantarum, Saccharomyces cerevisiae, B. velezensis, P. peoriae, B. altitudinis, Bacillus megaterium, Methylo bacterium* sp. 2A are some of the PGPR that solubilize phosphate (Ayyadurai et al., 2005; Islam et al., 2006; Bakthavatchalu et al., 2012; Dutta et al., 2015; Kang et al., 2015; Israr et al., 2016; Liu et al., 2017). Interestingly when compared to bacteria, fungi seem to have more advances as phosphate solubilising fungi PSF as it can reach to spread up to large area around the plants root and make the nutrient available to the plant. PSF utilize three primary mechanisms to facilitate phosphate solubilisation. These mechanisms encompass (a) the discharge of metabolites, (b) biochemical mineralization,

and (c) biological mineralization (Kaul et al., 2019). Solubilisation of inorganic phosphorus by PSF mainly occurs by the release of organic acids (glycolic acid, oxalic, tartaric, and citric acid, formic acid, gluconic acid, and fumaric acid), whereas organic phosphorus is solubilised by the release of various enzymes (phosphatases, phytases and phosphonatases) (Wang et al., 2018; Kaul et al., 2019; Daroodi et al., 2022), *Aspergillus hydei*, *Gongronella hydei*, *Penicillium solitum* and *Talaromyces yunnanensis* (Doillom et al., 2020). PSF have specialized in solubilising phosphate by releasing organic acids. These acids serve several functions, including (i) reducing pH levels, (ii) bolstering the chelation of cations, (iii) engaging in competition with P for soil adsorption sites, or (iv) generating metal complexes alongside insoluble p elements like calcium (ca), aluminium (Al), and iron (Fe). As a result, this process leads to the liberation of P (Kaur and Reddy, 2017)

Phytohormone production: Phytohormones are the chemical messengers that occur in low concentration and play a crucial role in the natural growth and development of plants. These phytohormones shape the plant, by affecting seed growth, time of flowering, sex of flowers, senescence of leaves, and fruits. They also affect gene expression and transcription levels, cellular division, and growth (Kundan et al., 2015). Microbes produce hormones identical to the hormones produced by the plants for their growth and development. Phytohormones produced PGPRs include Indole 3 acetic acid, cytokinins, gibberellins, and inhibitors of ethylene production (Prasad et al., 2019). Both plants and some microbes such as *Enterobacter cloacae,* which have an enzyme called indole pyruvate decarboxylase which can catalyse the decarboxylation of indole-3-pyruvic acid to yield indole-3-acetaldehyde and carbon dioxide (Koga et al., 1992).

IAA production: Phytohormones, especially auxins control several stages of plant growth and development such as cell elongation, cell division, tissue differentiation, and

aid apical dominance, formation of lateral roots and root hairs and the primary root length (Dahmani et al., 2020). Although plants produce a limited amount of endogenous IAA that is not directly utilized, the exogenous IAA derived from fungal and bacterial isolation can be applied in biological fertilizers to enhance results and provide optimal benefits (Gusmiaty et al., 2019; Dahmani et al., 2020). The microbial biosynthesis and the fundamental mechanism of auxins action on plant have undergone intense investigation (Spaepan and Vanderleyden, 2011). Among auxins, indole-3-acetic acid is an important phytohormone produced by several strain of PGPM and it is well known that treatment of IAA-producing GPPM increases the plant growth (Vessey, 2003; Kaymak, 2010; Amara et al., 2015; Kumar et al., 2017; Chouhan et al., 2022). Primarily, IAA is known to stimulate both rapid (e.g., increase in cell elongation) and long term (e.g., cell division and differentiation) responses in plants. Multiple pathways for IAA synthesis utilizing the amino acid tryptophan as a precursor have been described in bacteria and few in fungi (Spaepen and Vanderleyden, 2011; Ducaet al., 2014; Keswani et al., 2020). These include the Trp independent pathway, Trp dependent pathway (indole-3-acetamide (IAM), the indole-3-acetonitrile (IAN), the indole-3-pyruvate (PPyA), the tryptophan side-chain oxidase (TSO), and the tryptamine (TAM) pathways. Genetic analyses of the IAM and IPyA pathways have helped elucidate the role of bacterial IAA production in several different plant-microbe interactions (Manulis et al., 1998; Duca et al., 2014; Keswani et al., 2020). Some of the PGPR and PGPF producing IAA are *Enterobacter ludwigii*, *Pseudomonas fragi, Bacillus cereus, Rhizobium* sp*., Bacillus aerius, Pseudomonas fragi* and *Bacillus cereus* and *Bacillus amyloliquenfaciens, Bradyrhizobium japonicum* (Susilowati et al., 2018; Boiero et al., 2007), *Penicillium olsonii* (Tarroum et al., 2022), *Acrophialophor jodhpurensis* (Daroodi et al., 2022), *Aspergillus* sp., *Fusarium* sp.

(Gusmiaty et al., 2019), *Talaromyces trachyspermus* (Chouhan et al., 2022), *Trichoderma viride* (Kumar et al., 2017), *Bipolaris* sp. (Khan et al., 2022).

Gibberellic acidproduction: Gibberellins are plant hormones that influence and control plant developmental processes like stem elongation, germination, dormancy, flowering, sex expression and leaf and fruit senescence (Kundan et al., 2015). Gibberellins act as signalling molecules for host plants under stress andnon-stressconditions which protect plants from biotic and abiotic stress by modulating antioxidant levels by decreasing superoxide dismutase, flavonoids, and radical scavengers (Khatoon et al., 2020). Gibberellic acids are naturally produced by higher plants, fungi and bacteria and regulate plant growth and development. They are typical secondary metabolites in microorganisms. However, they act as endogenous hormones in higher organisms such as plants (Sharma et al., 2017). Some PGPR can synthesize GA_3 and compensate for the absence of plant gibberellins; in some cases, they can also stimulate the synthesis of plant's own gibberellins (Tsukanova et al., 2017). *Azospirillum lipoferum, Azotobacter chroococcum, Pseudomonas fluorescens, Bradyrhizobium japonicum* and *Bacillus megaterium* are some examples of PGPR that produces Gibberellic acid (Boiero et al., 2006; Lenin and Madhavan, 2012).

Cytokinin production: Cytokinins are phytohormones that promote cell division in plant roots and shoots and regulate cell growth and differentiation. Their main roles are to delay the senescence, countering the apical dominance induced by auxin and in conjunction with ethylene they promote abscission of leaves, flower parts and fruit (Kundan et al., 2015). The root of the plants in an environment when inoculated with cytokinin producing rhizobacteria, results in stimulating plant growth in a manner suggesting increased plant growth and improved soil health (Khatoon et al., 2020). Different PGPR

employs different mechanisms to change the plant cytokinin concentration (Tsukanova et al., 2017).

Ethylene and 1-Aminocyclopropane-1-Carboxylase production: Ethylene, the gaseous plant hormone influences plant growth by inhibiting the seed germination and root growth kinetics and it promotes fruit ripening. The unfavourable conditions like floods, extreme temperatures, exposure to radiation, and heavy metals cause physiological stress which induces ethylene synthesis in plants and consequently plant growth, development and yield are negatively impacted due to poor root growth (Andy et al., 2020). ACC deaminase production of PGPR elicits the growth promotion by decreasing the level of ethylene production (Dutta et al, 2015). In the presence of ACCD producing rhizobacteria, ACC secreted by plant roots in the rhizospheric soil gets degrade dandthen secretion of ACC from the roots is promoted, subsequently decreasing ACC contents both in roots as well as in leaves (Khatoon et al., 2020). The use of biofertilizers containing PGPR with ACC deaminase activity may improve plant growth and development by relieving harmful effects of salt stress ethylene. Besides this, heavy metal stress can also be alleviated using PGPR's (Das et al., 2013).

Ammonia production: Ammonia production by PGPR is one of the essential traits linked to plant growth promotion. In general, ammonia produced by PGPR has been shown to supply nitrogen to their host plants and thereby promote root and shoot elongation and their biomass (Bhattacharyya et al., 2020). The presence of ammonia producing Plant Growth Promoting bacteria (PGPR) and other prokaryotes is an indicative that the ammonification process is taking place in the plant rhizosphere which not only improves plant growth, through the supply of nitrogen, butalso, indirectly influences plant development by inhibiting plant pathogenic microbes (Abdelwahed et al.,

2021). The PGPR nitrogenous materials of peptones break down into ammonia, which is released into the soil and used by plants as their nutrient source (Vasanat et al., 2023)

Nitrogen fixation: The process of nitrogen fixation is truly remarkable, accounting for about two -third of the nitrogen fixed globally (Gouda et al., 2018). Despite atmospheric nitrogen comprising roughly 80% of the air we breathe, it is not readily available for plants uptake, making it the most limiting nutrient for the plant growth (Jha et al., 2011). Plants cannot directly convert atmospheric dinitrogen into ammonia for their growth, which is why they rely on biological nitrogen fixation (BNF). Globally, BNF contributes 180 x 106 metric tons per year, with 80% of that being attributed to symbiotic nitrogen fixation (Das et al., 2013). Both symbiotic and non-symbiotic PGPR can fix nitrogen. Non-symbiotic nitrogen fixers do not penetrate the root cells but forms a close association with the roots of the plants called non-specific or loose symbiosis (Goswami et al., 2016). The gene responsible for nitrogen fixation is called *nif* gene. By inoculating soil with nitrogen fixing PGPR, the amount of synthetic nitrogen being applied to the agricultural filed can be reducedand soil phytoremediation can be facilitated. *Azospirillum, Bacillus, Serratia, Enterobacter, Acinetobacter* etc. are some of thenitrogen fixing bacteria. These genera can colonize the rhizosphere and provide beneficial effects.

Siderophore production: Iron is known to be the fourth most abundant element on the earth's crust (Kour et al., 2019) and is vital for the growth and developmental processes of every living organism (Rana et al., 2020; Baron and Rigobelo, 2021). It regulates the biosynthesis of antibiotics, aromatic compounds, cytochromes, nucleic acids, pigments, porphyrins, siderophores, toxins, and vitamins (Saha et al., 2016). It occurs as Fe^{3+} in the aerobic environment which easily forms insoluble hydroxides and oxyhydroxides which are inaccessible to both plants and microbes (Ahemad, 2014). To satisfy nutritional requirements of iron, microorganisms have evolved highly specific pathways that employ

low molecular weight iron chelators termed siderophores. Siderophore can be defined as small peptidic molecules containing side chains and functional groups that can provide a high-affinity set ligands to coordinate ferric ions (Crosa and Walsh, 2002). Based on their iron-coordinating functional groups, structural features, and types of ligands, bacterial siderophores have been classified into four main classes- carboxylate, hydroxamates, phenol catecholates and pyoverdines in bacteria (Crowley, 2006) (**Figure 1.3**). In soil, siderophore production activity plays a central role in determining the ability of different microorganisms to improve plant development. Microbial siderophore enhance iron uptake by plants that can recognize the bacterial or fungal ferric-siderophore complex (Masalha et al., 2000: Katiyar and Goel, 2004; Dimkpa et al., 2009). It was Kloepper et al. (1980), who first described the microbial siderophore and gave evidence of its role as a biocontrol agent. Some of the PGPR and PGPF which act as biocontrol agents by producing siderophore are *Bacillus subtilis* (Hu et al., 2011; Patil et al., 2014), *Bacillus cereus* (Sherpa et al., 2021), *Pseudomonas fluroscens* and *Azospirillum lipoferum* (Bagmare et al., 2019), *Enterobacter* sp., *Pseudomonas* sp., *Enterobacter* sp., *Azospirillum brasilense* and *Brevibacillus brevi.* (Gupta and Gopal, 2008), *Providencia* sp., *Brevundmonas diminuta* (Rana et al., 2011), *Bacillus simensis* (Shen et al., 2022), *Alcaligenes faecalis* (Sayyed et al., 2010). *Azotobacter* sp. (Muthuselvan and Balagurunathan, 2013), *Laccaria laccata* and *Laccaria bicolor* (Haselwandter et al., 2013), *Rhizopus microspores* and *Penicillium bilaii* (Capon et al., 2007). Production of siderophore by PGPR depends on the factors like pH, amino acids, and carbon-nitrogen ratio (Hu et al., 2011). Although plants have their specific iron carriers called Phyto-siderophores, they can utilize the bacterial siderophore-iron complexes (Pathak et al., 2017). Some PGPR like *P. putida* utilizes the Siderophore produced by the other microorganisms to enhance the level of iron available in the natural habitat (Gouda et al.,

2018). Siderophores secreted by PGPB have a much higher affinity to sequester iron than those produced by fungi or the plant itself (Shah et al., 2021). Siderophores are produced by two different pathways NRPSs-dependent and non-NRPSs dependent (Shanmugaiah et al., 2015). Different methods to detect the production of siderophore by microbes have been developed. One of these methods is the detection of siderophores using the CAS reagent given by Schwyn and Neilands (1987). In CAS assay, competition is for iron uptake between siderophore and ferric complex of CAS dye (CAS-iron-detergent complex). Siderophore produced by the bacteria chelates the iron from the complex leaving the dye-free, which results in the change of the colour from blue to orange (Arora et al., 2017). Phyto-siderophores are produced by the gramineous monocots (Sayyed et al., 2013). Siderophore-producing microbes can thus be used in a variety of ways including bioremediation, sustainable agriculture as biosensorsand even in medicine (Arora et al., 2015). Research regarding the ability of siderophores to increase the iron uptake capacity of plants is however very limited and considerable research are further required in the context (Gouda et al., 2018). To date, there are about 500 known siderophores of which chemical structures of 270 of these compounds have been determined (Kundan et al., 2015). Siderophores thus not only help in enhancing plant growth but also play a very important role in providing iron to other organisms including humans (Arora, 2015). Production of the siderophore by the microbes may affect the other microbial population present within the same niche, which may inhibit the growth of some microbes (Hu et al., 2011) **(Figure 1.4**). Biological control of plant diseases is the suppression of populations of plant pathogens by living organisms (Kohl et al., 2019). PGPM produces a siderophore that binds with the ferric ion making it available to the other pathogenic microbes (Deb and Tatung, 2024). Singh et al. (2000), in their study have discussed that siderophore producing bacterial isolates were found to be effective in

reducing the growth of fungal pathogens like *Spedonium* sp., *Fusarium* sp., *Neurospora* sp., *Trichoderma* sp. and *Verticillium* sp. *Mycogone* sp. had increased the production of mushroom *Agaricus bisporus*. Similarly, *Bacillus subtilis* is found to have antifungal activity against *Fusarium oxysporum* and *Macrophomina phaseolina* in chickpea plant (Patil et al., 2014). Application of siderophore producing *Alcaligenes faecalis* has enhanced seed germination (8.75%), root length (9.35%), shoot length (16%) and chlorophyll content (8.0 %) in *Arachis hypogaea* (Sayyed et al., 2010). *Azotobacter* sp inhibits the growth of fungal pathogens *Fusariurm* sp., *Alternaria* sp., *Phytophthora* sp., *Rhizoctonia* sp., *Colletotrichum* sp., and *Curvularia* sp. by 20- 40% in dual culture technique and assay of cell free supernatant (Muthuselvan and Balagurunathan, 2013).

Indirect Mechanisms

The PGPR promote plant growth by indirect mechanisms by lessening or preventing them from phytopathogens and stressful conditions. A single bacterial strain can protect the plant in many ways by producing antibiotics, hydrogen cyanide, volatile organic compounds etc.

Salt tolerance: Soil Salinization decreases the production potential of up to 46 million ha per year and causes the annual loss in agricultural productivity estimated to be of US$ 31 million according to Food and Agricultural Organization (FAO). The salinity stress may also lead to generation of free radicals such as superoxide ions, hydrogen peroxide (H_2O_2) and singlet oxygen, decrease in plant defensive enzymes, imbalance in sodium hemostasis, decreased iron uptake, phenols and other trace elements (Sharma et al.,2021). Iron (Fe) bioavailability to plants is reduced in saline soils. Therefore, plants growing in arid soils face two major challenges for poor crop productivity: high salinity and Fe deficiency (Sultana et al., 2021). According to Molina et al. (2020), in their study they

suggested that certain *P. putida* strains possess accessory genes that consists of the specific biodegradative properties and can be exploited to remove pollutants via rhizoremediation. Association of *M. sativa* with *Pseudomonas* sp. have shown an efficient biological system for the bioremediation of Cr (VI)-contaminated soils (Tirry et al., 2021). Further, fungi also have the ability to accumulate compatible solutes, which helps them counteract osmotic imbalance between their cytoplasm and the external environments. Additionally, they express various Na+ transporters to regulate and minimize cytoplasmic Na^+ concentrations (Liu et al., 2022). In case of arbuscular mycorrhizal fungi (AMF), salt stress tolerance may be governed by the genes associated with water-channel proteins (aquaporins), Na^+/H^+ antiporters, Δ1-pyrroline-carboxylate synthetase (*LsP5CS)*; late embryogenesis abundant protein (*LsLea*) and ABA (*Lsnced*) (Saxena et al., 2022). The halotolerant fungal strains can stand a very high level of salinity presenting its potential to help the plants grow in such harsh condition. They help the plants to adapt to the harsh environment by providing better acquisition of essentials nutrients (phosphorus, nitrogen, potassium etc.), inducing chemical and physiological changes (Dar et al., 2023). For example, less than 150 mM and 300Mm of salt stress maize plants were inoculated with *Penicillium chrysogenum*, significant improvements were observed in various growth parameters compared to the group without it under both saline conditions. Specifically, the maize plants inoculated with PGPF exhibited higher shoot length, fresh and dry biomass; total chlorophyll and proline content (Galeano et al., 2023). *Penicillium olsonii* isolated from the rhizosphere of tobacco plants enhanced the plant salt tolerance by increasing the levels of total chlorophyll, proline, CAT and SOD activities. In addition, the treated plants accumulated less Na+ in their roots but more K^+ in their leaves. The A3CFF was also found to induce the expression of five salt stress related genes (NtSOS1, NtNHX1, NtHKT1, NtSOD, and NtCAT1) (Tarroum et al.,

2022). In the study conducted by Morsy et al. (2020), it was suggested that two endophytic fungus namely *Ampelomyces* sp. and *Penicillium* sp. promoted the growth of tomato plant under drought and salinity stress (300mM) respectively. Similarly, *Trichoderma longibrachiatum,* when inoculated on the wheat plant under salinity stress helped the plant to adapt to the stress and increase the relative water content of the leaves and the roots along with the chlorophyll content and root activity was observed over the control. Antioxidant enzymes-superoxide dismutase, peroxidase and catalase in the seedlings were also increased *(*Zhang et al., 2016). Certain fungi exhibit the remarkable ability to thrive in highly saline environment (hypersaline), showcasing their halotolerance. Among these fungi, *Hortaea werneckii* and *Wallemia ichthyophaga* stands out as particularly significant, offering substantial potential in the field of biotechnology as highlighted by Gostinčar et al. (2011). Other halo tolerant fungi are *Fusarium* sp. (Ramaiah et al., 2020), *Bipolaris* sp. (Khan et al., 2022), PGPR can help plants to grow in harsh condition like saline and drought condition. *P. monteilii* a PGPR when inoculated on the plants growing in extreme saline and drought condition shown to improve growth, increased in seedling growth (root length, shoot length, dry weight, and fresh weight) (Zhang et al., 2019). Salinity stress by PGPR has been studied by Grossi et al. (2020) on *Solanum tuberosum* L. cv. Desirée. Their study has shown that plantlets inoculated with bacterial isolate *Methylobacterium* sp. 2A have shown increased lateral root, a greater number of leaves.

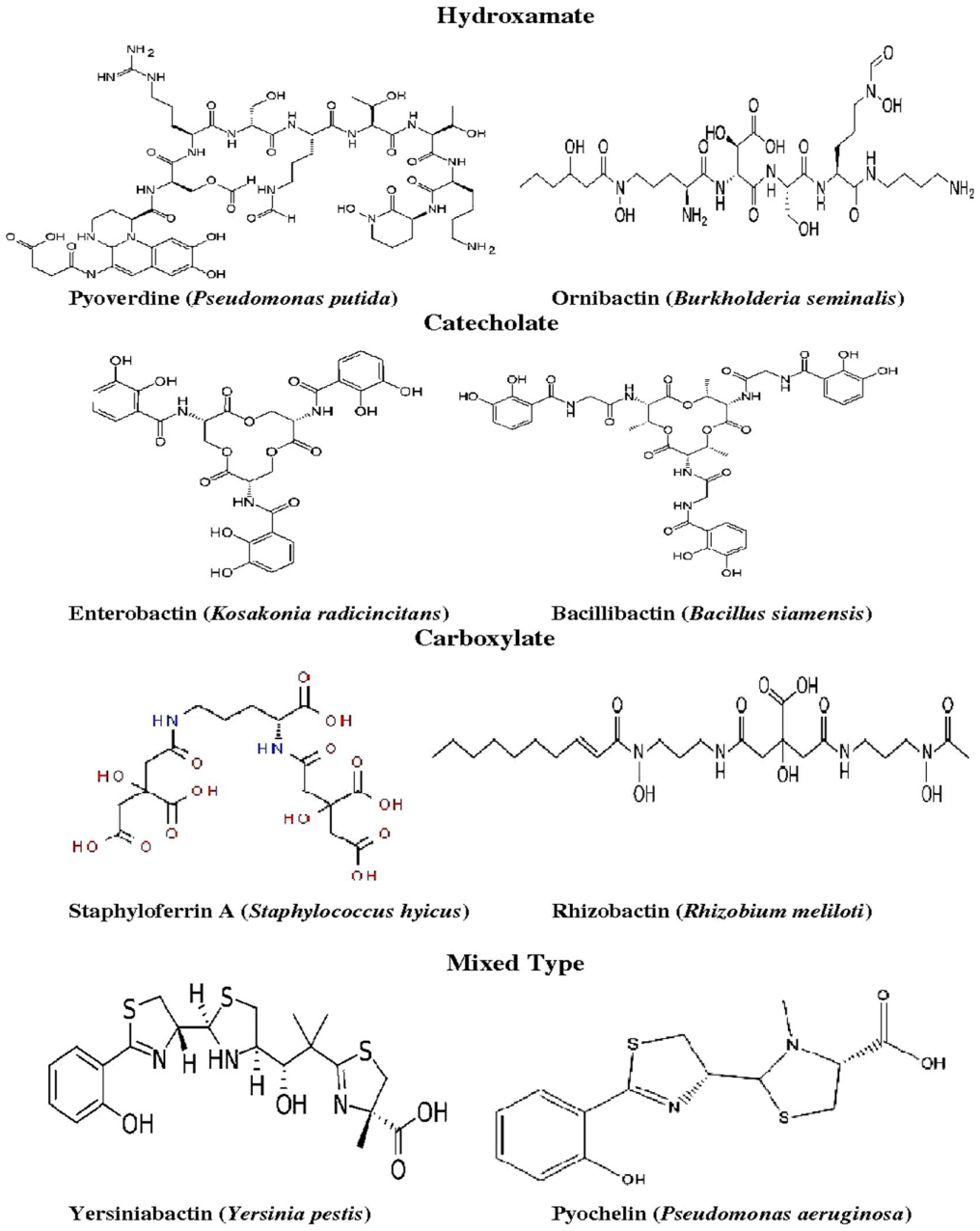

Figure 1.3: Representative example of the types of siderophore produced by bacteria based on their structural features, functional groups, and types of ligands. Four main types of bacterial siderophore are Hydroxamate (*Pseudomonas putida*), Catecholate (*Kosakonia radicincitans*), Carboxylate (*Rhizobium meliloti*), and Mixed type (*Yersinia pestis*).

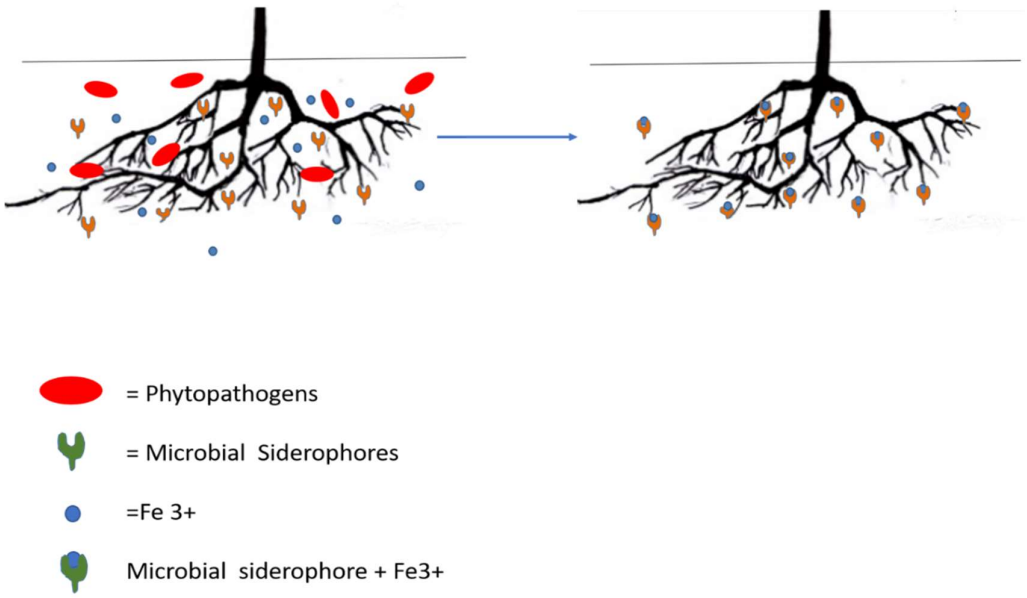

Figure 1.4: During deficiency of iron, microbial siderophores are produced by the PGPR. Microbial siderophore binds the ferric ion (Fe^{3+}) and convert it into Fe^{2+} which the soluble form. This form makes iron unavailable to the phytopathogens depriving them of iron nutrition. The ferrous form is then used up by the bacterial cell in the various growth processes. The siderophores are released to the environment again where they bind to the new ferric ions or get degraded.

Biocontrol agents: Chemicals such as pesticides, herbicides, fungicides etc. we use in the agricultural system has affected environment and human lives to a great extent. They are known for endocrine disruption, antagonization of natural hormones in the body, immune suppression, reproductive abnormalities, hormone disruption and cancer (Savita and Sharma, 2019). Biocontrol is the suppression of the population of pests and weeds by living organisms (Heimpel and Mills, 2007). It can be of any living form, few examples of microbes that can be biocontrol agents are bacteria and fungi. Microbial biocontrol agents (MBCAs) are applied to crops for biological control of plant pathogens where they act via a range of modes of action such as Inducing systemic resistance, nutrient competition, hyper-parasitism and antibiosis (Kohl et al., 2019). Many PGPRs can synthesize anti-fungal metabolites such as antibiotics, fungal cell wall-lysing enzymes, or

hydrogen cyanide, which suppress genetic modifications which could further contribute to the sustainable development of agriculture (Prasad et al., 2019). The HCN produced by PGPR not only acts as a biocontrol factor against phytopathogens but is also involved in geochemical processes in the substrate, such as the chelation of metals (Khatoon et al., 2020). Fluorescence Pseudomonads have received the most prominent attention as candidates for biocontrol agents because of their ability to colonize the surfaces and internal tissues of roots and stems (endo- and exo rhizosphere) at high densities. These bacteria can compete successfully with soil microorganisms and have a tremendous capacity to produce antifungal secondary metabolites (Bakthavatchalu et al., 2012). Antagonistic rhizobacteria play an important role in biological control by producing lytic enzymes and antibiotics and then inhibiting the growth of many pathogenic agents (Benaissa et al., 2019). Some PGPR like *B.cepacian* have been shown to have biocontrol characteristics to *Fusarium* spp., but also can stimulate the growth of maize under iron-poor conditions via siderophore production acting as a biofertilizer (Vessey, 2003) some of the antimicrobial compounds produced by the PGPR are 2,4-diacetylphloroglucinol (PHL), pyoluteorin (PLT), pyrrolnitrin (PRN), phenazine-1-carboxylic acid (PCA), 2-hydroxy phenazines and phenazine-1-carboxamide (PCN), DAPG and Phenazine,N, N-dimethyl hexadecylamine (DMHDA) (Bangeraand Thomashaw, 1996; Jha et al.,2011; Bakthavatchalu et al., 2012; Khatoon et al., 2020). *Bacillus* strains act as a biocontrol agent for tomato disease and are a resource arsenal for novel antimicrobial discovery, the genomes of 10 *Bacillus* and *Paenibacillus* strains with good antagonistic activity were sequenced via genome mining approaches (Zhou et al., 2021). Several fungal strains have also proven to be a great alternative to chemical pesticides and insecticides (**Table 1.4**). They can act as biocontrol agents (BCA) by different mechanisms such as direct antagonism (hyper parasitism), antibiosis and competition for micronutrients such as iron,

mycoparasitism, hydrolytic enzymes, induced resistance and rhizosphere competence (Tariq et al., 2020). The use of fungal strains has many benefits over the commercial harmful agrochemicals such as no development of resistance in the target, eco-friendly, renewable resource. Additionally, they exhibit a relatively rapid reproductive rate, encompassing both sexual and asexual processes, along with a brief generation time. They display specificity towards their targets. Furthermore, when devoid of a host, fungi possess the capability to endure within the surroundings by transitioning their parasitic behaviour to saprotrophic nourishment, thereby upholding a state of sustainability (Thambugala et al., 2020). Previous research has identified about 300 distinct genera or varieties belonging to 113 genera that functions as BCA against fungal pathogens affecting plants (Thambugala et al., 2020). *Trichoderma harzianum* was the first fungal strain to be officially available as biocontrol agent in the market when it was listed in United States Environmental Protection Agency (EPA) (Gawai, 2018). El-Maraghy et al. (2020), conducted a study to investigate the ability of ISR stimulation by PGPFs *Aspergillus falvus*, *Aspergillus niger*, *Penicillium citrinum*, *Penicillium chrysogenum* and *Trichoderma koningiopsis* in*Triticum aestivum* compared to benzothiadiazole (BTH), a chemical inducer. The study found that treatments with plant growth- promoting fungi (PGPF) led to the over expression of the defensive genes, resulting in fewer diseases symptoms compared to both the BTH and the control group. It is not only during the growing stage of the crop which gets affected by the phytopathogens but also after the crops has been harvested (Tariq et al., 2020). Many fungal strains have been selected and tested for biocontrol agents *in vitro* and in the field condition. Entomopathogenic fungus *Beauveria bassiana* is employed to manage harmful insects, including white flies, thrips, mites, aphids and their different life stages which cause damage to various crop plants. It is utilized as biological control to suppress these pests. Additionally, *Chaetomium*

cupreum is another fungus that offers protection to plants against fungal diseases like rust, early and late blight, leaf spot and as well as stem and tuber rot (Pirttilä et al., 2021). Study investigated by Kang et al. (2020), suggested the potential of endophytic *Klebsiella pneumoniae* as a rich source of herbicidal metabolites and effectiveness of its application to agricultural fields. In the work done by Delshadi et al. (2017), they have concluded that the use of bio-fertilizers, separately or in combination, increased the germination of *B. tomentellus* Boiss.

Table 1.4: List of some commercial biocontrol agents available in India

Biopesticides	Trade Name	Formulation	Targets	References
Trichoderma viride	Bioderma	1.0 % WP	Soil-borne pathogens	Singh et al., 2016
Beauveria bassiana	Myco-Jaal	2.15 % WP, 10 % SC or 1.0 %, 1.15 %	Coffee berry borer, diamond back moth, grasshoppers, white flies, aphids	Singh et al., 2016
Paecilomyces lilacinus	Yorker	1.0 %	White fly	Singh et al., 2016
Verticillium lecanii	Verisoft	1.15 %	White fly, coffee green bug, homopteran pests	Singh et al.,2016
Trichoderma harzianum	Maru sena 1	4g/kg seed	*Fusarium* spp., charcoal rot disease	Mawar et al., 2021
Aspergillus versicolor	Maru Sena 2	-	Soil borne pathogens	Mawar et al., 2021
Bacillus firmus	Maru Sena 3	-	Dry root rot	Mawar et al., 2021
Beauveria bassiana	Boverin	-	To control the Colorado potato beetle and the codling moth	Mishra and Arora, 2016

Note: WP: Wettable powder; SC: Suspension concentrates.

Bioremediation: Bioremediation is the clean-up of hazardous compounds accumulated in nature by using living organisms. Physicochemical technique for remediation of metal polluted soil is highly expensive and time consuming (Dharni et al., 2014). Use of PGPR can be done to remediate the polluted area. The plant growth-promoting rhizobacteria (PGPR) isolated from saline soil can overcome the detrimental effects of salt stress on plant (Sharma et al., 2021). Interactions among metals, microbes and plants have attracted

attention because of the biotechnological potential of microorganisms in mitigation of metal toxicity (Dharni et al. (2014). In the work done by Delshadi et al. (2017), they have concluded that the use of bio-fertilizers separately or in combination, increased the germination of *B. tomentellus* Boiss. Many PGPR are capable of bioremediation by taking up heavy metals, degrading pesticides and herbicides. For instance, *Variovorax boronicumulans*, which produce siderophore, ammonia, hydrogen cyanide and the phytohormone salicylic acid when applied to the soil contaminated with acrylamide, a neurotoxicant and carcinogen in animals, have resulted in complete degradation of the compound in just 4 days (Liu et al., 2013). Another bacteria *Pseudomonas aeruginosa* showed biosurfactant activity by both oil spread method and haemolytic activity (Bakthavatchalu et al., 2012). Therefore, using PGPR to remediate the affected land is a very promising aspect of these microbes.

Volatile organic compounds (VOCs): Microbial volatile organic compounds (mVOCs) are a type of volatile organic compound produced by microorganisms, especially bacteria and fungi, during their metabolism and are designated as lipophilic compounds with a low boiling point, low molecular mass (an average of 300 Da) and high vapor pressure (0.01 kPa) (Chandrasekaran et al., 2022). The VOCs produced by PGPM are heavily involved in improving plant growth and induce systemic resistance (ISR) towards pathogens (Vejan et al., 2016). There is enormous evidence suggesting that VOCs have dual direct and indirect action during plant growth-promoting activities (Santoyo et al., 2019). In rhizosphere, VOCs can induce plant growth directly or indirectly, by restricting the growth of potential phytopathogens (Khatoon et al., 2020). Bacterial species from diverse genera, including *Pseudomonas, Bacillus, Arthrobacter, Stenotrophomonas* and *Serratia* produce VOCs that impact plant growth (Gouda et al., 2018). *Enterobacter cloacae* enhanced the growth of *Arabidopsis thaliana* seedlings under both normal and

salt stress conditions by producing mVOC such as 2,3-butanediol and acetoin (Jana and Yaish, 2020). In one study, *Papiliotrema flavescens* VOCs triggered metabolic alterations, promoted auxin accumulation and distribution in the roots, and coordinated ethylene signalling, thus inhibiting primary root elongation and inducing lateral root formation in *Arabidopsis* (Liu et al., 2024a). VOCs (albuterol and 1,3-propanediole) produced by *B. subtilis* strain SYST2 increase the photosynthesis and the endogenous contents of gibberellin, auxin and cytokinin in tomato plants (Lone et al., 2015). VOCs produced by *Microbacterium aurantiacum* up-regulated 1286 genes which were involved in three biological processes: polysaccharide metabolic, polysaccharide catabolic and carbohydrate metabolic and down-regulated 1088. Out of 1286 up-regulated genes, 190 differentially expressed genes were mainly involved in plant hormone signal transduction, phenylpropyl biosynthesis, plant-pathogen interaction and flavonoid biosynthesis (Gao et al., 2022). These VOCs are species-specific and their emission depends on environmental conditions, such as growth medium, pH, temperature, incubation time and interaction with other microorganisms (Rani et al., 2023).

Induced systemic response (ISR): Infection by microbes e.g. bacteria, fungi, the virus can induce the plant to develop resistance to a future attack called induced systemic resistance and Induced systemic resistance induced by phytopathogens, immunizes plant against broad spectrum pathogens (Kumar and Verma, 2018a). The PGPM also activates plant defence resulting in systemic protection against plant pathogens; a phenomenon termed induced systemic resistance (ISR) (Bakthavatchalu et al., 2012). The whole impact of PGPR-induced elicitation responses in plants, whether, at the biochemical, molecular, or physical level may lead to protection against biotic and abiotic stresses andin a cumulative manner, constitutes the basis of eco-friendly stress management

strategy (Arya et al., 2018). The plant-microbe is known to interact with two major types of systemic resistance including systemic acquired resistance (SAR) and induced systemic resistance (ISR) after pathogen attack (Dixit et al., 2022). systemic acquired resistance (SAR) in plant tissues is induced via salicylic acid (SA) signalling which results in an accumulation of pathogenesis-related proteins (PR proteins), whereas, the exposure of roots to PGPM under influence of jasmonic acid (JA) and ethylene (ET) signalling, activation of NPR1 gene, transcription factors (MYB72 and MYC2) and callose formation induce induced systemic resistance (ISR) (Thomas and Singh, 2020). Strong evidences have shown that PGPM induce ISR. For instances, soybean seeds when coated with *Bacillus simplex* Sneb545 have shown resistance to *Heterodera glycines,* most destructive pathogens of soybean, as a result of induced systemic resistance (ISR) in the plants (Xing et al., 2020). *Trichoderma harzianum* and *Pseudomonas* sp. when applied assingle strains or in combination in cucumber against *Fusarium oxysporum* f. sp. *radicescucumerinum* and in *Arabidopsis thaliana* against *Botrytis cinereal* showed that both applied in combination induced a significantly higher level of resistance in cucumber, whereas in case of later both Ps14 and Tr6 triggered ISR against *B. cinerea* but their combination did not show enhanced effects indication both PGPM activate the same signalling pathway and thus have no enhanced effect in combination (Alizadeh et al., 2013).

Hydrogen cyanide (HCN) production: PGPR producing HCN can be used as both biofertilizer and biopesticides. It is involved in the chelation of metal ions and makes phosphate available to the plants. It is also proven to be an effective biopesticides because of its toxicity against phytopathogens (Singh et al., 2019). The HCN production is associated with bioremediation and as a bio control for growth enhancement and antagonistic activities (Vasant et al., 2023). A number of bacterial species produce

cyanide as a secondary metabolite is produced by bacteria through the metabolic pathway of bacterial cyanogenesis (Sehrawat et al., 2022). In the study conducted by Abd El-Rahman et al. (2019), 6 HCN producing PGPR (*Pseudomonas japonica, Bacillus megaterium, Pseudomonas* sp., *Pseudomonas tolaasii*, *Pseudomonas chlororaphis*, and *Pseudomonas mosselii*) were able to inhibit growth of *Agrobacterium tumefaciens* and affect viability of *Meloidogyne incognita* juveniles *in vitro* in tomato plant.The direct evidence for the role of cyanide in nematode suppression has been presented by Gallagher and Manoil (2001), by using cyanide-deficient bacterial mutants. When compared the killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* which was 100% and the isogenic noncyanogenic mutant who showed 13% in sealed plate assays. However, Genetic restoration of cyanide production in the mutant restored killing to 100% demonstrating the direct role of cyanide in nematode killing. It was reported that glycine is the precursor for cyanide and the process of conversion from glycine to cyanide depends on the presence of oxygen (Wissing, 1974). Even though, many studies have shown cyanogenic bacteria to be affective at reducing the pests and fungal phytopathogens, evidences have also been found indicating the growth suppression of certain plants such as potato, suggest that soil populations of cyanogenic Pseudomonads are an important variable to consider when optimizing growth of crop plants or the suppression of undesirable weeds. (Zdor, 2014).

Heavy metal resistance: In modern agricultural practice, heavy metal toxicity is one of the major abiotic stresses threatening sustainable agriculture, crop productivity and disturbs natural soil microbiota (Kisa et al., 2016). Soils contaminated by heavy metals as a result of mining activities are mostly covered only by sparse herbaceous vegetation with low productivity and species diversity (Baker et al., 2010). The sources of metals in the soil are diverse, including burning of fossil fuels, mining and smelting of metalliferous

ores, municipal wastes, fertilizers, pesticides, sewage sludge amendments, the use of pigments and batteries (Gaur and Adholeya, 2004).The agricultural sector suffers horribly from the increase over time of metal pollution, such as lead (Pb), cadmium (Cd), chromium (Cr), mercury (Hg) and Arsenic (As) causing a significant decrease in plant growth and crop yield (Tirry et al., 2021). These metals are commonly called heavy metals, although this term strictly refers to metallic elements with a specific mass higher than 5 gcm^{-3}, able to form sulphides (Adriano, 1986). It is well known that heavy metals cannot be chemically degraded and need to be physically removed or be immobilized (Kroopnick, 1994). In such circumstances, bioremediation is used as a novel technique which involves living organisms such as microbes for soil restoration (Husna et al., 2022). It is more cost effective and eco-friendly alternative and offers an effective way toremove heavy metals from soil, sediments and water through mechanisms such as bioaccumulation, biomineralization, biosorption and biotransformation, which microorganisms have developed to thrive in a heavy metal-rich environment (Ayangbenro et al., 2017). Compared to traditional chemical and physical remediation methods, this method is not only less expensive, and simpler but also more environmentally friendly (Liu and Tran, 2021). PGPM play a vital role in helping host plants adaptto suboptimal soil conditions and significantly improve phytoremediation efficiency. They achieve this by fostering plant growth, facilitating metal translocation within the plant, modifying metal bioavailability in soil and mitigating metal induced phytotoxicity (Kong and Glick, 2017). For instance, when *Sorghum bicolor* was inoculated with PGPRs (*Burkholderia* sp. and *Pseudomonas* sp.), it exhibited increased growth, Pb accumulation and Zn translocation from root to shoot (Wu et al., 2019). Some of the PGPR with high heavy metal tolerance are *Alcaligenes faecalis, Aeromonas* sp., *Staphylococcus aureus, Pseudomonas aeruginosa, Agrobacterium* sp., *Microbacterium*

schleiferi, Agromyces sp., and *Stenotrophomonas* sp. (Ibrahim et al., 2021; Yadav et al., 2022). Association of *M. sativa* with *Pseudomonas* sp. have shown an efficient biological system for the bioremediation of Cr (VI)-contaminated soils (Tirry et al., 2021). *Pseudomonas monteilii* when inoculated on the plants growing in extreme saline and drought condition shown to improve growth, increased in seedling growth (root length, shoot length, dry weight and fresh weight) (Zhang et al., 2019). Similarly, *Bacillus aryabhattai*, a halo tolerant siderophore producing rhizobacteria could produce a significant amount of siderophore (43%) even under 200mM saline conditions making it a good candidate for being used as biofertilizer in high salinity regions (Sultana et al., 2021). Furthermore, *Pseudomonas monteilii* and *Pseudomonas plecoglossicida* when tested have shown had high MIC values for Cr (VI) making it a good candidate forbiofertilizer in Cr contaminated areas (Dharni et al., 2014). El-Akhdar et al. (2020), in their study have also reported that inoculation of *Lelliottia amnigena* and *Bacillus halotolerans* have alleviate the stress of salt on *Triticum aestivum* L. PGPB employ various mechanisms to promote the phytoremediation process, including boosting plant metal tolerance and altering metal accumulation within plants. These actions collectively contribute to the successful and effective phytoremediation of contaminated environments (Kong and Glick, 2017). According to the study conducted by Guerrieri et al. (2021), tomato seedlings have demonstrated increase in the root length density and diameter class length parameters indicating towards its high IAA production by the inoculation of *Klebsiella variicola*. Fungal strains also aid in the alleviation of metal toxicity in plants (**Table 1.5**). For instance, ectomycorrhizas has been demonstrated in a number of experiments (Jentschke and Godbold, 2000). *Umbelopsis, Pochonia, Pseudogymnoascus, Trichocladium* and *Ilyonectria* have been reported as tolerant taxa to multiple metals (Torres-Cruza et al., 2018). The AMF enhance plant resistance and heavy

metal tolerance, but their influence on plants growing on contaminated medium largely depends on plant species, fungal species, and the type of associated heavy metal (Khalid et al., 2021). *Diversispora spurcum* and *Funneliformis mosseae* cause retention of heavy metals in the underground part of plants and thus have been shown to reduce zinc (Zn), lead (Pb)and cadmium (Cd) contents in the shoot in comparison to the roots in maize plants (Zhan et al., 2018). *Aspergillus* sp. was spotted to yield a strong tolerance towards Cu, Pb, As and Zn in the moderate level of heavy metal contamination and positively correlated with Ni and Cr in the severe level of heavy metal contamination (Lin et al., 2020). Hence, it is proven that microorganisms associated with plant roots may influence heavy metal availability and uptake by plants in the rhizosphere (Edelstein and Ben-Hur, 2017). However, this can only be achieved when the fungus can maintain the growth of its mycelium. Eventually this improved nutrition should lead to a better health and growth of trees associated with the most tolerant isolates (Colpaert, 2008).

Table 1.5: Report on fungi as heavy metal tolerant plant growth promoting fungi

Heavy Metal Tolerant Fungi	Test Plants	Elements	Effect on Plant Growth	References
Piriformospora indica	*Medicago sativa* L.	Cd	Significantly increased biomass and nutrients uptake and minimized the Cd concentration in the shoots.	Sepehri and Khatabi, 2021
Aspergillus welwitschiae	*Glycine max* (L.) Merr.	Cr-VI, As-V	Showed higher root shoot length and fresh/dry weight and strengthened the antioxidant system of the host by increasing enzymatic antioxidants, i.e., catalases (CAT) by 1.58 and 1.11 fold, ascorbic acid oxidase (AAO) by 6.75 and 7.94 fold, peroxidase activity (POD) by 1.12 and 1.37 fold, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) by 1.42 and 1.25 fold at 50 µg/mL of chromate and arsenate.	Husna et al., 2022
Rhizophagus irregularis	*Agrostis capillaries* L.	Pb, Cd, Zn, Cu	Showed generally higher chlorophyll concentrations and	Doubková

			a lower carotenoids/chlorophyll ratio. However, shoot and root dry weight as well as the number of tillers were substantially reduced by increasing substrate contamination.	and Sudová, 2016
Glomus mosseae	*Phaseolus vulgaris* L. and *Triticum aestivum* L.	Zn, Cu, Pb, Cd	Significantly increased root and shoot dry weight, chlorophyll content and total lipid in wheat plants. It also significantly increased root and shoot dry weight, protein content and the activity of antioxidant enzymes in red kidney plants	Rabie, 2005
Trichoderma virens	*Zea mays* L.	Cd, As, Zn, Pb, Cu	Significantly increased the dry biomass of maize roots (64%) and shoots (56%). Chlorophyll, total soluble sugars (reducible and nonreducible), starch, and protein contents increased by 46%, 28%, 30%, and 29%, respectively.	Babu et al., 2014
Penicillium simplicissimum	*Vigna radiata* L.	Cu, Pb	Biosorption and bioaccumulation of metals by live cells reduced Cu and Pb toxicity, observed from good root and (4.00-4.28 cm) and shoot (8.07-8.36 cm) growth of *Vigna radiate.*	Chen et al., 2018
Phialocephala fortinii, Rhizodermea veluwensis, and *Rhizoscyphus* sp.	*Clethra barbinervis*Sieb old & Zucc.	Cu, Zn, Pb.	Enhanced the growth of *C. barbinervis* seedlings, increased K uptake in shoots and reduced the concentrations of Cu, Ni, Zn, Cd, and Pb in roots.	Yamaji et al., 2016
Claroideogloms etunicatum	*Zea mays* L.	La	Significantly increased nutrition uptake (K, P, Ca and Mg content) in shoot of maize by 27.40–441.77% and decreased shoot La concentration by 51.53% in maize, but increased root La concentration by 30.45%.	Hao et al., 2021
Rhizophagus intraradices	*Oryzae sativa* L.	As	The ratios of inorganic/organic As concentrations in rice grains of all cultivars were significantly reduced and involved the transformation of inorganic As into less toxic organic form dimethylarsinic	Li et al., 2016

			acid (DMA) in rice.	
Glomus mosseae	*Sesbania rostrate* Bremek & Oberm., *Sesbani cannabina* (Retz.) Poir., *Medicago sativa* L.	Cu, Zn	Significantly stimulated the formation of root nodules and increased the N and P uptake and decreased the concentration of metals, such as Cu, in the shoots of the three legumes.	Lin et al., 2007
Pseudomonas fluorescence and *Trichoderma* sp.	*Cicer arietinum* L.	Cd	25µg Cd/Kg + PGPR-7 + T4 treatment caused maximum increase in germination percentage (10%), root dry biomass (71.4%) and vigour index (33%), chl-a (38%), chl-b (41%) and carotenoid content (52%)	Syed et al., 2023
Fusarium sp. and *Penicillium* sp.	*Brassica napus* L.	Pb, Cd	Significantly increased the rape biomass and promoted the extraction efficacy of Pb and Cd,	Shi et al., 2017
Gaeumannomyces cylindrosporus	*Zea mays* L.	Pb	Improved efficiency of photosynthesis and enhanced resistance to Pb	Ban et al., 2017
Serendipita Vermifera sp.	*Populus* sp. clone INRA 717-1B4	Cd, Zn, Pb, Cu	Increased root tip number and shoot biomass	Lacercat-Didier et al., 2016
Exophiala pisciphila	*Zea mays* L.	Zn, Pb, Cd	Increased growth, alleviated heavy metal toxicity	Li et al., 2011
Neotyphodium coenophialum	*Lolium arundinaceum* (Schreb.) Darbysh.	Cd	Increased tiller number and biomass, enhanced Cd accumulation and transport from root to shoot	Ren et al., 2011
Serendipita indica	*Ocimum basilicum* L.	Pb, Cu	Increased root and shoot dry weights, reduced metal contents in shoot	Sabra et al., 2018
Mucor sp.	*Brassica campestris* L.	Cr, Zn, Mn, Cu, Co	Increased resistance to multi-metal contamination	Zahoor et al.,2017
Penicillim oxalicum and Fusarium solani	*Triticum aestivum* L.	Cu, Cd	Positively influenced the germination and growth of wheat and increased root and shoot length of the plants.	Akbar et al., 2022

Note: Cd: Cadmium; Cr: Chromium; As: Arsenic; Pb: Lead; Zn: Zinc; Cu: Copper; La: anthanum; Mn: Manganese; Co: Cobalt.

Wild *Musa* species of Nagaland

Northeast region of India is endowed with large number of wild *Musa* species. Nagaland along with other north-eastern states is one of the regions where different types of wild and cultivated species of banana are being harboured. It lies between 26°00ˈNorth latitude and 94°20ˈ East longitude. So far 12 wild banana species have been reported till date from Nagaland (Deb et al., 2023; Dey et al., 2014; Gogoi and Borah, 2013; Gogoi et al., 2013; Joe et al., 2014; Joe et al., 2013) is which are *Musa aurantiaca* G. mann ex Baker, *Musa balbisiana* Colla, *Musa cheesmanii* N. W. Simmonds, *Musa flaviflora* N.W. Simmonds, *Musa itinerans* Cheesman, *Musa manii*H. Wendl. ex Baker, *Musa markkuana* (M. Sabu, A. Joe & Sreejith) Hareesh, A. Joe & M. Sabu, *Musa markkui* Gogoi & Borah, *Musa nagalandiana* S. Dey & Gogoi, *Musa nagesium* Prain, *Musa sikkimensis* Kurz and *Musa velutina* H. Wendl. & Drude.

In the wild, *Musa* sp. grows robustly without even any proper care, also they are found to be less affected by the pest and the diseases as compared to its cultivated counterparts. One of the factors responsible for this healthy growth is the interactions of microorganisms found in the rhizospheric soil of the plant. Banana is one of the easily accessible fruit because of its cheap price. It is counted as one of the most important horticultural crops. However, it requires high amount of nutrients (Mia et al., 2010). The wild *Musa* spp. grows healthy without any care compared to cultivated ones. The healthy growth is probably due to the close associations of different types of growth promoting microbes in the rhizospheric region, besides other factors. Due to the above-mentioned reasons, it is of interest to explore plant growth microorganisms associated with the rhizosphere of this group of plant to understand the PGP ability and test them in other crop plants to develop suitable bioinoculant formulations. For the purpose, five crop plants *viz., Cicer arietinum* L., *Phaseo vulgaris* L., *Helianthus annuus* L., *Brassica*

juncea (L.) Czern. and *Beta vulgaris* L. were considered as test plants. PGPR isolates were tested on *C. arietinum* L.*, P. vulgaris* L., *Brassica juncea* (L.) Czern.*,* and *H. annuus*L.*,* whereas*,* PGPF isolates were testedon *P. vulgaris* L. and *B. vulgaris* L. Brief report on the test plants are given below:

1. *Cicer arietinum* L. is an important pulse crop grown and consumed all over the world. It is a good source of carbohydrates and protein, and protein quality is considered to be better than other pulses (Jukanti et al., 2012). However, abiotic stresses such as drought, salinity, water logging, high temperature and chilling frequently limit the growth and productivity of chickpeas (Jha et al., 2014). Since it is one of the most demanded cereals, farmers tend to apply large quantities of artificial fertilizers for its production, disregarding the potentially hazardous effects of it on the environment.

2. *Brassica juncea* (L.) Czern. has gained recognition as one of the most effective plants for accumulating heavy metals and aiding in soil phytoremediation. According to a study conducted by Rizwan et al. (2018), *Brassica* sp. exhibits tolerance to cadmium (Cd) by stimulating its antioxidant defence system, compartmentalizing the heavy metal into metabolically inactive parts, using Osmo-protectants and accumulating total amino-acids. In a hydroponic culture experiment by Ishikawa et al. (2006), also when exposed to 150µg/ml of Cd/Cu contamination the growth of *B. juncea* growth was less affected as compared to the treatment which was not given PGPR dose (Tatung and Deb, 2024)

3. *Helianthus annuus* L. is considered a hyper-accumulator with the ability to tolerate various heavy metals in the soil and remove them from the ecosystem. Its efficacy to remediate Cd and Pb was studied and proven to be favourable to accumulate the heavy metal from the soil (Alaboudi et al., 2018). When given Cd/Cu stress (µg/ml) *H. annuus* growth was found to be less affected when given PGPR consortia treatment as compared to the control treatment (Tatung and Deb, 2024). It is also an important oilseed crop currently cultivated throughout the world and contains mineral elements and

phytochemicals such as dietary fibre, manganese, vitamins, tocopherols, phytosterols, triterpene glycosides, α-tocopherol, glutathione reductase, flavonoids, phenolic acids, carotenoids, peptides, chlorogenic acid, caffeic acid, alkaloids, tannins, saponins and these compounds contribute to their functional and nutraceutical development (Adeleke and Babalola, 2020).

4. *Phaseolus vulgaris* L. is one of the most important food legumes for direct consumption in the world and over 12 million tons of dry beans are produced annually world-wide, with a total production value of US million $5717 (FAO). It is the most widely produced grain legume and ranked third after soybean and groundnut for oilseed and grain legumes combined (Myers and Kmiecik, 2017). Besides providing nutrients such as multifaceted carbohydrates, elevated proteins, dietary fibre, minerals and vitamins, these also contain rich variety of polyphenolic compounds with prospective health benefits (Hayat et al., 2014). It is consumed mainly by its dry grains, peel beans (seeds in physiological maturity) and green pods are consumed as vegetables. The seeds can be used in multiple ways, such as whole unprocessed seeds, as part of mixes, canned goods, or as a substitute for gluten-free wheat flour (Rodríguez et al., 2022).

5. *Beta vulgaris* L. is a potential cash crop of immense commercial importance. The crop finds much industrial utilization owing to its rich carbohydrate storage reserves (Mukherjee and Gantait, 2023). Consumption of beetroot reduce the risk of obesity, diabetes mellitus, cardiovascular disease, demonstrates benefits for cancer treatment and protection against heart disease (Nikan and Manayi, 2019).

Considering the above factors for my Doctoral Research, the present study was undertaken on isolation of PGPR and PGPF strains from the *Musa* rhizosphere and screening for plant growth promoting traits. After the potential PGPM were obtained the effects of its inoculation was observed in plants which include *C. arietinum* L., *B. juncea* (L.) Czern. *P. vulgaris* L., *H. annuus* L., and *B. vulgaris* L. I have worked on the following objectives:

I. Collection of the *Musa* rhizospheric soil sample from three districts of the Nagaland *viz*., Mokokchung, Zunheboto, and Wokha.

II. Physicochemical analysis of the soil sample collected.

III. Isolation of the plant growth promoting microorganisms (PGPM) from *Musa* rhizosphere and their biochemical characterization.

IV. Screening for growth promoting traits which includes phosphate solubilisation, ammonia production, amylase production, Indole 3 acetic acid production, siderophore production, heavy metal stress tolerance, and salinity stress tolerance.

V. Molecular characterization of the PGPM isolates.

VI. Effects of PGPM inoculation on growth traits of test plants.

Chapter – 2

Isolation and Biochemical Analysis of Bacterial Isolates from Wild *Musa* Rhizosphere

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Introduction

Bacteria are a dominant group among the soil microorganism community, approximately one gram of soil contains 108-109 bacteria, 106-108 archaea, 107-108 actinomycetes, 105-106 fungi, 103-106 algae, 103-105 protozoa and 10 nematodes (Rughöft et al., 2016). Bacteria that positively affect plant growth are categorized as plant growth-promoting bacteria (PGPB), often interchangeably called plant health-promoting bacteria (PHPB). They can be applied as biofertilizers and biocontrol agents in agriculture to reduce the use of chemical fertilizers and pesticides (Tatung and Deb, 2021). They enhance the plant's growth and development by either direct (phosphate solubilisation, IAA production, nitrogen fixation) or indirect method (biocontrol activity, induced systemic resistance, ammonia production) (Tatung and Deb, 2023). PGPR are hence considered to be a great alternative to synthetic fertilizers and agrochemicals and

have been commercialized by many companies (Backer et al., 2018). These bacteria have demonstrated their ability to survive in harsh environments like heavy metal polluted areas and have aided plants in adapting to such conditions (Tatung and Deb, 2021, 2023). PGPR are widely distributed in nature and belong to different genera of bacteria, such as *Bacillus, Pseudomonas, Azospirillum, Azotobacter, Rhizobium* and many others. *Pseudomonas plecoglossicida,* when inoculated on rose-scented geranium increased dry weight of the shoot (44%), root (48%), chlorophyll (31%) and essential oil yield (43%) (Dharni et al., 2014). Hence, utilizing PGPR as biofertilizers in adaptive agriculture and to enhance the salinity tolerance of non-halophytic crops, as well as for heavy metal phytoremediation in heavy metal contaminated soils, has been proposed (Dharni et al., 2014; Sharma et al., 2021; Idaszkin et al., 2021). PGPR play a vital role in helping host plants adapt to suboptimal soil conditions and significantly improve phytoremediation efficiency. They achieve this by fostering plant growth, facilitating metal translocation within the plant, modifying metal bioavailability in soil and mitigating metal induced phytotoxicity (Kong and Glick, 2017). For instance, when *Sorghum bicolor* was inoculated with PGPRs (*Burkholderia* sp. and *Pseudomonas* sp.), it exhibited increased growth, Pb accumulation, and Zn translocation from root to shoot (Wu et al., 2019). Some of the PGPR with high heavy metal tolerance are *Alcaligenes faecalis, Aeromonas* sp., *Staphylococcus aureus, Pseudomonas aeruginosa, Agrobacterium* sp., *Microbacterium schleiferi, Agromyces* sp., and *Stenotrophomonas* sp. (Ibrahim et al., 2021; Yadav et al., 2022). Inoculation of *M. sativa* with *Pseudomonas* sp. exhibited increase in root (95.4%) and shoot dry weight (97.6%) along with improved chlorophyll content in the presence of Cr (VI)-contamination as compared to non-inoculated treatment (Tirry et al., 2021).

Considering the above-mentioned facts, in this chapter, study was undertaken to isolate the rhizospheric bacterial isolates from the rhizosphere of *Musa* plants and their biochemical characteristics was studied with the following objectives:

Objectives

- Collection of rhizospheric soil sample from *Musa* plants from three districts of Nagaland and physicochemical analysis.
- Isolation of rhizobacterial strains using serial dilution and spread plate technique.
- Morphological characterization of the purified bacterial strains.
- Biochemical analysis of the bacterial strains such as gram staining, starch hydrolysis test, ammonia production test, citrate utilization test, methyl red test, catalase test.

Material and Methods

Rhizospheric soil sample collection

The rhizospheric soil samples were collected from three different districts of Nagaland (Mokokchung, Wokha, and Zunheboto) (**Figure 2.1**). Soil was dugout from 5-30 cm depth with intact roots from the banana rhizosphere. Roots were shaken a little bit and the soil adhering to the roots was then collected in a sterile polythene bag. The sample collected was used immediately or kept in a refrigerator at 4°C for further study. Physicochemical analysis of soil for the following study were analysed for organic carbon using Walkley and Black (1934), available nitrogen using Modified Kjeldahl method (Goyal et al., 2022), available phosphorus via Bray's method for acidic soil (Gutiérrez Boem et al., 2011), available potassium (Hanway and Heidel, 1952) and pH of the soil (Systronics μpH digital meter). The moisture content of the soil was done by oven dry method (**Table 2.1**).

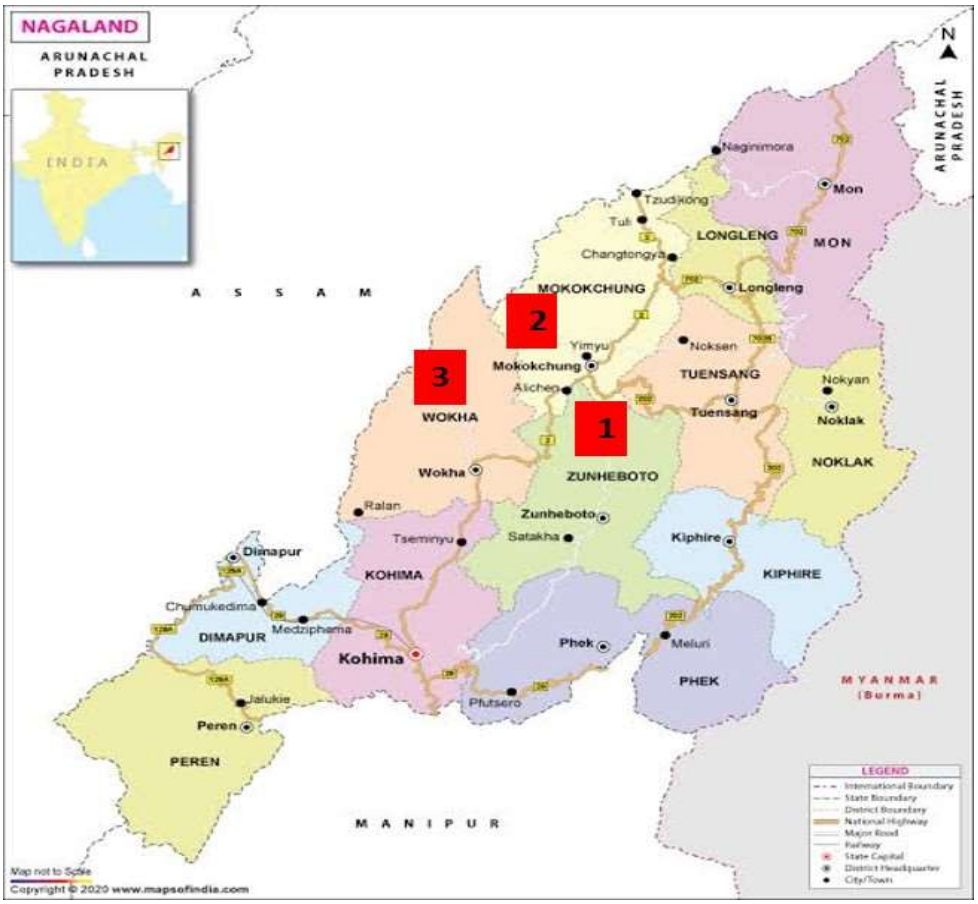

Figure 2.1: Map of Nagaland showing the three study sites; Zunheboto district (1), Mokokchung district (2) and Wokha district (3). (Source: https://www.mapsofIndia.com/maps/nagaland/nagaland.htm).

Serial dilution technique

The isolation of bacteria was conducted following a standard microbiological procedure using the serial dilution technique (Koch, 1883). For serial dilution technique, one gram of soil was taken which was mixed with 9ml of sterilized distilled water. Then 1ml of suspension was taken from the first test tube ($10^{-1)}$ with the help of pipette and

transferred to the second test tube (10^{-2}) and mixed well. Next 1ml of suspension was taken from second tube and transferred to 10^{-3} and then 10^{-4} to 10^{-5}. This was continued for up to 10^{-6}. After dilutions were prepared 40 µl was transferred to the dilution nutrient agar plate and spread using a sterile glass L rod. Plates were sealed with Parafilm and incubated at 28±2ºC for 24-48 h (**Figure 2.2).**

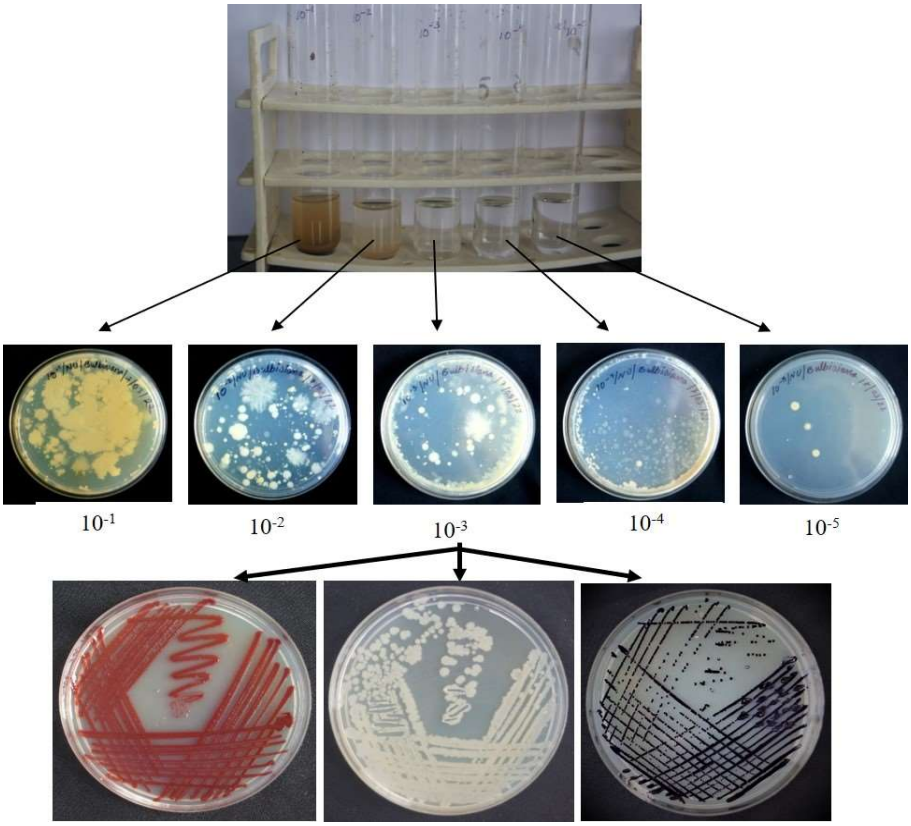

Figure 2.2: Isolation of rhizobacterial strains from *Musa* (*M. itinerans, M. balbisiana*, *M. flaviflora*, and *M. velutina*) rhizospheric soil sample collected from three districts of Nagaland.

Isolation of rhizobacterial strains

The bacterial isolates were carefully chosen and purified based on their colony morphology which included characteristics such as colony colour, transparency, elevation, margin and colony form (**Figure 2.3 and Table 2.2**). After selection isolates were purified by streaking single colony on single petri plates. After streaking till 3rd

generation, the purified isolates were stored as slants and glycerol stocks for further studies. Biochemical activities of bacterial isolates *viz.*, gram staining, motility test, catalase test, citrate utilization test, methyl red, starch hydrolysis test and sugar fermentation test (**Table 2.3)** were done for bacterial isolates

Biochemical analysis of the bacterial isolates

Gram staining

For gram staining a glass slide was taken and cleaned with 75% ethyl alcohol. The slide was marked with the isolates name to identify the slide. After that a drop of sterile water was placed on the slide with the help of a dropper. With a sterilized toothpick a bacterial colony was taken from the bacterial culture and smeared on the slide (on the water drop). It was then allowed to air dry and after that it was heat fixed by passing over the flame 3-4 times. Next step was to pour crystal violet on the slide and keeping it for about 30 sec. After 30 sec, slide was rinsed with sterile water in such a way that most of the dye gets off. After that gram iodine was poured and kept for 1 min. Then, washed with 95% alcohol and rinsed with sterile water. Finally, the slide was flooded with the counter stain, 'Safranin' and kept for 30 sec and rinsed with sterile water. Once the slide was ready, a cover slip was placed on it, few drops of immersion oil was placed on top of the cover slip and examined under microscope [**Figure 2.4 (A-iand A-ii)**]. Gram-positive isolates gave purple colour cells, while, Gram-negative isolates gave red colour.

Motility test

With a sterilized toothpick, a young culture growing on agar medium was taken and stabbed to a semisolid medium (composition g/l; Peptone from casein 20.0; peptone from meat 6.6; ammonium iron (II) citrate 0.2; sodium thiosulfate 0.2; agar-agar 3.0.), down the centre of the tube to about half the depth of the medium. It was then incubated at 28±2º C for 24 to 48 h. Non-motile bacteria gave growths that were confined to the

stab-line, with sharp defined margins and left the surrounding medium clearly transparent. Motile bacteria gave diffuse, hazy growths that spread throughout the medium rendering it slightly opaque [**Figure 2.4 (B-i and B-ii)].**

Starch hydrolysis

For starch hydrolysis test, a single colony was taken and streaked inoculated onto the medium (Media composition: Beef extract 1.5 gL^{-1}; NaCl 5.0 gL^{-1}; Yeast extract 1.5 gL^{-1}; Peptic digest of animal tissue 5.0 gL^{-1}; Starch soluble 2.0 gL^{-1}; Agar 15 gL^{-1}) using a sterile toothpick. The culture plates were then incubated for 48 h at 28±2ºC. Following incubation, the surface of the plates was flooded with iodine solution (Composition gL^{-1}; Iodine: 12.5g, Potassium iodide: 60g, distilled water: 1 L) with a dropper for 30 sec. Excess iodine was discarded and the bacterial growth was examined for the clear zone around the line of the culture. A clear halo zone around the line of growth after addition of iodine solution indicated that the organisms have hydrolysed starch, while a blue, purpleor black coloration of the medium (depending on the concentration of the iodine) indicated negative results [**Figure 2.4 (C-i and C-ii)].**

Catalase test

For catalase test, a cleaned glass slide was taken and wiped with 75% alcohol (v/v). After that small amount of bacterial colony was taken using a sterilized toothpick and placed onto the microscope slide. However, during this process no agar must be picked up with the colony. After the colony was taken and placed on the glass slide a drop of 3% hydrogen peroxide (H_2O_2) was poured onto the organisms by using a dropper. The formation of bubbles indicated the positive result for catalase test [**Figure 2.4 (D-i and D-ii)].**

Methyl red test

For methyl red test, MRVP broth was prepared and inoculated with pure culture of the bacteria. Cultures were incubated at 28±2°C for a minimum of 48 h in ambient air. After incubation, 5-6 drops of methyl red reagent per 5 ml of broth was added and change in the Colour was observed. Change in Colour to bright red was considered as positive, while, negative result gave yellow colour [**Figure 2.4 (E-i and E-ii)].**

Citrate utilization test

For citrate utilization test, all the compositions of the media were taken in a conical flask with distil water were added to it ($(NH_4)H_2PO_4$ ($1.0gL^{-1}$); $MgSO_4$ ($0.2gL^{-1}$); K_2HPO_4 ($1.0gL^{-1}$); $Na_3C_6H_5O_72H_2O$ ($2.0gL^{-1}$); NaCl ($5.0gL^{-1}$); Bromothymol blue ($0.008gL^{-1}$); Agar ($15gL^{-1}$). The solution was heated to bring it to a boiling point in order to dissolve the medium completely. The dissolved media was then dispensed into tubes and sterilized in an autoclave at 121 psi for 15 min. Once the autoclaving process was complete, the tubes were taken out and cooled at a slanted position. A well isolated colony was then taken from 18-24 h culture with a sterile toothpick. The citrate agar tubes were inoculated by streaking the surface of the slant. The cap of the test tube was left loosened to ensure adequate aeration and then incubated aerobically at 28±2°C for up to 4 days. The test tubes were examined daily for 4 days before discarding the result as a negative. The change in colour, if present, was observed. A positive result was demonstrated by growth with a colour change from green to blue along the slant. No change in colour indicated negative result [**Figure 2.4 (F-i, F-ii, and F-iii)].**

Carbohydrate fermentation test

Phenol red carbohydrate broth (Composition gL^{-1}; peptone: 10g, beef extract: 3g, sodium chloride: 5g, phenol red indicator: 0.08g, Sugar: 5g, distilled water: 1L) was prepared and poured into test tubes. Broth was then autoclaved at 121°C for 10 min to sterilize as these carbohydrates are subject to breakdown by autoclaving. The prepared

broth medium was light red in colour and the final *pH* was adjusted to 7.4±0.2. The preferred carbohydrate concentration was 1%. Using a sterilized toothpick, a single colony of the test bacteria was inoculated in each test tube and incubated at 28±2^{o}C for 18-24 h. Culture was kept for longer periods to confirm the result. Post incubation cultures turned yellow by reacting with the phenol red indicator indicated that there is drop in the *pH* because due to the production of acid by the fermentation of the carbohydrate (sugar) present in the medium. While, the tube containing medium that remained red, indicating the bacteria cannot ferment that particular carbohydrate source present in the medium [**Figure 2.4 (G-i, G-ii, and G-iii**)].

Statistical Analysis

The SPSS software was used for statistical analysis of the experimental data. All the reported results are the mean of the three replicates and deviations were calculated as the standard error of the mean (SEM).

Results

Physicochemical analysis of rhizospheric soil sample collected

Soil samples from the rhizosphere were collected from three districts in Nagaland: Mokokchung, Zunheboto, and Wokha (**Table 2.1**). In Wokha, samples were gathered from four distinct locations: SITE 1 (26.281483N, 94.374400E), SITE 2 (26.287055N, 94.364751E), SITE 3 (26.239112N, 94.315846E) and SITE 4 (26.215632N, 94.305030E). Mokokchung contributed samples from nine locations: SITE 1 (26.4828164N, 94.3913688E), SITE 2 (26.4658641N, 94.3801225E), SITE 3 (26.4861618N, 94.3541044E), SITE 4 (26.43483N, 94.43353E), SITE 5 (26. 43383N, 94.43317E), SITE 6 (26.48441N, 94.34359E), SITE 7 (26.4861618N, 94.3541044E), SITE 8 (26.4658641N, 94.3801225E) and SITE 9 (26.48447N, 94.34347E). Zunheboto provided samples from three locations: SITE 1 (26.20469N, 94.48372E), SITE 2

(26.20717N, 94.48594E), and SITE 3 (26.23487N, 94.28033E). The *pH*, temperature and moisture content of the soil samples were measured immediately upon bringing to the laboratory. The *pH* values ranged from the lowest recorded at 4.38±0.42 to the highest at 6.01±0.07. Among the soil samples collected from Wokha, SITE 4 exhibited the most acidic pH (5.02±0.2), followed by SITE 3 (5.55±0.02), SITE 1 (5.70±0.03) and SITE 2 (6.01±0.07). In Mokokchung, the most acidic samples were found at SITE 8 (4.38±0.42), followed by SITE 9 (4.40±0.23), SITE 1 (4.50±0.09), SITE 4 (4.57±0.08), SITE 7 (4.79±0.18), SITE 2 (4.79±0.23), SITE 5 (4.89±0.34), SITE 3 (4.99±0.12), and SITE 6 (5.04±0.12). In Zunheboto, the most acidic soil was observed at SITE 1 (4.78±0.09), followed by SITE 3 (4.94±0.45) and SITE 2 (4.97±0.07).

The temperature of the soil samples varied, ranging from 26.08±0.34ºC in Mokokchung to 30.58±1.78ºC in Wokha. In Wokha, temperatures were recorded as follows: SITE 1 (27.98±1.34ºC), SITE 2 (28.76±2.76ºC), SITE 3 (28.76±2.76ºC) and SITE 4 (29.76±0.98ºC). Mokokchung's soil samples exhibited temperatures at SITE 1 (27.65±1.45ºC), SITE 2 (27.65±0.99ºC), SITE 3 (28.98±2.52ºC), SITE 4 (26.08±0.34ºC), SITE 5 (26.18±0.98ºC), SITE 6 (29.89±0.99ºC), SITE 7 (28.56±0.56 ºC), SITE 8 (28.78±1.56ºC) and SITE 9 (28.78±1.56ºC). Soil samples from Zunheboto showed temperatures at SITE 1 (27.19±1.9ºC), SITE 2 (27.30±2.5 ºC) and SITE 3 (26.20±3.54ºC).

The moisture content of soil samples from Wokha were as follows: SITE 1 (39.02±1.09), SITE 2 (39.49±0.99), SITE 3 (40.01±1.04) and SITE 4 (38.09±0.01). In Mokokchung, the moisture content varied across SITE 1 (35.78±1.34), SITE 2 (34.65±1.45), SITE 3 (40.45±2.87), SITE 4 (40.24±2.21), SITE 5 (40.27±1.1), SITE 6 (37.56±0.99), SITE 7 (0.23±0.89), SITE 8 (0.23±0.99) and SITE 9 (0.24±1.32). Soil

samples from Zunheboto exhibited moisture content at SITE 1 (40.23±3.89), SITE 2 (40.21±2.66) and SITE 3 (40.26±3.01).

For further biochemical analysis such as available nitrogen, available phosphorus, available potassium, organic carbon, and soil electrical conductivity, the soil samples were dried and analyzed. In Wokha, the available nitrogen content was recorded as follows: SITE 1 had 170.03±1.23 Kg/ha, SITE 2 had 176.05±2.23 Kg/ha, SITE 3 had 169.03±1.9 Kg/ha and SITE 4 had 169.09±1.98 Kg/ha. In Mokokchung, the values for available nitrogen were as follows: SITE 1 had 198.67±1.89 Kg/ha, SITE 2 had 197.59±0.54 Kg/ha, SITE 3 had 212.78±0.98 Kg/ha, SITE 4 had 200.54±1.45 Kg/ha, SITE 5 had 200.64±1.76 Kg/ha, SITE 6 had 163.02±0.77 Kg/ha, SITE 7 had 163.02±0.56 Kg/ha, SITE 8 had 163.02±1.89. For the soil samples from Zunheboto, the available nitrogen content was found to be: SITE 1 had 212.78±3.56 Kg/ha, SITE 2 had 213.18±0.45 Kg/ha and SITE 3 had 413.82±5.09 Kg/ha.

Available phosphorus of soil samples from Wokha were 3.45±1.23Kg/ha (SITE 1), 3.23±0.22 Kg/ha (SITE 2), 3.36±0.94 Kg/ha (SITE 3) and 3.45±1.35 Kg/ha (SITE 4) and from Mokokchung district values were 9.67±2.00 Kg/ha (SITE 1), 9.89±4.09 Kg/ha (SITE 2), 10.99±1.34 Kg/ha (SITE 3), 10.87±1.99 Kg/ha (SITE 4), 10.97±0.67 Kg/ha (SITE 5), 19.64±0.99 Kg/ha (SITE 6), 28.31±3.89 Kg/ha (SITE 7), 3.31±3.33 Kg/ha (SITE 8) and 3.29±0.78 Kg/ha (SITE 9). For Zunheboto district values were 36.56±0.88 Kg/ha (SITE 1), 35.71±1.67 Kg/ha (SITE 2) and 9.63±3.21 Kg/ha (SITE 3). Available potassium form soil samples collected from Wokha were 76.03±1.11 Kg/ha (SITE 1), 77.01±3.67 Kg/ha (SITE 2), 69.01±2.89 Kg/ha (SITE 3) and 67.05±1.45 Kg/ha (SITE 4) and for Mokokchung districts soil samples values were 490.12±4.76 Kg/ha (SITE 1), 510.05±6.1 Kg/ha (SITE 2), 523.02±3.78 Kg/ha (SITE 3), 520.01±2.99 Kg/ha (SITE 4), 527.05±3.54 Kg/ha (SITE 5), 611.595±2.00 Kg/ha (SITE 6), 273.984±1.09 Kg/ha (SITE

7), 78.01±0.99 Kg/ha (SITE 8) and 77.03±0.67 Kg/ha (site 9). In case of Zunheboto values were 657.02±3.88 Kg/ha (SITE1), 645.01±2.78 Kg/ha (SITE 2) and 361.984±2.43 Kg/ha (SITE 3). Organic carbon content of the soil samples from Wokha were 0.24±0.09 (SITE 1), 0.35±0.45 (SITE 2), 0.32±0.22 (SITE 3) and 0.25±0.01 (SITE 4) and from Mokokchung district values were 0.65±0.01 (SITE 1), 0.63±0.67 (SITE 2), 0.63±0.22 (SITE 3), 0.65±0.12 (SITE 4), 0.67±0.23 (SITE 5), 0.43±1.23 (SITE 6), 0.23±0.89 (SITE 7), 0.23±0.99 (SITE 8) and 0.24±1.32 (SITE 9). For soil sample from Zunheboto district values were 0.67±1.56 (SITE 1), 0.64±0.07 (SITE 2) and 0.71±0.02 (SITE 3).

The Wokha district soil samples had soil electrical conductivity values of 0.087±0.01 dS/mat SITE 1, 0.078±0.01 dS/mat SITE 2, 0.076±0.02 dS/mat SITE 3 and 0.079±0.01 dS/mat SITE 4. SITE 1 (0.032±0.01dS/m), SITE 2 (0.034±0.01dS/m), SITE 3 (0.032±0.02dS/m), SITE 4 (0.034±0.03dS/m), SITE 5 (0.035±0.02dS/m), SITE 6 (0.033±0.01dS/m), SITE 7 (0.025±0.02dS/m) and SITE 9 (0.026±0.01dS/m) exhibited the highest electrical conductivity among the soil samples from Mokokchung. The EC values of the soil samples from Zunheboto were 0.087±0.02 dS/m (SITE 1), 0.089±0.03 dS/m (SITE 2) and 0.682±0.01 dS/m (SITE 3).

Table 2.1: Physicochemical analysis of rhizospheric sol sample

District	Sites	Coordinates	pH± SE*	Temp. (°C) ± SE*	Available nitrogen (Kg/ha) ± SE*	Available phosphorus (Kg/ha) ± SE*	Available potassium (Kg/ha) ± SE*
Wokha	SITE 1	26.281483N, 94.374400E	5.70±0.03	27.98±1.34	170.03±1.23	3.45±1.23	76.03±1.11
	SITE 2	26.287055N, 94.364751E	6.01±0.07	28.76±2.76	176.05±2.23	3.23±0.22	77.01±3.67
	SITE 3	26.239112N, 94.315846E	5.55±0.02	30.58±1.78	169.03±1.9	3.36±0.94	69.01±2.89
	SITE 4	26.215632N, 94.305030E	5.02±0.2	29.76±0.98	169.09±1.98	3.45±1.35	67.05±1.45
Mokokchung	SITE 1	26.4828164N, 94.3913688E	4.50±0.09	27.65±1.45	198.67±1.89	9.67±2.00	490.12±4.76
	SITE 2	26.4658641N, 94.3801225E	4.79±0.23	27.65±0.99	197.59±0.54	9.89±4.09	510.05±6.1
	SITE 3	26.4861618N, 94.3541044E	4.99±0.12	28.98±2.52	212.78±0.98	10.99±1.34	523.02±3.78
	SITE 1	26.43483N, 94.43353E	4.57±0.08	26.08±0.34	200.54±1.45	10.87±1.99	520.01±2.99

	SITE 2	26.43383N, 94.43317 E	4.89±0.34	26.18 ±0.98	200.64±1.76	10.97±0.67	527.05±3.54	0.67±0.23	40.27±1.1	0.035±0.02
	SITE 6	26.48441N, 94.34359 E	5.04±0.12	29.89±0.99	163.02±0.77	19.64±0.99	611.595±2.00	0.43±1.23	37.56± 0.99	0.033±0.01
	SITE 7	26.4861618 N, 94.3541044 E	4.79±0.18	28.56±0.56	163.02±0.56	28.31±3.89	273.984±1.09	0.23±0.89	34.67±2.21	0.033±0.01
	SITE 8	26.4658641 N, 94.3801225 E	4.38±0.42	28.78±1.56	163.02±1.89	3.31±3.33	78.01±0.99	0.23±0.99	33.78±2.34	0.025±0.02
	SITE 9	26.48447N, 94.34347 E	4.40±0.23	27.78±1.87	164.01±3.98	3.29±0.78	77.03±0.67	0.24±1.32	34.98±1.76	0.026±0.01
Zunheboto	SITE 1	26.20469N, 94.48372E	4.78±0.09	27.19±1.9	212.78±3.56	36.56±0.88	657.02±3.88	0.67±1.56	40.23±3.89	0.087±0.02
	SITE 2	26.20717N, 94.48594 E	4.97±0.07	27.30±2.5	213.18±0.45	35.71±1.67	645.01±2.78	0.64±0.07	40.21±2.66	0.089±0.03
	SITE 3	26.23487N,94.28033ˈE	4.94±0.45	26.20±3.54	413.82± 5.09	9.63±3.21	361.984±2.43	0.71±0.02	40.26±3.01	0.682±0.01

* ±SE: Standard error from mean.

Isolation of the bacterial isolates

A total of 136 culturable bacterial isolates were obtained and purified. After purification, all the isolates were assigned the strain numbers and preserved in 20% glycerol at -80°C. Isolates were also stored as slants of pure culture and stored at 4°C.

Morphological characteristics of the bacterial isolates

Once the mixed colony was obtained by using serial dilution and spread plate technique, isolation of the pure cultures was obtained. For isolation, Bacterial strains were selected based on distinct colony size, shape, colour, margin and elevation of colonies. Morphological characteristics of bacterial isolates have been documented in **Table 2.2 and Figure 2.3**. It showed that, the isolates had varied colony colours most common being off-white with 99 isolates (LUMB1, LUMB3, LUMB4, LUMB5LUMB6, LUMB7, LUMB8, LUMB9, LUMB10, LUMITI1, LUMITI2, LUMITI3, LUMITI6, LUMITI7, LUMBB1, LUMBB3, LUMBB4, LUMBB6, LUMBB7, LUMBB8, LUMBB9, LBS12A, LBS36, LBS25B, LBS44A, LBS44B, 5E20, 5E8, 25E12, 5E24, 5E5, 25R23, 5R1, 5E22, 5R16, EZ11, E24,25E18, 5R15, 5R12, 25EZ1, 5E9, 5E28, 25E16, 25R10, RZ27, 25R2, EZ30, 5R9, 5R10, 25Z13, 25E22, 25E5, EZ27, 25EZ2, 25E20, 5E11, 5E3, 25EZ6, 25EZ17, RZ5, RZ20, 5EZ13, 5R3, 25R1, 25E3, TSU2(4), TSU1(5), TSU4, TSU8, TSU6, TSU7, TSU11, TSU12, TSU9, TSU2(1), TSU3(7), TSU3(6), TUL3, TUL4, TUL5, TUL6, TUL8, TUL9, TUL10, TUL11, TUL12, 5NC8, 5NC10, 5NC14, 25NC8, 5NC6, 25NC12, 25NC9, 25NC3, 5NC17, 25NC12, 5NC1, 5NC20 and NC7), ten isolates had white colony (LUMITI4, RZ7, TSU2(2), TSU3(2), TSU3(4), TUL1, TUL2, 5NC13, 25NC10 and 5NC26), two isolates had purple colony (NC11 and LUMB2), twenty-two isolates had yellowish colony (LBS11, LBS18, LBS25A, LBS16, LBS21, LBS13, LBS14, LBS29, LBS23, LBS7, LBS4, LBS31, LBS15, LBS19, LBS12, LBS17, LBS22, 25E10, 5R5, RZ23 and TSU3), two isolates had dark yellow colony (LUMITI5 and LUMBB2), one isolate had red colour (TSU1).

Additionally, various types of colony margin was shown with seventy-four isolates having entire margin (LUMB1, LUMB2, LUMB3, LUMB5, LUMB6, LUMB8, LUMB9, LUMITI3, LUMITI4, LUMITI5, LUMBB2, LUMBB3, LUMBB4, LUMBB7, LUMBB9, LBS12A, LBS36, LBS16, 5E20,5E8, 25E12, 5E24, 5E5, 5R1, 5R5, 5E22, 25E18, 5R15, 5R12, 25EZ1, RZ23, 5E9, 5E28, RZ27, EZ30, 5R9, 5R10, 25Z13, 25E22, 25E5, EZ27, 25EZ2, 5E11, 5E3, 25EZ6, 25EZ17, 25R1, TSU1, TSU2(4), TSU6, TSU7, TSU11, TSU12, TSU9, TSU2(1), TSU3(7) TSU3(6), TUL3, TUL4, TUL5, TUL6, TUL8, TUL10, TUL11, NC11, 5NC10, 5NC14, 25NC8, 25NC12, 25NC9, 25NC3, 5NC17, 25NC12 and 5NC26), 47 isolates had undulate margin (LUMB4, LUMB7, LUMITI2, LUMITI6, LUMITI7, LUMBB6, LBS11, LBS25A, LBS25B, LBS44A, LBS44B, LBS21, LBS13, LBS14, LBS23, LBS7, LBS4, LBS31, LBS15, LBS19, LBS12, LBS17, 25E10, 5R16, EZ11, E24, 25E16, 25R10, 25R2, RZ5, RZ20, 5EZ13, 25E3, TSU3, TSU8, TSU2(2), TSU3(2), TSU3(4), TUL1, TUL2, TUL9, 5NC8, 5NC6, 5NC13, 25NC10, 5NC1and 5NC20), three isolates had filiform margin (LUMITI1, 25R23 and LUMBB1), nine isolates had lobate margin (LUMB10, LUMBB8, LBS18, LBS29, LBS22, RZ7, TSU1(5), TSU4 and TUL12), two had serrated margin (25E20 and 5R3) and one had wavy margin (NC7). In case of colony elevation, seventeen had raised elevation (LUMB1, LUMB3, LUMB5, LUMB6, LUMB9, LUMITI3, LUMITI4, LUMBB4, LUMBB8, LUMBB9, 5R5, 25R2, EZ30, 5R9, 25E5, 5NC14 and 25NC8), eighty eight isolates had flat isolates (LUMB2, LUMB4, LUMB7, LUMB10, LUMITI1, LUMITI2, LUMITI5, LUMITI6, LUMITI7, LUMBB1, LUMBB2, LUMBB3, LBS12A, LBS18, LBS25A, LBS36, LBS16, LBS44A, LBS13, LBS29, LBS19, LBS12, LBS17, LBS22, 5E20, 5E8, 25E12, 5E24, 5E5, 25R23, 25E10, 5E22, 5R16, EZ11, E24, 25E18, 5R15, 25EZ1, RZ23, 5E9, 5E28, 25E16, RZ7, 25R10, 25Z13, 25E22, 25EZ2, 5E11, 5E3, 25EZ6, 25EZ17, RZ5, RZ20, 5EZ13, 5R3, 25R1, TSU2(4), TSU3, TSU1(5), TSU4, TSU8, TSU9, TSU2(1), TSU2(2), TSU3(2), TSU3(4), TUL1, TUL2, TUL9, TUL12,

NC11, 5NC8, 5NC6, 5NC13, 25NC12, 25NC9, 25NC3, 5NC17, 25NC12, 25NC10, 5NC1, 5NC20 and 5NC26), seven isolates had umbonate elevation (LUMB8, 5R1, 5R12, 5R10, EZ27, 25E20 and 25E3), twenty five isolates convex (LUMBB7, LBS11, LBS25B, LBS44B, LBS21, LBS14, LBS23, LBS7, LBS4, LBS31, LBS15, TSU1, TSU6, TSU7, TSU11, TSU12, TSU3(7), TSU3(6), TUL3, TUL4, TUL5, TUL8, TUL10, 5NC10 and NC7), three had bulging margin (RZ27, TUL6 and TUL11). Shape of the colony were also varied with seventy-six isolates having round shape (LUMB1, LUMB2, LUMB3, LUMB5, LUMB6, LUMB8, LUMB9, LUMITI3, LUMITI4, LUMITI5, LUMITI6, LUMBB2, LUMBB3, LUMBB4, LUMBB7, LUMBB9, LBS36, LBS16, 5E20, 5E8, 25E12, 5E24, 5E5, 25R23, 25E10, 5R1, 5R5, 5E22, E24, 25E18, 5R15, 5R12, RZ23, 5E9, 5E28, RZ27, EZ30, 5R9, 5R10, 25Z13, 25E22, 25E5, EZ27, 25EZ2, 25E20, 5E11, 5E3, 25EZ6, 25EZ17, RZ5, 25R1, TSU1, TSU2(4), TSU8, TSU6, TSU7, TSU11, TSU12, TSU9, TSU2(1), TSU3(7)TSU3(6), TUL3, TUL4, TUL5, TUL6, TUL8, TUL10, TUL11, NC11, 5NC10, 5NC14, 25NC8, 25NC12, 25NC9, 25NC3, 5NC17, 25NC12 and 5NC26), forty seven isolates had irregular shape (LUMB4, LUMB7, LUMB10, LUMITI1, LUMITI2, LUMITI7, LUMBB6, LUMBB8, LBS11, LBS18, LBS25A, LBS25B, LBS44A, LBS44B, LBS21, LBS13, LBS14, LBS29, LBS23, LBS7, LBS4, LBS31, LBS15, LBS19, LBS12, LBS17, LBS22, 5R16, EZ11, 25EZ1, 25E16, RZ7, 25R10, 25R2, RZ20, 5EZ13, 5R3, 25E3, TSU1(5), TSU4, 5NC8, 5NC6, 5NC13, 25NC10, 5NC1, 5NC20 and NC7), one had filamentous shape (LUMBB1) and one had rhizoid shape (TSU3).Almost all the isolates were opaque except one isolate (EZ27) which was transparent.

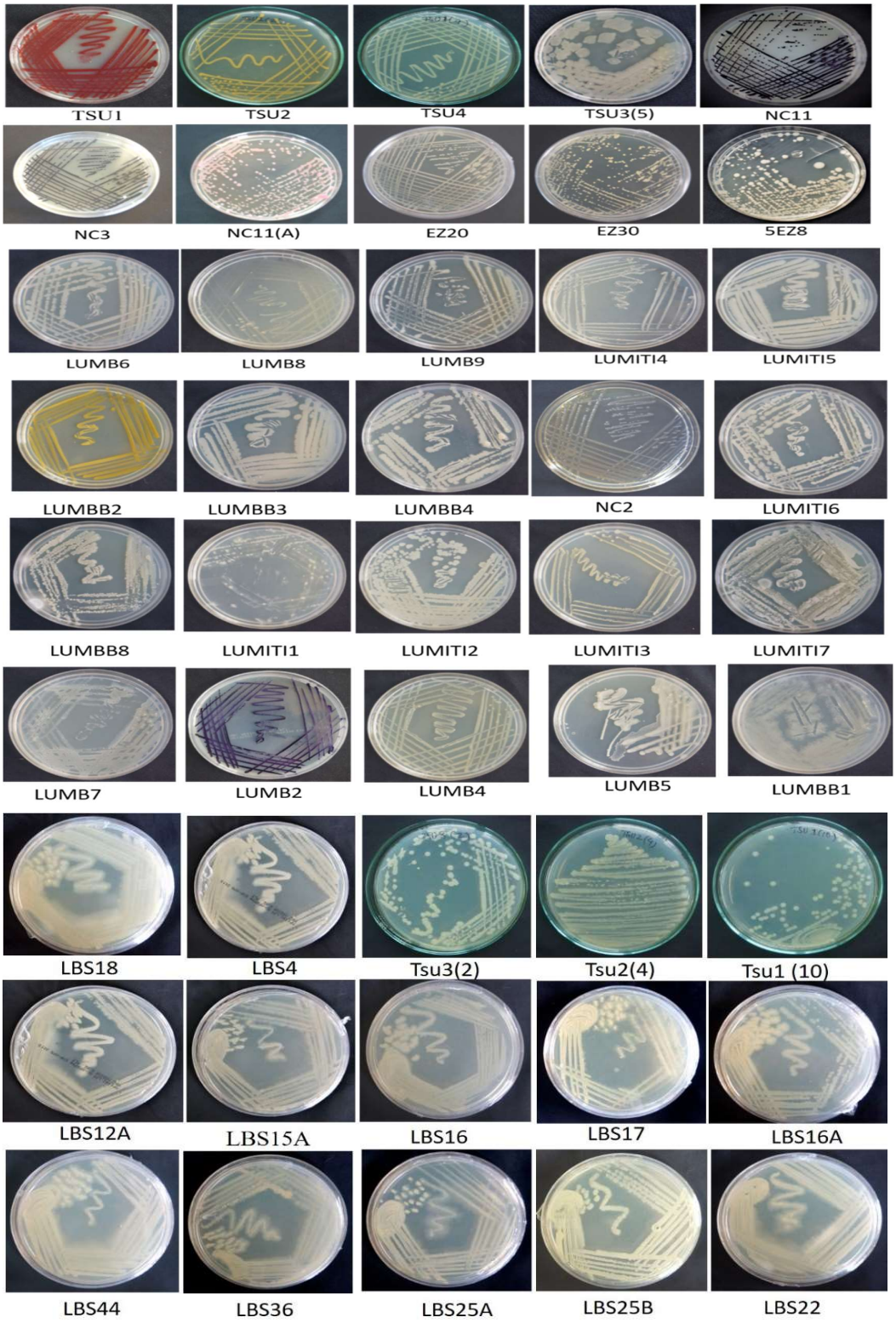

Figure 2.3: Pictures of representative pictures of some of the purified bacterial isolates on nutrient agar plates.

Table 2.2: Colony morphology of the bacterial strains isolated from *Musa* rhizosphere

Sl. No.	Bacteria Isolates	Margin	Elevation	Shape	Colour	Transparency
1	LUMB1	Entire	Raised	Round	Off-White	Opaque
2	LUMB2	Entire	Flat	Round	Purple	Opaque
3	LUMB3	Entire	Raised	Round	Off-White	Opaque
4	LUMB4	Undulate	Flat	Irregular	Off-White	Opaque
5	LUMB5	Entire	Raised	Round	Off-White	Opaque
6	LUMB6	Entire	Raised	Round	Off White	Opaque
7	LUMB7	Undulate	Flat	Irregular	Off-White	Opaque
8	LUMB8	Entire	Umbonate	Round	Off-White	Opaque
9	LUMB9	Entire	Raised	Round	Off-White	Opaque
10	LUMB10	Lobate	Flat	Irregular	Off-White	Opaque
11	LUMITI1	Filiform	Flat	Irregular	Off-White	Opaque
12	LUMITI2	Undulate	Flat	Irregular	Off-White	Opaque
13	LUMITI3	Entire	Raised	Round	Off-White	Opaque
14	LUMITI4	Entire	Raised	Round	White	Opaque
15	LUMITI5	Entire	Flat	Round	Dark Yellow	Opaque
16	LUMITI6	Undulate	Flat	Round	Off-White	Opaque
17	LUMITI7	Undulate	Flat	Irregular	Off-White	Opaque
18	LUMBB1	Filiform	Flat	Filamentous	Off-White	Opaque
19	LUMBB2	Entire	Flat	Round	Dark Yellow	Opaque
20	LUMBB3	Entire	Flat	Round	Off-White	Opaque
21	LUMBB4	Entire	Raised	Round	Off-White	Opaque
22	LUMBB6	Undulate	Flat	Irregular	Off-White	Opaque
23	LUMBB7	Entire	Convex	Round	Off-White	Opaque
24	LUMBB8	Lobate	Raised	Irregular	Off-White	Opaque
25	LUMBB9	Entire	Raised	Round	Off-White	Opaque
26	LBS11	Undulate	Convex	Irregular	Yellowish	Opaque
27	LBS12A	Entire	Flat	Regular	Off-white	Opaque
28	LBS18	Lobate	Flat	Irregular	Yellowish	Opaque
29	LBS25A	Undulate	Flat	Irregular	Yellowish	Opaque
30	LBS36	Entire	Flat	Round	Off-White	Opaque
31	LBS25B	Undulate	Convex	Irregular	Off-White	Opaque
32	LBS16	Entire	Flat	Round	Yellowish	Opaque
33	LBS44A	Undulate	Flat	Irregular	Off-White	Opaque
34	LBS44B	Undulate	Convex	Irregular	Off-White	Opaque
35	LBS21	Undulate	Convex	Irregular	Yellowish	Opaque
36	LBS13	Undulate	Flat	Irregular	Yellowish	Opaque
37	LBS14	Undulate	Convex	Irregular	Yellowish	Opaque
38	LBS29	Lobate	Flat	Irregular	Yellowish	Opaque
39	LBS23	Undulate	Convex	Irregular	Yellowish	Opaque
40	LBS7	Undulate	Convex	Irregular	Yellowish	Opaque
41	LBS4	Undulate	Convex	Irregular	Yellowish	Opaque
42	LBS31	Undulate	Convex	Irregular	Yellowish	Opaque
43	LBS15	Undulate	Convex	Irregular	Yellowish	Opaque
44	LBS19	Undulate	Flat	Irregular	Yellowish	Opaque
45	LBS12	Undulate	Flat	Irregular	Yellowish	Opaque
46	LBS17	Undulate	Flat	Irregular	Yellowish	Opaque

47	LBS22	Lobate	Flat	Irregular	Yellowish	Opaque
48	5E20	Entire	Flat	Round	Off-white	Opaque
49	5E8	Entire	Flat	Round	Off-white	Opaque
50	25E12	Entire	Flat	Round	Off-white	Opaque
51	5E24	Entire	Flat	Round	Off-white	Opaque
52	5E5	Entire	Fat	Round	Off-white	Opaque
53	25R23	Filiform	Flat	Round	Off-white	Opaque
54	25E10	Undulate	Flat	Round	Yellowish	Opaque
55	5R1	Entire	Umbonate	Round	Off-white	Opaque
56	5R5	Entire	Raised	Round	Yellowish	Opaque
57	5E22	Entire	Flat	Round	Off-white	Opaque
58	5R16	Undulate	Flat	Irregular	Off-white	Opaque
59	EZ11	Undulate	Flat	Irregular	Off-white	Opaque
60	E24	Undulate	Flat	Round	Off-white	Opaque
61	25E18	Entire	Flat	Round	Off-white	Opaque
62	5R15	Entire	Flat	Round	Off-white	Opaque
63	5R12	Entire	Umbonate	Round	Off-white	Opaque
64	25EZ1	Entire	Flat	Irregular	Off-white	Opaque
65	RZ23	Entire	Flat	Round	Yellowish	Opaque
66	5E9	Entire	Flat	Round	Off-white	Opaque
67	5E28	Entire	Flat	Round	Off-white	Opaque
68	25E16	Undulate	Flat	Irregular	Off-white	Opaque
69	RZ7	Lobate	Flat	Irregular	White	Opaque
70	25R10	Undulate	Flat	Irregular	Off-white	Opaque
71	RZ27	Entire	Bulging	Round	Off-white	Opaque
72	25R2	Undulate	Raised	Irregular	Off-white	Opaque
73	EZ30	Entire	Raised	Round	Off-white	Opaque
74	5R9	Entire	Raised	Round	Off-white	Opaque
75	5R10	Entire	Umbonate	Round	Off-white	Opaque
76	25Z13	Entire	Flat	Round	Off-white	Opaque
77	25E22	Entire	Flat	Round	Off-white	Opaque
78	25E5	Entire	Raised	Round	Off-white	Opaque
79	EZ27	Entire	Umbonate	Round	Off-white	Transparent
80	25EZ2	Entire	Flat	Round	Off-white	Opaque
81	25E20	Serrated	Umbonate	Round	Off-white	Opaque
82	5E11	Entire	Flat	Round	Off-white	Opaque
83	5E3	Entire	Flat	Round	Off-white	Opaque
84	25EZ6	Entire	Flat	Round	Off-white	Opaque
85	25EZ17	Entire	Flat	Round	Off-white	Opaque
86	RZ5	Undulate	Flat	Round	Off-white	Opaque
87	RZ20	Undulate	Flat	Irregular	Off-white	Opaque
88	5EZ13	Undulate	Flat	Irregular	Off-white	Opaque
89	5R3	Serrated	Flat	Irregular	Off-white	Opaque
90	25R1	Entire	Flat	Round	Off-white	Opaque
91	25E3	Undulate	Umbonate	Irregular	Off-white	Opaque
92	TSU1	Entire	Convex	Round	Red	Opaque
93	TSU2(4)	Entire	Flat	Round	Off -white	Opaque
94	TSU3	Undulate	Flat	Rhizoid	Yellowish	Opaque
95	TSU1(5)	Lobate	Flat	Irregular	Off-White	Opaque

96	TSU4	Lobate	Flat	Irregular	Off-White	Opaque
97	TSU8	Undulate	Flat	Round	Off-white	Opaque
98	TSU6	Entire	Convex	Round	Off-White	Opaque
99	TSU7	Entire	Convex	Round	Off-white	Opaque
100	TSU11	Entire	Convex	Round	Off-white	Opaque
101	TSU12	Entire	Convex	Round	Off-white	Opaque
102	TSU9	Entire	Flat	Round	Off-white	Opaque
103	TSU2(1)	Entire	Flat	Round	Off-white	Opaque
104	TSU2(2)	Undulate	Flat	Undulate	White	Opaque
105	TSU3(7)	Entire	Convex	Round	Off-White	Opaque
106	TSU3(2)	Undulate	Flat	Undulate	White	Opaque
107	TSU3(6)	Entire	Convex	Round	Off-White	Opaque
108	TSU3(4)	Undulate	Flat	Undulate	White	Opaque
109	TUL1	Undulate	Flat	Undulate	White	Opaque
110	TUL2	Undulate	Flat	Undulate	White	Opaque
111	TUL3	Entire	Convex	Round	Off-White	Opaque
112	TUL4	Entire	Convex	Round	Off-White	Opaque
113	TUL5	Entire	Convex	Round	Off-White	Opaque
114	TUL6	Entire	Bulging	Round	Off-white	Opaque
115	TUL8	Entire	Convex	Round	Off-White	Opaque
116	TUL9	Undulate	Flat	Undulate	Yellowish	Opaque
117	TUL10	Entire	Convex	Round	Off-White	Opaque
118	TUL11	Entire	Bulging	Round	Off-white	Opaque
119	TUL12	Lobate	Flat	Lobate	Off-White	Opaque
120	NC11	Entire	Flat	Round	Purple	Opaque
121	5NC8	Undulate	Flat	Irregular	Off-White	Opaque
122	5NC10	Entire	Convex	Round	Off-White	Opaque
123	5NC14	Entire	Raised	Round	Off-White	Opaque
124	25NC8	Entire	Raised	Round	Off-White	Opaque
125	5NC6	Undulate	Flat	Irregular	Off-White	Opaque
126	5NC13	Undulate	Flat	Irregular	White	Opaque
127	25NC12	Entire	Flat	Round	Off-White	Opaque
128	25NC9	Entire	Flat	Round	Off-White	Opaque
129	25NC3	Entire	Flat	Round	Off-White	Opaque
130	5NC17	Entire	Flat	Round	Off-White	Opaque
131	25NC12	Entire	Flat	Round	Off-White	Opaque
132	25NC10	Undulate	Flat	Irregular	White	Opaque
133	5NC1	Undulate	Flat	Irregular	Off-White	Opaque
134	5NC20	Undulate	Flat	Irregular	Off-White	Opaque
135	5NC26	Entire	Flat	Round	White	Opaque
136	NC7	Wavy	Convex	Irregular	Off-white	Opaque

Table 2.3: Biochemical analysis of the bacterial isolates

Bacteria Isolates	Gram Staining	Motility	Starch Hydrolysis	Catalase Test	Methyl Red Test	Citrate Utilization Test	Sugar Fermentation Test				
							Sucrose	Maltose	Mannitol	Glucose	Sorbitol
LUMB1	+	-	+	+	-	-	+	+	-	+	-
LUMB2	-	-	-	-	-	+	-	-	-	-	-
LUMB3	-	-	-	+	+	+	-	+	-	-	+
LUMB4	+	+	-	-	-	-	+	+	-	+	-
LUMB5	+	-	-	-	+	-	-	-	-	-	-
LUMB6	-	-	-	+	-	-	-	-	-	-	-
LUMB7	+	-	+	+	-	-	-	-	-	-	-
LUMB8	+	+	-	-	+	+	+	+	+	+	+
LUMB9	-	+	-	+	+	+	-	-	-	-	-
LUMB10	+	+	+	-	-	-	-	-	-	-	+
LUMITI1	+	+	+	+	-	-	-	+	-	-	-
LUMITI2	+	+	+	-	-	-	-	+	-	-	-
LUMITI3	+	-	-	-	-	-	-	-	-	-	-
LUMITI4	-	-	+	+	-	+	-	-	+	+	-
LUMITI5	+	-	+	-	-	-	-	-	-	-	-
LUMITI6	+	-	+	+	-	+	-	+	-	+	+
LUMITI7	+	-	+	-	-	-	+	-	-	+	+
LUMBB1	+	-	+	-	+	+	-	-	-	+	-
LUMBB2	+	+	+	+	-	-	-	-	-	+	-
LUMBB3	+	+	+	+	-	-	-	-	-	-	-
LUMBB4	+	+	+	-	-	-	-	+	-	-	-
LUMBB6	+	+	-	+	+	+	-	-	-	+	+
LUMBB7	+	+	+	+	-	+	-	+	-	-	-
LUMBB8	+	-	+	+	-	+	-	-	-	-	-
LUMBB9	+	-	-	+	-	+	+	+	-	+	+
LBS11	+	-	-	+	-	+	-	-	-	-	+
LBS12A	-	+	+	+	-	+	-	-	+	-	-
LBS18	+	+	+	+	+	-	+	+	+	+	-
LBS25A	+	-	-	+	-	-	+	-	+	+	+
LBS36	+	+	+	+	+	-	-	+	+	+	-
LBS25B	+	-	-	+	-	-	+	+	+	+	-
LBS16	-	-	-	+	-	-	+	+	+	+	-
LBS44A	-	+	-	+	+	+	-	-	-	-	-
LBS44B	-	-	-	+	-	-	+	+	+	+	-
LBS21	+	+	-	-	-	+	+	+	+	+	-
LBS13	+	+	+	+	+	-	+	+	+	+	-
LBS14	-	-	-	+	-	+	+	-	+	+	+
LBS29	+	-	-	+	+	-	+	-	+	+	+
LBS23	+	-	-	+	-	+	-	-	+	+	-
LBS7	-	-	-	+	-	-	+	-	+	+	+
LBS4	+	-	-	+	-	-	-	+	-	-	+
LBS31	+	-	-	+	-	-	-	-	-	-	-
LBS15	-	-	-	+	+	-	+	+	+	-	+
LBS19	+	+	-	+	+	+	-	+	+	+	-

LBS12	-	-	-	+	-	-	-	+	+	+	-
LBS17	+	-	+	+	+	-	+	-	-	+	-
LBS22	-	-	-	+	-	-	-	-	-	-	-
5E20	+	-	+	+	+	-	-	-	-	-	-
5E8	-	-	-	+	+	-	+	+	-	+	-
25E12	-	-	-	+	+	-	+	-	-	+	-
5E24	-	-	-	+	+	-	-	-	-	-	-
5E5	-	-	-	+	+	-	-	-	-	-	-
25R23	-	-	-	+	+	-	+	+	-	+	-
25E10	+	-	-	+	-	+	+	-	-	+	-
5R1	-	-	-	+	+	-	-	-	-	-	-
5R5	-	-	-	+	+	-	-	-	-	-	-
5E22	-	-	-	+	+	-	+	-	-	+	-
5R16	+	-	-	+	-	+	-	-	-	-	-
EZ11	-	+	-	+	-	+	-	-	+	+	-
E24	-	-	-	+	+	-	+	-	-	+	-
25E18	+	+	-	+	-	-	-	-	-	-	-
5R15	+	+	-	+	-	-	-	-	-	-	-
5R12	+	+	-	+	+	+	+	+	-	+	-
25EZ1	+	+	-	+	+	-	+	-	-	+	-
RZ23	-	+	-	+	-	-	+	+	+	+	+
5E9	+	-	-	+	+	-	+	-	-	+	-
5E28	-	+	-	+	-	-	-	-	-	-	-
25E16	-	+	+	+	-	+	-	-	-	-	-
RZ7	+	+	-	+	+	-	+	+	-	+	-
25R10	+	+	-	+	+	+	+	-	-	+	-
RZ27	-	+	+	+	-	-	+	-	-	+	-
25R2	-	+	-	+	-	+	+	-	-	+	-
EZ30	-	+	-	+	-	+	-	-	+	+	-
5R9	+	-	-	+	+	+	+	-	-	+	-
5R10	-	+	-	-	-	+	-	-	-	-	-
25Z13	-	-	-	-	+	+	-	-	-	-	-
25E22	-	+	-	+	+	+	+	+	-	+	-
25E5	-	-	+	+	-	+	+	-	-	+	-
EZ27	-	+	-	+	-	+	+	+	+	+	+
25EZ2	-	+	-	+	-	+	+	-	-	+	-
25E20	+	+	-	+	+	+	-	-	-	-	-
5E11	+	+	-	+	+	+	-	-	-	+	-
5E3	+	+	-	+	+	+	+	-	-	+	-
25EZ6	-	+	-	+	-	-	-	-	-	+	-
25EZ17	-	+	-	+	+	+	+	-	-	+	-
RZ5	-	+	-	+	-	+	+	-	-	-	-
RZ20	-	+	-	+	-	+	-	-	+	+	-
5EZ13	-	+	-	+	-	+	-	-	-	+	-
5R3	-	+	+	+	-	-	-	-	-	-	-
25R1	-	+	-	+	-	+	+	-	-	+	-
25E3	-	+	-	+	-	+	+	+	+	+	+
TSU1	-	+	-	+	-	+	+	+	+	+	+
TSU2(4)	-	+	-	+	+	+	+	+	-	+	+
TSU3	+	+	+	+	-	+	-	+	-	-	+
TSU1(5)	+	+	+	+	+	-	+	-	-	-	+
TSU4	+	+	-	+	+	-	+	+	-	+	-

TSU8	+	+	+	+	+	-	+	+	+	+	+
TSU6	-	-	-	+	+	-	+	+	+	+	+
TSU7	-	-	-	-	-	+	-	-	-	+	-
TSU11	-	-	-	-	+	+	+	+	-	+	+
TSU12	-	+	-	-	+	+	+	+	+	+	+
TSU9	-	-	-	-	+	-	-	-	-	-	-
TSU2(1)	-	-	-	-	+	-	-	-	-	-	-
TSU2(2)	-	-	+	+	+	-	+	+	+	-	+
TSU3(7)	-	+	+	+	+	+	+	+	+	+	+
TSU3(2)	-	+	+	+	+	+	+	+	+	+	+
TSU3(6)	-	+	-	-	+	+	+	+	+	+	+
TSU3(4)	-	+	+	+	+	+	+	+	+	+	+
TUL1	+	+	+	+	+	-	-	-	-	-	+
TUL2	+	+	+	+	+	-	-	+	-	+	-
TUL3	+	+	+	+	+	-	-	+	-	+	+
TUL4	+	+	+	+	+	-	-	+	-	+	-
TUL5	+	+	+	+	+	-	-	+	-	+	+
TUL6	-	+	+	+	-	+	+	-	-	+	-
TUL8	+	+	-	-	-	+	-	+	-	+	-
TUL9	+	+	+	-	-	-	-	+	-	+	+
TUL10	+	+	-	-	+	+	-	+	-	+	-
TUL11	-	+	+	+	-	+	+	-	-	+	-
TUL12	+	+	-	+	+	+	+	+	-	+	-
NC11	-	+	-	+	-	-	+	+	+	+	+
5NC8	-	+	-	-	-	-	-	-	+	+	-
5NC10	-	+	-	-	-	+	-	+	-	+	-
5NC14	-	+	-	-	-	+	-	+	-	+	+
25NC8	+	+	-	-	-	+	-	+	-	+	-
5NC6	+	-	+	-	-	-	-	+	-	+	+
5NC13	+	-	+	-	+	-	+	+	+	+	+
25NC12	-	-	+	-	-	-	-	+	-	+	-
25NC9	-	+	-	-	-	-	-	+	-	+	-
25NC3	+	+	-	+	-	-	-	+	-	+	+
5NC17	+	+	-	+	+	+	-	+	-	+	-
25NC12	+	-	-	+	+	+	-	+	-	+	+
25NC10	-	-	-	+	-	-	-	+	-	+	-
5NC1	-	-	+	-	-	-	+	-	-	+	-
5NC20	-	+	+	-	-	-	+	+	-	+	-
NC26	-	-	-	-	-	+	+	+	+	+	+
NC7	-	+	-	-	-	+	+	+	+	+	+

Note: '+': Indicates the positive test; '- ': Indicates the negative test.

Biochemical characterization of the bacterial isolates

The pure bacterial isolates were analyzed by biochemical tests such as citrate utilization, methyl red test, starch hydrolysis, catalase test, gram staining, motility test, sugar fermentation test. **Table 2.3** presents the biochemical characterization of the selected bacterial isolates.

Gram staining

Gram staining was done to check whether the bacterial isolates are gram positive or negative. It is the first step in the preliminary identification of bacteria. This process differentiates bacteria by the chemical and physical properties of the cell wall. Out of 136 bacterial isolates, sixty-six isolates were gram positive (thick wall) (LUMB1, LUMB4, LUMB5, LUMB7, LUMB8, LUMB10, LUMITI1, LUMITI2, LUMITI3, LUMITI5, LUMITI6, LUMITI7, LUMBB1, LUMBB2, LUMBB3, LUMBB4, LUMBB6, LUMBB7, LUMBB8, LUMBB9, LBS11, LBS18, LBS25A , LBS36, LBS25B , LBS21, LBS13, LBS29, LBS23, LBS4, LBS31, LBS19, LBS17, 5E20, 25E10, 5R16, 25E18, 5R15, 5R12, 25EZ1, 5E9, RZ7, 25R10, 5R9, 25E20, 5E11, 5E3, TSU3, TSU1(5), TSU4, TSU8, TUL1, TUL2, TUL3, TUL4, TUL5, TUL8, TUL9, TUL10, TUL12, 25NC8, 5NC6, 5NC13, 25NC3, 5NC17 and 25NC12) and the remaining 70 isolates were gram negative (thin peptidoglycan layer) (NC7, NC26, 5NC20, 5NC1, 25NC10, 25NC9, 25NC12, 5NC14, 5NC10, 5NC8, NC11, TUL11, TUL6, TSU3(4), TSU3(6), TSU3(2), TSU3(7), TSU2(2), TSU2(1), TSU9, TSU12, TSU11, TSU7, TSU6, TSU2(4), TSU1, 25E3, 25R1, 5R3, 5EZ13, RZ20, RZ5, 25EZ17, 25EZ6, 25EZ2, EZ27, 25E5, 25E22, 25Z13, 5R10, EZ30, 25R2, RZ27, 25E16, 5E28, RZ23, E24, EZ11, 5E22, 5R5, 5R1, 25R23, 5E5, 5E24, 25E12, 5E8, LBS22, LBS12, LBS15, LBS7, LBS14, LBS44B, LBS44A, LBS16, LBS12A, LUMITI4, LUMB9, LUMB6, LUMB3 and LUMB2). Gram positive strains were indicated by appearance of purple colour cells under microscope because of the thick layer of cell wall. The isolates which produced pink colour pigment, rod shaped, motile which confirms the gram-negative character [**Figure 2.4 (A-i and A-ii) and Table 2.3].**

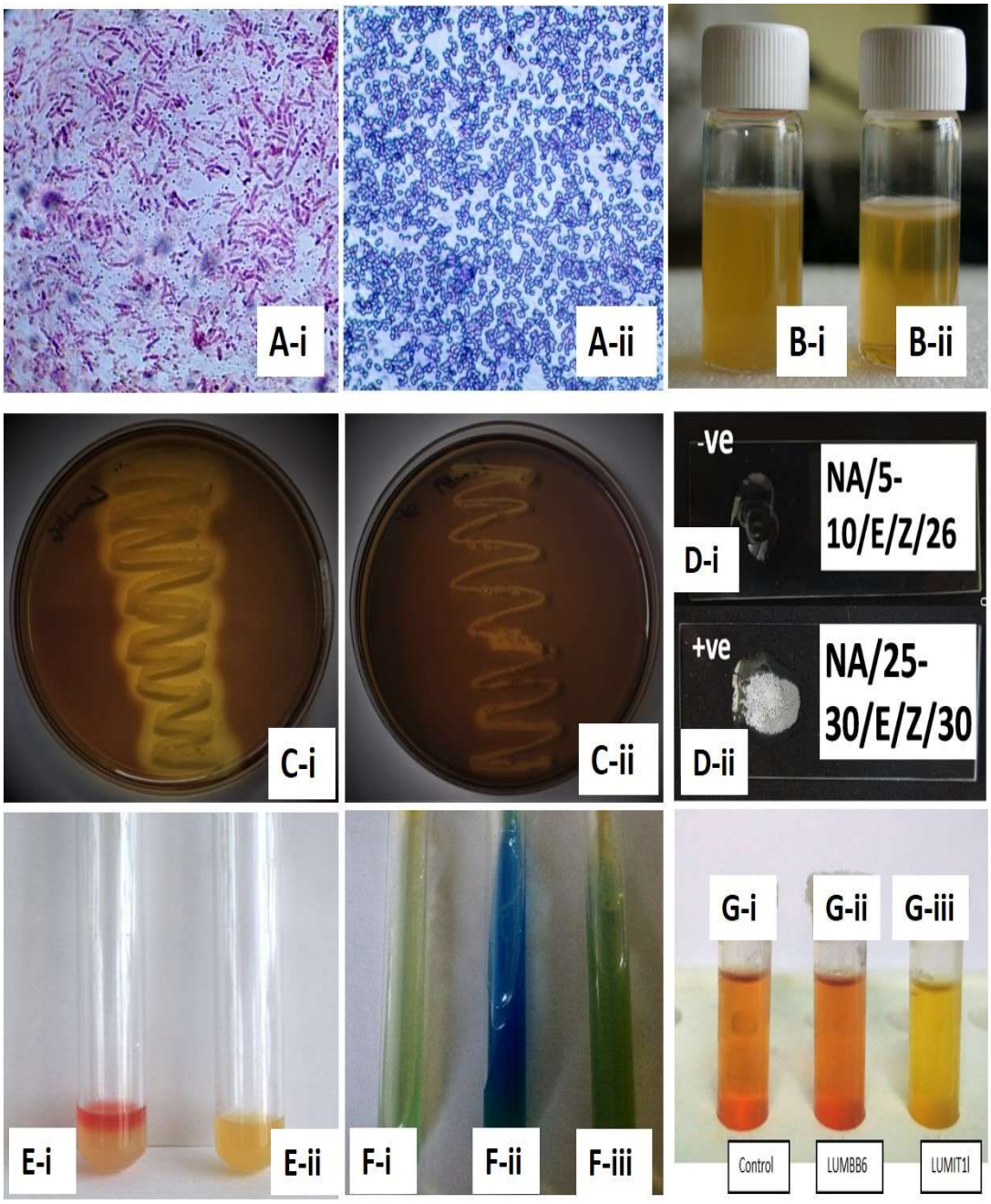

Figure 2.4: Biochemical characterization of the bacterial isolates; **A**. Gram positive, **B.** gram negative, **C.** Motility tests, **D**. Starch positive test, **E**. Starch negative tests, **F**. Catalase negative test, **G**. Catalase positive test, **H**. Methyl red test and **I**. Citrate utilization test.

Motility test

Motility test was done to check whether the bacterial isolates are motile or non-motile, based on their cellular movement. The motility test aids in the characterization or identification of pathogens or non-pathogens (as motility is one of the virulent factors) and facilitates species-level differentiation. Out of 136 bacterial isolates, 78 were motile (NC7, 5NC20, 5NC17, 25NC3, 25NC9, 25NC8, 5NC14, 5NC10, 5NC8, NC11, TUL12, TUL11, TUL10, TUL9, TUL8, TUL6, TUL5, TUL4, TUL3, TUL2, TUL1, TSU3(4), TSU3(6), TSU3(2), TSU3(7), TSU12, TSU8 , TSU4, TSU1(5), TSU3, TSU2(4), TSU1, 25E3, 25R1, 5R3, 5EZ13, RZ20, RZ5, 25EZ17, 25EZ6, 5E3, 5E11, 25E20, 25EZ2, EZ27, 25E22, 5R10, EZ30, 25R2, RZ27, 25R10, RZ7, 25E16, 5E28, RZ23, 25EZ1, 5R12, 5R15, 25E18, EZ11, LBS19, LBS13, LBS21, LBS44A, LBS36, LBS18, LBS12A, LUMBB7, LUMBB6, LUMBB4, LUMBB3, LUMBB2, LUMITI2, LUMITI1, LUMB10, LUMB9, LUMB8 and LUMB4) and the remaining 58 isolates (LUMB1, LUMB2, LUMB3, LUMB5, LUMB6, LUMB7, LUMITI3, LUMITI4, LUMITI5, LUMITI6, LUMITI7, LUMBB1, LUMBB8, LUMBB9, LBS11, LBS25A, LBS25B, LBS16, LBS44B, LBS14, LBS29, LBS23, LBS7, LBS4, LBS31, LBS15, LBS12, LBS17, LBS22, 5E20, 5E8, 25E12, 5E24, 5E5, 25R23, 5R1, 5R5, 5E22, 5R16, E24, 5E9, 5R9, 25Z13, 25E5, TSU6, TSU7, TSU11, TSU9, TSU2(1), TSU2(2), 5NC6, 5NC13, 25NC12, 25NC12, 25NC10, 5NC1 and NC26) were non-motile (**Figure 2.4 (B-i and B-ii) and Table 2.3.**

Starch hydrolysis test

Starch is a complete polysaccharide found abundantly in plants and usually deposited in the form of large granules in the cytoplasm of the cell. Starch molecule are too large to enter the bacterial cell, so only bacteria that secret exoenzymes (a-amylase

and oligo-1,6-glucosidase) are able to hydrolyzed starch into sub-units (maltose, glucose), which can enter directly into the glucolytic pathway. Starch hydrolysis test was done to determine whether the bacterial isolates are able to break starch down into individual glucose molecules using the enzymes. Out of 136 bacterial isolates, 45 isolates were positive (LUMB1, LUMB7, LUMB10, LUMITI1, LUMITI2, LUMITI4, LUMITI5, LUMITI6, LUMITI7, LUMBB1, LUMBB2, LUMBB3, LUMBB4, LUMBB7, LUMBB8, LBS12A, LBS18, LBS36, LBS13, LBS17, 5E20, 25E16, RZ27, 25E5, 5R3, TSU3, TSU1(5), TSU8, TSU2(2), TSU3(7), TSU3(2), TSU3(4), TUL1, TUL2, TUL3, TUL4, TUL5, TUL6, TUL9, TUL11, 5NC6, 5NC13, 25NC12, 5NC1 and 5NC20) and the remaining 91 were negative (LUMB2, LUMB3, LUMB4, LUMB5, LUMB6, LUMB8, LUMB9, LUMITI3, LUMBB6, LUMBB9, LBS11, LBS25A, LBS25B, LBS16, LBS44A, LBS44B, LBS21, LBS14, LBS29, LBS23, LBS7, LBS4, LBS31, LBS15, LBS19, LBS12, LBS22, 5E8, 25E12, 5E24, 5E5, 25R23, 25E10, 5R1, 5R5, 5E22, 5R16, EZ11, 25E18, 5R15, 5R12, 25EZ1, RZ23, E24, 5E9, 5E28, RZ7, 25R10, 25R2, EZ30, 5R9, 5R10, 25Z13, 25E22, EZ27, 25EZ2, 25E20, 5E11, 5E3, 25EZ6, 25EZ17, RZ5, RZ20, 5EZ13, 25R1, 25E3, TSU1, TSU2(4), TSU4, TSU6, TSU7, TSU11, TSU12, TSU9, TSU2(1), TSU3(6), TUL8, TUL10, TUL12, NC11, 5NC8, 5NC10, 5NC14, 25NC8, 25NC9, 25NC3, 5NC17, 25NC12, 25NC10, NC26 and NC7). The negative responds are because the bacteria are not able to release enzyme that are needed for the breakdown of starch (**Figure 2.4 (C-i and C-ii) and Table 2.3).**

Catalase test

Catalase test was done to check whether the bacterial isolates produce catalase enzyme. Catalase enzyme is a common enzyme that is found in all living beings that survive in oxygen and catalyzes the decomposition of hydrogen peroxide, releasing water

and oxygen. Catalase is an essential enzyme in pathogenic organisms as it protects the organism from oxidative damage from the reactive oxygen species. Out of 136 bacterial isolates, 111 were positive (LUMB1, LUMB3, LUMB6, LUMB7, LUMB9, LUMITI1, LUMITI4, LUMITI6, LUMBB2, LUMBB3, LUMBB6, LUMBB7, LUMBB8, LUMBB9, LBS11, LBS12A, LBS18, LBS25A, LBS36, LBS25B, LBS16, LBS44A, LBS44B, LBS21, LBS13, LBS14, LBS29, LBS23, LBS7, LBS4, LBS31, LBS15, LBS19, LBS12, LBS17, LBS22, 5E20, 5E8, 25E12, 5E24, 5E5, 25R23, 25E10, 5R1, 5R5, 5E22, 5R16, EZ11, E24, 25E18, 5R15, 5R12, 25EZ1, RZ23, 5E9, 5E28, 25E16, RZ7, 25R10, RZ27, 25R2, EZ30, 5R9, 25E22, 25E5, EZ27, 25EZ2, 25E20, 5E11, 5E3, 25EZ6, 25EZ17, RZ5, RZ20, 5EZ13, 5R3, 25R1, 25E3, TSU1, TSU2(4), TSU3, TSU1(5), TSU4, TSU8, TSU6, TSU2(2), TSU3(7), TSU3(2), TSU3(4), TUL1, TUL2, TUL3, TUL4, TUL5, TUL6, TUL11, TUL12, NC11, 25NC3, 5NC17, 25NC12 and 25NC10)and the remaining 25 bacterial isolates (LUMB2, LUMB4, LUMB5, LUMB8, LUMB10, LUMITI2, LUMITI3, LUMITI5, LUMITI7, LUMBB1, LUMBB4, LBS21, 5R10, 25Z13, TSU7, TSU11, TSU12, TSU9, TSU2(1), TSU3(6), TUL8, TUL9, TUL10, 5NC8, 5NC10, 5NC14, 25NC8, 5NC6, 5NC13, 25NC12, 25NC9, 5NC1, 5NC20, NC26 and NC7) were negative. The negative responds of the bacteria are due to its inability to produce catalase enzyme (**Figure 2.4. (D-i and D-ii) and Table 2.3**).

Methyl red test

Methyl red test was conducted to find out the performance of acid fermentation of the bacteria. Colour of the test tube turned bright red indicated isolates were methyl red positive. The presence of extreme acidity in acid fermentation, hence methyl red was used as a *pH* indicator. In our study 60 isolates (LUMB3, LUMB5, LUMB8, LUMB9, LUMBB1, LUMBB6, LBS18, LBS36, LBS44A, LBS13, LBS29, LBS15, LBS19,

LBS17, 5E20, 5E8, 25E12, 5E24, 5E5, 25R23, 5R1, 5R5, 5E22, E24, 5R12, 25EZ1, 5E9, RZ7, 25R10, 5R9, 25Z13, 25E22, 25E20, 5E11, 5E3, 25EZ17, TSU2(4), TSU1(5), TSU4, TSU8, TSU6, TSU11, TSU12, TSU9, TSU2(1), TSU2(2), TSU3(7), TSU3(2), TSU3(6), TSU3(4), TUL1, TUL2, TUL3, TUL4, TUL5, TUL10, TUL12, 5NC13, 5NC17 and 25NC12) were positive for methyl red test and 76 (LUMB1, LUMB2, LUMB4, LUMB6, LUMB7, LUMB10, LUMITI1, LUMITI2, LUMITI3, LUMITI4, LUMITI5, LUMITI6, LUMITI7, LUMBB2, LUMBB3, LUMBB4, LUMBB7, LUMBB8, LUMBB9, LBS11, LBS12A , LBS25A , LBS25B , LBS16, LBS44B , LBS21, LBS14, LBS23, LBS7, LBS4, LBS31, LBS12, LBS22, 25E10, 5R16, EZ11, 25E18, 5R15, RZ23, 5E28, 25E16, RZ27, 25R2, EZ30, 25E5, EZ27, 25EZ2, 25EZ6, RZ5, RZ20, 5EZ13, 5R3, 25R1, 25E3, TSU1, TSU3, TSU7, TUL6, TUL8, TUL9, TUL11, NC11, 5NC8, 5NC10, 5NC14, 25NC8, 5NC6, 25NC12, 25NC9, 25NC3, 25NC10, 5NC1, 5NC20, NC26 and NC7) were negative (**Figure 2.4 (E-i and E-ii) and Table 2.3).**

Citrate utilization test

The citrate utilization test was conducted to check the potential of isolates to utilize citrate as its carbon and energy source. After incubation the bacteria, 64 isolates (LUMB2, LUMB3, LUMB8, LUMB9, LUMITI4, LUMITI6, LUMBB1, LUMBB6, LUMBB7, LUMBB8, LUMBB9, LBS11, LBS12A, LBS44A, LBS21, LBS14, LBS23, LBS19, 25E10, 5R16, EZ11, 5R12, 25E16, 25R10, 25R2, EZ30, 5R9, 5R10, 25Z13, 25E22, 25E5, EZ27, 25EZ2, 25E20, 5E11, 5E3, 25EZ17, RZ5, RZ20, 5EZ13, 25R1, 25E3, TSU1, TSU2(4), TSU3, TSU7, TSU11, TSU12, TSU3(7), TSU3(2), TSU3(6), TSU3(4), TUL6, TUL8, TUL10, TUL11, TUL12, 5NC10, 5NC14, 25NC8, 5NC17, 25NC12, NC26 and NC7) changed to intense blue in colour, which indicates that they were able to utilize the citrate present in the media hence citrate positive. Whereas, 72

cultures (LUMB1, LUMB4, LUMB5, LUMB6, LUMB7, LUMB10, LUMITI1, LUMITI2, LUMITI3, LUMITI5, LUMITI7, LUMBB2, LUMBB3, LUMBB4, LBS18, LBS25A, LBS36, LBS25B, LBS16, LBS44B, LBS13, LBS29, LBS7, LBS4, LBS31,LBS15, LBS12, LBS17, LBS22, 5E20, 5E8, 25E12, 5E24, 5E5, 25R23, 5R1, 5R5, 5E22, E24, 25E18, 5R15, 25EZ1, RZ23, 5E9, 5E28, RZ7, RZ27, 25EZ6, 5R3, TSU1(5), TSU4, TSU8, TSU6, TSU9, TSU2(1), TSU2(2), TUL1, TUL2, TUL3, , TUL5, TUL9, NC11, 5NC8, 5NC6, 5NC13, 25NC12, 25NC9, 25NC3, 25NC10, 5NC1 and 5NC20) remained green in colour could not utilize the citrate hence negative (**Figure 2.4 (F-i, F-ii, and F-iii) and Table 2.3).**

Sugar fermentation test

Carbohydrate Fermentation Test is the biochemical test used to assess the ability of bacteria to ferment a specific carbohydrate and to differentiate bacteria based on their carbohydrate fermentation pattern and identify them**.** Not all bacterial groups have the same nutritional requirement and biochemical properties. Different bacteria might have different enzyme systems making them different in substrate-utilizing ability. During the fermentation process, the carbohydrate molecules are anaerobically catabolized into organic acids. Thus, produced acid decreases the pH of the medium and compels the pH indicator to change its colour from red to yellow. In our study, 63 isolates were able to ferment sucrose, 65 could ferment maltose, 38 could ferment mannitol, 93 could ferment glucose and 42 could ferment sorbitol (**Figure 2.4 (G-i, G-ii, and G-iii**) **and Table 2.3**).

Discussion

Plant growth-promoting Rhizobacteria (PGPR) are beneficial microorganisms that live in the soil and interact with plant roots can enhance plant growth by various mechanisms, such as improving nutrient uptake (Qingwei et al., 2023), producing plant

hormones (Etesami et al., 2015), siderophore production, and by suppressing soil-borne diseases (Tatung and Deb, 2023; Qingwei et al., 2023). Thus, the meticulous screening and selection of potent PGPR strains and their incorporation into integrated agricultural practices are paramount for bolstering crop growth and yield while upholding agro-ecosystem sustainability (Rana et al., 2011). In our current investigation, we isolated and subjected 136 bacterial isolates to morphological and biochemical characterization. The selection of bacterial isolates was based on discernible differences in colony size, shape, colour, margin and elevation. Our documentation revealed a spectrum of colony colours, including off-white, white, purple, yellowish, dark yellow and red. Moreover, various colony margins were observed, such as entire, undulate, filiform, lobate, serrated and wavy. Elevations of colonies ranged from raised to flat, with some exhibiting umbonate, convex, or bulging margins. Colony shapes varied from round to irregular, filamentous, and rhizoid, with the majority appearing opaque, except for one transparent isolate. Subsequently, the pure bacterial isolates underwent biochemical tests, including citrate utilization, methyl red test, starch hydrolysis, catalase test, gram staining, motility test and sugar fermentation test, to further elucidate their characteristics.

To preliminarily identify the PGPR strains, a series of biochemical studies were conducted, and the results are presented in **Table 2.3.** Gram staining serves as the initial step in distinguishing individuals within colony patterns, although it holds little taxonomic significance (Di Franco et al., 2002). Out of 136 isolates, 66 were Gram-positive, while the remaining 70 were Gram-negative. This finding underscores the predominance of Gram-negative bacteria in the *Musa* rhizosphere environment, consistent with previous studies (Apastambh et al., 2016). The rhizosphere of several crop species shows greater association with gram-negative rhizobacteria as compared

togram positive rhizobacteria (Gupta et al., 2022a). The variations in Gram staining reactions are typically attributed to differences in the chemical structure of bacterial cell walls, with Gram-positive bacteria possessing more peptidoglycan and fewer lipids compared to Gram-negative bacteria (Al-Mulla and Khalifa, 2020). The rhizodeposition stimulates gram negative bacteria and makes them motile whereas this deposition inhibits the activity on gram positive bacteria. Moreover, gram negative bacteria are attracted by the root exudates which in turn increase their population around the roots and in turn release substances which can be absorbed by roots for plant growth promotion whereas gram positive bacteria are aerobic bacteria due to deficiency of oxygen around the roots their population around the roots decreases (Gupta et al., 2022a). Moreover, bacteria may exhibit characteristic shapes such as bacilli or cocci, further highlighting the diversity within bacterial divisions (Lone et al., 2015; Lihan et al., 2022).

Motility is essential for chemotactic responses and colonization, thereby facilitating a close relationship with the plant host and modulating defence responses against adverse conditions (Vandebroek et al., 1998; Hardoim et al., 2008; Lugtenberg and Kamilova, 2009). Additionally, among the tested isolates, 58 were non-motile (LUMB1, LUMB2, LUMB3, LUMB5, LUMB6, LUMB7, LUMITI3, LUMITI4, LUMITI5, LUMITI6, LUMITI7, LUMBB1, LUMBB8, LUMBB9, LBS11, LBS25A, LBS25B, LBS16, LBS44B, LBS14, LBS29, LBS23, LBS7, LBS4, LBS31, LBS15, LBS12, LBS17, LBS22, 5E20, 5E8, 25E12, 5E24, 5E5, 25R23, 5R1, 5R5, 5E22, 5R16, E24, 5E9, 5R9, 25Z13, 25E5, TSU6, TSU7, TSU11, TSU9, TSU2(1), TSU2(2), 5NC6, 5NC13, 25NC12, 25NC12, 25NC10, 5NC1 and NC26). The ability to move is crucial for different rhizospheric species to colonize the rhizosphere and gives them a competitive edge in a variety of environments (Santoyo et al., 2021). Motility also helps in

establishing primary bacteria-root interaction in response to compounds secreted by the host roots (Liu et al., 2024b). In the present study, 78 isolates exhibited motility (NC7,5NC20, 5NC17, 25NC3, 25NC9, 25NC8, 5NC14, 5NC10, 5NC8, NC11, TUL12, TUL11, TUL10, TUL9, TUL8, TUL6, TUL5, TUL4, TUL3, TUL2, TUL1, TSU3(4), TSU3(6), TSU3(2), TSU3(7), TSU12, TSU8 , TSU4, TSU1(5), TSU3, TSU2(4), TSU1, 25E3, 25R1, 5R3, 5EZ13, RZ20, RZ5, 25EZ17, 25EZ6, 5E3, 5E11, 25E20, 25EZ2, EZ27, 25E22, 5R10, EZ30, 25R2, RZ27, 25R10, RZ7, 25E16, 5E28, RZ23, 25EZ1, 5R12, 5R15, 25E18, EZ11, LBS19, LBS13, LBS21, LBS44A, LBS36, LBS18, LBS12A, LUMBB7, LUMBB6, LUMBB4, LUMBB3, LUMBB2, LUMITI2, LUMITI1, LUMB10, LUMB9, LUMB8 and LUMB4). In comparison to non-motile bacteria, motile bacteria are more effectively involved in plant-beneficial nutrient transformation activities including nitrogen and phosphorus cycling due to their selective enrichment in rhizosphere soil and their capacity to perceive and negotiate chemical gradients (Wu et al., 2023).

Starch, being a large molecule, and cannot cross the bacterial cell wall for use as a carbon source. Enzymes that hydrolyze starch into glucose are necessary to convert starch into usable energy and one such enzyme is amylase (Shohaib et al., 2020). A-amylase and B-amylase are known to make up the starch-hydrolyzing enzyme amylase. While microbes, animals, and plants all synthesis a-amylases, plants are the primary source of b-amylase (Das and Verma, 2010). In the present study, 45 isolates (LUMB1, LUMB7, LUMB10, LUMITI1, LUMITI2, LUMITI4, LUMITI5, LUMITI6, LUMITI7, LUMBB1, LUMBB2, LUMBB3, LUMBB4, LUMBB7, LUMBB8, LBS12A, LBS18, LBS36, LBS13, LBS17, 5E20, 25E16, RZ27, 25E5, 5R3, TSU3, TSU1(5), TSU8, TSU2(2), TSU3(7), TSU3(2), TSU3(4), TUL1, TUL2, TUL3, TUL4, TUL5, TUL6, TUL9, TUL11,

5NC6, 5NC13, 25NC12, 5NC1 and 5NC20) tested positive for starch hydrolysis, while 91 were negative (LUMB2, LUMB3, LUMB4, LUMB5, LUMB6, LUMB8, LUMB9, LUMITI3, LUMBB6, LUMBB9, LBS11, LBS25A, LBS25B, LBS16, LBS44A, LBS44B, LBS21, LBS14, LBS29, LBS23, LBS7, LBS4, LBS31, LBS15, LBS19, LBS12, LBS22, 5E8, 25E12, 5E24, 5E5, 25R23, 25E10, 5R1, 5R5, 5E22, 5R16, EZ11, 25E18, 5R15, 5R12, 25EZ1, RZ23, E24, 5E9, 5E28, RZ7, 25R10, 25R2, EZ30, 5R9, 5R10, 25Z13, 25E22, EZ27, 25EZ2, 25E20, 5E11, 5E3, 25EZ6, 25EZ17, RZ5, RZ20, 5EZ13, 25R1, 25E3, TSU1, TSU2(4), TSU4, TSU6, TSU7, TSU11, TSU12, TSU9, TSU2(1), TSU3(6), TUL8, TUL10, TUL12, NC11, 5NC8, 5NC10, 5NC14, 25NC8, 25NC9, 25NC3, 5NC17, 25NC12, 25NC10, NC26 and NC7). Microbial enzymes such as amylase play an inevitable role in mobilizing the inorganic and organic nutrients present in soil (Prasad and Raghuwanshi, 2022). Some of the amylase producing bacteria are *B. subtilis*, *B. stereothermophilus*, *B. licheniformis*, *Pseudomonas* and *Clostridium* (Meshesha and Namo, 2023).

The catalase test is a biochemical test for aerobic organisms that enable us to notice the production of catalase enzymes in the microbes. This enzyme is the most common enzyme that is found in all living organisms that mainly survive in oxygen and catalyzes the breakdown of hydrogen peroxide, releasing water and oxygen (Khatoon et al., 2022). Several pathogens produce catalase in order to defend themselves against attacks by hydrogen peroxide, a weapon commonly used by the host's immune system, in addition to oxidative stress. A previous report has in fact demonstrated that a catalase-deficient mutant pathogen was more susceptible than its wild-type strain to the oxidative stress induced by hydrogen peroxide and immune cell attacks (Iwase et al., 2013). It is thus useful to measure the catalase activity of pathogens in order to gain a better

understanding of the underlying mechanisms of their pathogenicity, including their resistance towards oxidative stress (Iwase et al., 2013). In the present study, 111 isolates were catalase test positive (LUMB1, LUMB3, LUMB6, LUMB7, LUMB9, LUMITI1, LUMITI4, LUMITI6, LUMBB2, LUMBB3, LUMBB6, LUMBB7, LUMBB8, LUMBB9, LBS11, LBS12A, LBS18, LBS25A, LBS36, LBS25B, LBS16, LBS44A, LBS44B, LBS21, LBS13, LBS14, LBS29, LBS23, LBS7, LBS4, LBS31, LBS15, LBS19, LBS12, LBS17, LBS22, 5E20, 5E8, 25E12, 5E24, 5E5, 25R23, 25E10, 5R1, 5R5, 5E22, 5R16, EZ11, E24, 25E18, 5R15, 5R12, 25EZ1, RZ23, 5E9, 5E28, 25E16, RZ7, 25R10, RZ27, 25R2, EZ30, 5R9, 25E22, 25E5, EZ27, 25EZ2, 25E20, 5E11, 5E3, 25EZ6, 25EZ17, RZ5, RZ20, 5EZ13, 5R3, 25R1, 25E3, TSU1, TSU2(4), TSU3, TSU1(5), TSU4, TSU8, TSU6, TSU2(2), TSU3(7), TSU3(2), TSU3(4), TUL1, TUL2, TUL3, TUL4, TUL5, TUL6, TUL11, TUL12, NC11, 25NC3, 5NC17, 25NC12, and 25NC10) and 25 (LUMB2, LUMB4, LUMB5, LUMB8, LUMB10, LUMITI2, LUMITI3, LUMITI5, LUMITI7, LUMBB1, LUMBB4, LBS21, 5R10, 25Z13, TSU7, TSU11, TSU12, TSU9, TSU2(1), TSU3(6), TUL8, TUL9, TUL10, 5NC8, 5NC10, 5NC14, 25NC8, 5NC6, 5NC13, 25NC12, 25NC9, 5NC1, 5NC20, NC26 and NC7)were found negative. Additionally, catalase enzyme is essential to many biotechnological applications. It is especially significant in bioremediation because it acts as a marker for soil hydrocarbon breakdown. This role is essential to the bioremediation of pollution caused by crude oil (Daunoras et al., 2024). Hence, suggesting bacterial strains with catalase enzyme to be a great option as bioremediatory.

To distinguish between the two major types of facultative anaerobic enteric bacteria based on the production of acid, the methyl red test is employed. Some bacteria utilize glucose and convert it into various acids, such as lactic acid (LA), acetic acid (AA)

and formic acid (FA), ultimately lowering the medium's *pH* to 4.4 or below (Shoaib et al., 2020). This decrease in pH causes a colour change in the methyl red indicator added to the media after incubation, turning it red at pH 4.4 and yellow at *pH* 6.2. If the organism produces a significant amount of organic acids, including formic acid, acetic acid, lactic acid, and succinic acid, through glucose fermentation, the broth medium will remain red after the addition of methyl red, indicating a positive test for mixed acid fermentation. In contrast, MR-negative organisms further metabolize the initial fermentation products via decarboxylation, resulting in the production of neutral acetyl methylcarbinol (acetoin), which raises the *pH* towards neutrality (pH 6.0 or above). For organisms that do not produce acid end products, the broth medium will change to a yellow colour, indicating a negative test. In our study, 60 isolates (LUMB3, LUMB5, LUMB8, LUMB9, LUMBB1, LUMBB6, LBS18, LBS36, LBS44A, LBS13, LBS29, LBS15, LBS19, LBS17, 5E20, 5E8, 25E12, 5E24, 5E5, 25R23, 5R1, 5R5, 5E22, E24, 5R12, 25EZ1, 5E9, RZ7, 25R10, 5R9, 25Z13, 25E22, 25E20, 5E11, 5E3, 25EZ17, TSU2(4), TSU1(5),TSU4, TSU8, TSU6, TSU11, TSU12, TSU9, TSU2(1), TSU2(2), TSU3(7), TSU3(2), TSU3(6), TSU3(4), TUL1, TUL2, TUL3, TUL4, TUL5, TUL10, TUL12, 5NC13, 5NC17 and 25NC12) tested positive for the methyl red test, while 76 tested negative (LUMB1, LUMB2, LUMB4, LUMB6, LUMB7, LUMB10, LUMITI1, LUMITI2, LUMITI3, LUMITI4, LUMITI5, LUMITI6, LUMITI7, LUMBB2, LUMBB3, LUMBB4, LUMBB7, LUMBB8, LUMBB9, LBS11, LBS12A , LBS25A , LBS25B , LBS16, LBS44B , LBS21, LBS14, LBS23, LBS7, LBS4, LBS31, LBS12, LBS22, 25E10, 5R16, EZ11, 25E18, 5R15, RZ23, 5E28, 25E16, RZ27, 25R2, EZ30, 25E5, EZ27, 25EZ2, 25EZ6, RZ5, RZ20, 5EZ13, 5R3, 25R1, 25E3, TSU1, TSU3, TSU7, TUL6, TUL8, TUL9, TUL11, NC11,

5NC8, 5NC10, 5NC14, 25NC8, 5NC6, 25NC12, 25NC9, 25NC3, 25NC10, 5NC1, 5NC20, NC26 and NC7).

Citrate agar is utilized to assess an organism's capacity to utilize citrate as an energy source. The medium consists of citrate as the sole carbon source and inorganic ammonium salts ($NH_4H_2PO_4$) as the sole nitrogen source. Bacteria capable of growth on this medium produce an enzyme called citrate-permease, which catalyzes the conversion of citrate to pyruvate. Pyruvate can then enter the organism's metabolic pathway for energy production. Citrate utilization test is also utilized to ascertain if the rhizobacterial isolates are more adapted to aerobic or anaerobic conditions (Islam et al., 2016). As the bacteria metabolize citrate, the ammonium salts are hydrolyzed to ammonia, resulting in an increase in alkalinity. This *pH* shift causes the bromthymol blue indicator in the medium to change from green to blue when the *pH* exceeds 7.6. It is believed that both flagellar motility and citrate consumption are important for the competitive colonization of roots by roots and the upkeep of bacteria in roots (Islam et al., 2016). In the current study, 64 isolates tested positive (LUMB2, LUMB3, LUMB8, LUMB9, LUMITI4, LUMITI6, LUMBB1, LUMBB6, LUMBB7, LUMBB8, LUMBB9, LBS11, LBS12A, LBS44A, LBS21, LBS14, LBS23, LBS19, 25E10, 5R16, EZ11, 5R12, 25E16, 25R10, 25R2, EZ30, 5R9, 5R10, 25Z13, 25E22, 25E5, EZ27, 25EZ2, 25E20, 5E11, 5E3, 25EZ17, RZ5, RZ20, 5EZ13, 25R1, 25E3, TSU1, TSU2(4), TSU3, TSU7, TSU11, TSU12, TSU3(7), TSU3(2), TSU3(6), TSU3(4), TUL6, TUL8, TUL10, TUL11, TUL12, 5NC10, 5NC14, 25NC8, 5NC17, 25NC12, NC26 and NC7) for citrate utilization, while 72 tested negative (LUMB1, LUMB4, LUMB5, LUMB6, LUMB7, LUMB10, LUMITI1, LUMITI2, LUMITI3, LUMITI5, LUMITI7, LUMBB2, LUMBB3, LUMBB4, LBS18, LBS25A, LBS36, LBS25B, LBS16, LBS44B, LBS13, LBS29, LBS7, LBS4,

LBS31,LBS15, LBS12, LBS17, LBS22, 5E20, 5E8, 25E12, 5E24, 5E5, 25R23, 5R1, 5R5, 5E22, E24, 25E18, 5R15, 25EZ1, RZ23, 5E9, 5E28, RZ7, RZ27, 25EZ6, 5R3, TSU1(5), TSU4, TSU8, TSU6, TSU9, TSU2(1), TSU2(2), TUL1, TUL2, TUL3, , TUL5, TUL9, NC11, 5NC8, 5NC6, 5NC13, 25NC12, 25NC9, 25NC3, 25NC10, 5NC1 and 5NC20).

Sugar fermentation test is used to detect bacteria that ferment various sugars (e.g. Glucose, sucrose, maltose, and mannitol) as well as convert pyruvate (the end product of glycolysis) into gaseous by-products (e.g. hydrogen and CO_2). Bacteria in an effort to generate energy can ferment various simple sugars and this serves as basis for their identification in the laboratory. Plant root secretes 5–21% of photosynthetic matter such as carbohydrates, proteins, secondary metabolites, etc., into the rhizospheric soil environment, generally known as root exudates (Upadhyay et al., 2022). Sugars are the most important regulators that facilitate many physiological processes, such as photosynthesis, seed germination, flowering, senescence, and many more under various abiotic stresses (Sami et al., 2016). Among these, sugars play a crucial role in regulating various physiological processes such as photosynthesis, seed germination, flowering, and senescence, especially under different environmental stresses (Sami et al., 2016). Sugars, particularly sucrose, a commonly secreted disaccharide by plant roots, facilitate the movement of bacteria in the rhizosphere and promote root colonization. For example, sucrose induces solid surface motility (SSM) and root colonization by *Bacillus subtilis* through a signaling cascade, involving the extracellular synthesis of polymeric levan, leading to increased production of surfactin and enhanced flagellation of the cells (Tian et al., 2021). Plant Growth Promoting Rhizobacteria (PGPR) mediated alterations in carbohydrate metabolism significantly contribute to enhancing plant growth, stress

resilience and defense mechanisms. By regulating sugar levels, photosynthetic efficiency and carbohydrate distribution, PGPR optimize various plant physiological processes, ultimately improving crop productivity and sustainability (Su et al., 2024). Therefore, understanding the bacterial utilization of different sugar forms holds great importance in augmenting plant growth.In our study, 63 isolates were able to ferment sucrose (LUMB1, LUMB4, LUMB8, LUMITI7, LUMBB9, LBS18, LBS25A, LBS25B, LBS16, LBS44B, LBS21, LBS13, LBS14, LBS29, LBS7, LBS15, LBS17, 5E8, 25E12, 25R23, 25E10, 5E22, E24, 5R12, 25EZ1, RZ23, 5E9, RZ7, 25R10, RZ27, 25R2, 5R9, 25E22, 25E5, EZ27, 25EZ2, 5E3, 25EZ17, RZ5, 25R1, 25E3, TSU1, TSU2(4), TSU1(5), TSU4, TSU8, TSU6, TSU11, TSU12, TSU2(2), TSU3(7), TSU3(2), TSU3(6), TSU3(4), TUL6, TUL11, TUL12, NC11, 5NC13, 5NC1, 5NC20, NC26 and NC7), 65 could ferment maltose (LUMB1, LUMB3, LUMB4, LUMB8, LUMITI1, LUMITI2, LUMITI6, LUMBB4, LUMBB7, LUMBB9, LBS18, LBS25A, LBS25B, LBS16, LBS44B, LBS21, LBS13, LBS14, LBS29, LBS7, LBS15, LBS17, 5E8, 25E12, 25R23, 25E10, 5E22, E24, 5R12, 25EZ1, RZ23, 5E9, RZ7, 25R10, RZ27, 25R2, 5R9, 25E22, 25E5, EZ27, 25EZ2, 5E3, 25EZ17, RZ5, 25R1, 25E3, TSU1, TSU2(4), TSU1(5), TSU4, TSU8, TSU6, TSU11, TSU12, TSU2(2), TSU3(7), TSU3(2), TSU3(6), TSU3(4), TUL6, TUL11, TUL12, NC11, 5NC13, 5NC1, 5NC20, NC26 and NC7), 38 could ferment mannitol (LUMB8, LUMITI4, LBS12A, LBS18, LBS25A, LBS36, LBS25B, LBS16, LBS44B, LBS21, LBS13, LBS14, LBS29, LBS23, LBS7, LBS15, LBS19, LBS12, EZ11, RZ23, EZ30, EZ27, RZ20, 25E3, TSU1, TSU8, TSU6, TSU12, TSU2(2), TSU3(7), TSU3(2), TSU3(6), TSU3(4), NC11, 5NC8, 5NC13, NC26 and NC7), 93 could ferment glucose (LUMB1, LUMB4, LUMB8, LUMITI4, LUMITI6, LUMITI7, LUMBB1, LUMBB6, LUMBB9, LBS18, LBS25A, LBS36, LBS25B, LBS16, LBS44B, LBS21, LBS13,

LBS14, LBS29, LBS23, LBS7, LBS19,LBS12, LBS17, 5E8, 25E12, 25R23, 25E10, 5E22, EZ11, E24, 5R12, 25EZ1, RZ23, 5E9, RZ7, 25R10, RZ27, 25R2, EZ30, 5R9, 25E22, 25E5, EZ27, 25EZ2, 5E11, 5E3, 25EZ6, 25EZ17, RZ20, 5EZ13, 25R1, 25E3, TSU1, TSU2(4), TSU4, TSU8, TSU6, TSU7, TSU11, TSU12, TSU3(7), TSU3(2), TSU3(6), TSU3(4), TUL2, TUL3, TUL4, TUL5, TUL6, TUL8, TUL9, TUL10, TUL11, TUL12, NC11,5NC8, 5NC10, 5NC14, 25NC8, 5NC6, 5NC13, 5NC17, 25NC12, 25NC10, 5NC1, 5NC20, NC26 and NC7), and 42 could ferment sorbitol (LUMB3, LUMB8, LUMB10, LUMITI6, LUMITI7, LUMBB6, LUMBB9, LBS11, LBS25A, LBS14, LBS29, LBS7, LBS4, LBS15, RZ23, EZ27, 25E3, TSU1, TSU2(4), TSU3, TSU1(5), TSU8, TSU6, TSU11, TSU12, TSU2(2), TSU3(7), TSU3(2), TSU3(6), TSU3(4), TUL1, TUL3, TUL5 and TUL9).

Conclusion

Based on the analysis of the bacterial strains isolated form *Musa* rhizosphere, it can be concluded that the *Musa* rhizosphere hosts a diverse array of bacterial strains, as evidenced by their varied colony morphologies and biochemical reactions. In terms of sugar utilization as carbon source, glucose emerged as the preferred for the majority of the bacterial strains, indicating its importance in the rhizosphere ecosystem. The majorities of the isolates were negative for citrate and methyl red tests, suggesting limited utilization of citrate and mixed acid fermentation pathways. However, a significant proportion of isolates were positive for catalase activity, indicating their ability to metabolize hydrogen peroxide. Additionally, the starch hydrolysis test revealed that fewer isolates possess the enzymatic machinery required to break down starch, indicating a lesser prevalence of this metabolic trait among rhizosphere bacteria. A notable proportion of isolates exhibited motility and were gram-negative, highlighting the prevalence of

mobile, gram-negative bacteria within the *Musa* rhizosphere. Overall, these findings underscore the complexity and heterogeneity of the microbial community inhabiting the *Musa* rhizosphere, with implications for nutrient cycling, plant-microbe interactions, and ecosystem functioning. Further research into the specific roles and interactions of these diverse bacterial strains will contribute to a deeper understanding of rhizosphere ecology and agricultural sustainability.

Chapter - 3

Screening of the Plant Growth Promoting Traits of PGPR Isolates and Their Molecular Characterization

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Introduction

In agricultural cultivation, chemical fertilizers are frequently employed to increase yields. Nonetheless, this approach disregards the rhizosphere's and roots' biological potential. (Meena et al., 2017). Microorganisms have been shown in numerous studies to improve plant nutrition and lower pesticide usage (Aloo et al., 2019). PGPR are beneficial microbes that can enhance plant growth and health by various mechanisms. They improve or enhance the plants growth by different mechanisms such as phosphate solubilisation, phytohormones, siderophore production, stress tolerance (Tatung and Deb, 2021). Successful plant colonization by PGPR relied on multiple factors including but not limited to withstand environmental stress, efficient chemotaxis effective communication within the bacterial communities and between bacteria and plants carried

out by synthesized plant hormones and quorum sensing (Mwita et al., 2016). Rhizobacteria plays an important role in maintaining soil fertility and improving plant growth and development. This growth enhancement takes place by several mechanisms as seen above, but there are also some harmful aspects of PGPR (Suslow and Schroth, 1982; Alstrom and Burns, 1989; Saharan and Nehra, 2011). For example, the production of cyanide is a well-known characteristic of certain *Pseudomonas* species. The cyanide production by the bacteria is considered as growth promoting as well as growth inhibiting characteristic because on one hand, cyanide act as a biocontrol agent against certain pathogen (Martinez-Viveros et al., 2010) while on the other hand, it can also cause adverse effects on plant growth (Bakker and Shippers, 1987). Similarly, the auxin production by the PGPR can also cause positive as well as negative effect on plant growth (Eliasson et al., 1989; Kim and Mulkey, 1997; Vacheron et al., 2013). The effectiveness of auxin depends upon its concentration. For example, at low concentration, it enhances plant growth (Patten and Glick, 2002), but, at higher level it inhibits root growth (Xie et al., 1996). PGPR supports sustainable agriculture development and protect the environment (Das et al., 2013). However, microbes to be used as biofertilizer must come under the category of generally recognized as safe and have low harmful effects on human health (Kang et al., 2015). Adesemoye et al. (2008) showed by their study that inoculation of the corn plant with PGPR and AMF has increased yield and promoted plant growth. They also concluded that these microbial inoculants were very much effective in removing the build-up of N, P, and K in agricultural soils. So PGPR are a great alternative to move in the direction of a sustainable agricultural system.

While soils contain a considerable amount of phosphorus in both organic and inorganic forms, its availability is limited due to its predominantly insoluble nature

(Sharma et al., 2013). Phosphate solubilising bacteria provide an eco-friendly solution by converting insoluble phosphates into forms accessible to plants (Pathak et al., 2018). *Pseudomonas chlororaphis* and *Pseudomonas fluorescens* markedly improved plant height, shoot and root dry weight and phosphorus and nitrogen uptake in walnut seedlings, with the greatest increases observed when these strains were combined with *Bacillus cereus* and tricalcium phosphate (TCP) addition (Yu et al., 2011b). Among the types of auxins, indole acetic acid (IAA) plays a crucial role. IAA plays a dual role in promoting plant growth and participating in biocontrol activity by working with glutathione- S-transferases in defence- related plant reactions, and it also inhibits the germination of spores and the growth of mycelium of various pathogenic fungi (Bakthavatchalu et al., 2012). Additionally, several environmental factors can influence the biosynthesis of this phytohormone, with high pH and the presence of large quantities of tryptophan leading to increased production (Dosselaere and Vanderleyden, 2001). *In vitro* studies have shown that some microorganisms can produce small amounts of auxins in the absence of L-TRP. However, in its presence; the microorganism produces much greater quantities of auxins (Khalid et al., 2004; Zahir et al., 2010).

Siderophore producing microbes have proven to be excellent biocontrol agents, enhancing plant growth and development (Deb and Tatung, 2024). More than 90% of siderophore-producing bacterial isolates belong to the gram-negative bacteria: *Enterobacter* and *Pseudomonas* dominate. Few gram-positive genera such as *Bacillus* and *Rhodococcus* can produce siderophores (Shah et al., 2021). Siderophore producing bacterial strains possess iron regulated outer membrane proteins (IROMPs) on their cell surface that transport ferric iron complex to the respective cognate membrane, iron thus becomes available for metabolic processes (Sayyed et al., 2013). Ammonia

production by fungal isolate has been reported for biocontrol activities and supply for nitrogen which ultimately results in plants growth and development (Khalil et al., 2021). Wang et al. (2022c), reported that *Burkholderia* sp. which produces catecholate type siderophores, effectively binds Fe^{3+}, Zn^{2+}, and Cd^{2+}, increasing the germination rate of *Lollium perenne* and promoting germination under Cu^{2+}, and Zn^{2+} stress. Additionally, siderophilic bacteria (*Bacillus* sp.) significantly promoted the germination of pepper and maize seeds and the development of shoots and leaves in *Gynura divaricata*, also enhancing root elongation (Wang et al., 2022d). Environmental stress negatively impacts food productivity by generating reactive oxygen species (ROS), which damage cell organelles and biomolecules, leading to apoptosis (Karnwal et al., 2023). Other environmental stress is heavy metal contamination and salinity stress. Heavy metals are typically present in the environment at trace levels, ranging from parts per billion (ppb) to less than 10ppm (Manoj et al., 2020).Salt stress causes imbalance ion hemostasis (Na^+ and K^+), disrupts mineral absorption and leads to oxidative stress though the production and accumulation of reactive oxygen species (ROS). Accumulation of ROS is extremely harmful and potentially lethal for plants (Neshat et al., 2022). Reports by Karnwal et al. (2023), suggest that PGPR can mitigate the adverse effects of salinity, drought, heavy metals, floods and other stresses on plants by inducing the activity of antioxidant enzymes such as catalase, peroxidase and superoxide dismutase. For instance, inoculating wheat plants with *B. megaterium*, *B. tequilensis* and *P. putida* improved relative water content, photosynthetic pigments, reduced malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) concentrations, lowered electrolyte leakage and enhanced enzymatic activity for ROS scavenging (Haroon et al., 2021).

This chapter dealt with purification of bacterial isolates, screening for various growth promoting traits such as phosphate solubilisation, IAA production, siderophore production, salinity tolerance test, heavy metal tolerance test and ammonia production test and molecular characterization of PGPR isolates with the following objectives:

- Qualitative and quantitative estimation of phosphate solubilisation, IAA production and siderophore production by the bacterial isolates.
- Salinity and heavy metal stress tolerance test.
- Qualitative analysis of ammonia production by the bacterial isolates.
- Molecular characterization of selected PGPR strains.

Material and Methods

PGPR can enhance the plants growth through both direct and indirect methods. Direct methods involve activity such as phosphate solubilisation and production of indole 3-acetic acid (IAA). On the other hand, indirect methods encompass the production of siderophore, induced systemic resistance, and the ability to confer biotic and abiotic stress such as tolerance to heavy metal and salinity.

Qualitative assay of phosphate solubilisation activity

Bacterial isolates were grown on National Botanical Research Institute's phosphate (NBRIP) growth medium to solubilise phosphate supplemented in the media with media composition gL^{-1}; 10g glucose, 5g $Ca_3(PO_4)_2$, 5g $MgCl_2$, $6H_2O$, 0.25g $MgSO_4$, $7H_2O$, 0.2KCl, 0.2g and 0.1g $(NH_4)_2SO_4$ and 15g agar (You et al., 2020). Bacterial isolates were incubated at 28±2º C for 7 days after being inoculated on agar plates. Isolates with a clear halo zone around the colony were found to be phosphate solubilising. The following formula was used to calculate the phosphate solubilising index (PSI):

$$\text{Phosphate solubilizing index} = \frac{\text{Colony diameter + Halo zone}}{\text{Colony diameter}}$$

Quantitative assay of phosphate solubilisation

Quantitative analysis for phosphate solubilisation was also done in liquid media as described by Pande et al. (2017), 'P' solubilisation was estimated in 40 ml of NBRIP broth (g/litre; 10g glucose, 5g $Ca_3(PO_4)_2$, 5g $MgCl_2,6H_2O$, 0.25g $MgSO_4, 7H_2O$, 0.2g KCl, 0.1g $(NH_4)_2SO_4$, pH 7.0) in 250 ml conical flask. The broth without culture inoculated has served as control. The test strains were grown overnight and incubated in NBRIP broth for 12 days at 28±2ºC.

After incubation, 1 ml of supernatant was taken out on 2nd, 4th, 6th, 8th, 10th, and 12th day. The supernatant was obtained by centrifugation at 10,000 rpm for 20 min and the 600µl of filtered supernatant was mixed with 1500µl of Barton's reagents and volume was made up to 5ml with double distilled water (2.9 ml). After 10 min the intensity of yellow colour was read on spectrophotometer (Thermo Scientific Multiskan Go) at 430nm and the amount of P solubilised was extrapolated from standard curve. The experiments were conducted in triplicates and values were expressed as their mean.

Preparation of standard solution

To prepare the standard, 0.02195 g of potassium dihydrogen orthophosphate/ monopotassium phosphate (KH_2PO_4) was added in 1000 ml of double distilled water and labelled as stock p solution to get 50µg/ml. A further dilution of 0, 10, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500,550 and 600µg/ml was made to get standard curve of KH_2PO_4. After 10 min the intensity of yellow colour developed was read at 430 nm spectrophotometrically. Standard curve was prepared by plotting absorbance at 430nm vs. concentration of P.

Preparation of Barton's reagent

For the preparation of Barton's reagent two solutions were prepared as follows:

Solution 1: 25 gm Ammonium molybdate was dissolved in 400 ml distilled water.

Solution 2: 1.25grms Ammonium metavandate was dissolved in 300 ml of boiling water and cooled and then 250 ml of concentrated HNO_3 added. After the solutions were prepared, both were mixed and the volume was made up to 1 litter with distilled water.

Qualitative assay of indole acetic acid (IAA) production

Ten ml of nutrient broth supplemented with tryptophan (0.1%, w/v) was inoculated with freshly grown bacterial culture and incubated in a shaking incubator at 28±2ºC for 7 days to estimate IAA production (Kumar et al., 2012a). Ten ml nutrient broth with 0.1% tryptophan without inoculation was considered as control. After 7 days 1 ml of the culture broth was taken and centrifuged for 10 min at 10,000 rpm. After that 1 ml of the supernatant was taken and transferred to glass vial containing 2 ml of the Salkowski reagent. The mixture was then incubated for 25 min and the formation of pink colour solution was observed which indicated the production of IAA by the isolate.

Quantitative assay of indole acetic acid (IAA) production

Isolates were kept for 12 days inside the shaker incubator at 28±2º C. On 4^{th}, 6^{th}, 8^{th}, 10^{th} and 12^{th} day, 1 ml of the culture was taken and centrifuged at 10,000 rpm for 10 min. Supernatant was then transferred to a vial containing 2 ml of the Salkowski reagent to test tubes labelled with each strain (Kumar et al., 2012a). After 25 min of incubation, cultures showing pink colour formation were identified as positive for IAA production. 200 µl of the mixture of reagent and bacterial culture supernatant was transferred to a 96 well microplate and the O.D. was measured at 530nm. The absorbance at 530nm was

plotted against the concentration for the standard curve, and the concentration (µg/ml) was obtained by plotting the absorbance of the bacterial strains.

Preparation of Salkowski reagent

For Salkowski reagent two solutions are required first 35% perchloric acid and then ferric chloride solution.

Preparation of 35% perchloric acid from 70% acid

To make Salkowski reagent first 35% of perchloric acid was prepared. To get 300 ml of 35% perchloric acid, 200 ml of distilled was added in 100 ml of 70% perchloric acid.

Preparation of 0.5M ferric chloride solution

In 10 ml of distilled water, 1.35g of ferric chloride was dissolved to get0.5M of ferric chloride solution. In every 50 ml of 35% perchloric acid add 1 ml of 0.5M ferric chloride solution.

Preparation of standard curve

Standards for IAA of varying concentrations from 0-200µg/ml were prepared to measure the IAA produced by the bacterial isolates over a 12-day period. Since IAA is not soluble in water, it was initially dissolved in acetone. Specifically, 10 mg (0.01g) of IAA was added to 10 ml of acetone in a glass beaker. The mixture was stirred thoroughly with a metal spatula until the IAA was completely dissolved, resulting in a stock solution of 1000ug/ml. A series of vials were labelled for the dilution series. To prepare standards, 1 ml of the 1000µg/ml stock solution was transferred to a vial containing 10ml media and mixed well by inversion to obtain a 100µg/ml standard. For the 50µg/ml standard, 0.5ml of the 1000µg/ml stock solution was transferred to another vial containing 10ml of media. This process was repeated to prepare other standards, ranging from 0 to 200µg/ml. Once the standards were prepared, 2ml of the Salkowski reagent was added to each vial. The solutions were then incubated at room temperature for 25 min. The colour changes across

the concentration gradient were noticeable within minutes, and the optical density (O. D.) was read at 530nm to determine the concentration of IAA produced by the bacterial isolates.

Analysis of siderophore production

Preparation of CAS agar medium

Chrome Azurol Sulfonate (CAS) agar medium was prepared following Srimathi and Suji (2018) with suitable modification. To prepare CAS agar media first CAS reagent was prepared. Reagent was first prepared in the form of three different solutions and finally mixed together to get the final reagent. For Solution 1, 0.06 g of CAS powder was dissolved in 50 ml of distil water; while for Solution2, 0.0027 g of $FeCl_3$, $6H_2O$ was dissolved in 10 ml of 10 mM HCl. To make 10mM HCL solution, 36.46 ml of HCL was taken and made up to 100 ml with distilled water. And for Solution 3, 0.073 g of hexadecyl trimethyl ammonium (HDTMA) was dissolved in 40ml of ddH_2O and kept in over at 50ºC for 5 min to completely dissolve the HDTMA. Finally, Solution 1 was blended with 9 ml of Solution 2 and then its mixture was added with Solution 3 and a blue colour solution was resulted, which is called the CAS reagent. The final solution was autoclaved and stored in a plastic holder/bottle in the fridge at 4ºC for future use. The resulted reagent is mixed with culture media to the blue colour media for detection of siderophore production by bacterial and fungal isolates. The CAS agar medium was prepared using the reagent prepared and sterilized. To make the CAS agar media, nutrient agar (NA) was used. Nutrient gar media was prepared by boiling and later autoclaving to sterilize it. The reagent was also sterilized in the autoclave along with the media in a separate conical flask. After the sterilization, both the media and reagent were brought to laminar air chamber flow and cooled down at 50ºC. Once the media and the reagent were

cooled down at desirable temperature, both the medium and reagent were mixed after stirring lightly. While adding the blue dye (reagent) solution it was slowly poured along the glass wall with enough agitation to mix thoroughly and poured onto the sterilized petri plates.

Qualitative estimation of siderophore production

This assay was performed following the protocol of Hu and Xu, (2011). CAS agar plates were prepared by mixing 100ml CAS reagent in 900ml sterilized NA agar medium. Four bacterial strains were spot inoculated on each plate. An un-inoculated plate was taken as control. After inoculation, plates were incubated at 28±2ºCfor 7 days and observed for the formation of orange zone around the bacterial colonies. Formation of orange halo zone around the bacterial colony indicated positive result.

Quantitative estimation of siderophore production

Isolates were grown in 20 ml of CAS nutrient broth for 14 days at 28±2º C. On the 2^{nd}, 4^{th}, 6^{th}, 10^{th} and 12^{th} day 2ml broth was taken from each culture vial and centrifuged at 1000 rpm for 10 min.200 µl of the supernatant from each culture was taken and the absorbance was read at 630nm using micro plate reader. Siderophore produced by strains was measured in percent siderophore unit (PSU) following formula given by Payne (1993):

$$\text{Siderophore production (PSU)} = \frac{(A_r - A_s) \times 100}{A_r}$$

Where, $\mathbf{A_r}$= absorbance of reference (CAS solution and uninoculated broth), and $\mathbf{A_s}$ = absorbance of sample (CAS solution and cell-free supernatant of sample).

Ammonia production

Purified bacterial isolates were grown in 5ml nutrient broth and incubated in shaking incubator at 28±2ºC for 48 h. Then 10 ml of peptone water broth g/l (peptone: 20 g and sodium chloride: 1 g) was inoculated with the freshly grown isolates for 48 h at room temperature with constant shaking. One ml of the Nessler's reagent (Mercuric chloride: 10 gm/l Potassium iodide: 70 gram/l, and Sodium hydroxide 160gm/l) was added in the broth and incubated for 10 min. Positive ammonia production cases were considered where a faint yellow colour developed post Nessler's reagent addition and quantity of ammonia production is directly proportional with the depth of the yellow colour. Isolates giving light yellow colour after adding the reagent was considered as isolates producing a small amount of ammonia whereas isolate producing dark yellow and orange colour indicated a medium and high amount of ammonia production, respectively.

Salinity tolerance test

The bacterial isolates were screened for salinity resistant test by following the method used by Sharma et al. (2021). Pure isolates of the bacteria were grown on nutrient agar media with different concentration of salt (2%, 4%, 6%, 8%, 10%, 12% and 14%). The inoculated plates were incubated at 28±2º C for three days. The growth of the isolates the plates indicated their resistance.

Heavy metal tolerance test

Bacterial isolates were tested for $Cd^{2+}(CdCl_2, H_2O)$, $Cu^{2+}(CuSO_4)$, Ni^{2+} $(NISO_4, H_2O)$, As^{3+} $(NaAsO_2)$, $Sb^{3+}(K_2(SbO)_2C_8H_4O_{40}, 3H_2O)$, $Zn^{2+}(ZnSO_4,7H_2O)$, and $Cr^{2+}(K_2CrO_4)$ tolerance using the minimum inhibitory concentration (MIC), as described by Yadav et al. (2022). On the nutrient agar plates supplemented with different

concentrations (30-10010g/ml) of heavy metal, the bacterial isolates were streaked. The appearance of bacterial growth after incubating the plates at room temperature for 24-48h at 28±2ºC was used to determine heavy metal tolerance. All experiments were carried out in triplicate. The concentration of heavy metals on NA plates was gradually increased until the strains could no longer grow on the plates. By streaking on the plates, the culture growing on the last concentration was transferred to the higher concentration. When the isolates failed to grow, the MIC was determined.

Molecular identification of the bacterial isolates

Some of the isolates were selected for molecular characterization. For molecular identification of the bacterial isolates, colony PCR was done. Bacterial isolates were freshly streaked and incubated for 24 h in nutrient agar media. To pick the colony, sterilized toothpicks were used. A loop full of bacterial colony was taken and suspended in 60 µl of triton x 100 buffer in PCR tubes. It was then boiled for 10-15 min. After boiling the tube was then taken out and kept in the freezer for 2-3 min. It was then centrifuged for 3-4 min at 10, 0000 rpm for 3 min. The PCR amplification of the target sequence was carried out with 0.6µl of dNTPs, 3µl of buffer, 21.7µl of sterile deionized water, 3µl of the template, 0.6µl of both primers [1492R (5'GGTTACCTTGTTACGACTT3') reverse primer and 18F (5'AGAGTTTGATCCTCAG3') forward primer] (Thanh and Diep, 2014) and 1µl of the *Taq* DNA polymerase. The reaction was performed in the Bio-Rad thermal cycler with 95ºC for the early denaturation stage followed by 30 cycles of 94ºC for 50 sec, 55ºC for 90 sec, 72ºC for 1 min, and last extension step at 72ºC for 3 min. PCR product of the 16S *rRNA* was confirmed on 1% (w/v) agarose gel. The gene sequences obtained were equated with others in the GenBank databases using the NCBI BLAST. Sequences were

then submitted to the NCBI GenBank database and accession numbers were obtained. A phylogenetic tree was made using MEGA11 software.

Statistical Analysis

The SPSS software was used for statistical analysis of the experimental data. All the reported results are the mean of the three replicates and deviations were calculated as the standard error of the mean (SEM).

Results

Phosphate solubilisation

Out of 136 isolates, 94 isolates were able to display the capability to solubilise phosphates when cultured on National Botanical Research Institute's phosphate medium (NBRIP) as evidenced by the formation of a distinct halo zone surrounding the bacterial colony (**Figure 3.1**). Among the positive isolates, the highest phosphate solubilising index (PSI) was observed by LUMB8 (4.23±0.19), LBS16 (4.09±0.22), 5R3 (4.09±0.22), 25EZ18 (4.07±0.03), LBS14 (4.02±0.24), LBS21 (3.95±0.16), EZ30 (3.60±0.06), TSU1 (3.55±0.17), 25Z13 (3.43±0.03), EZ11 (3.43±0.07), TSU8 (3.17±0.09), TSU7 (3.08±0.21), TSU2(2) (3.23±0.15), TSU6 (2.92±0.08), EZ20 (2.91±0.24), LUMB4 (2.81±0.18) and EZ27 (2.80±0.3). Other positive isolates showed PSI ranging from 1.12 to 2.91±0.24 (**Table 3.1**).

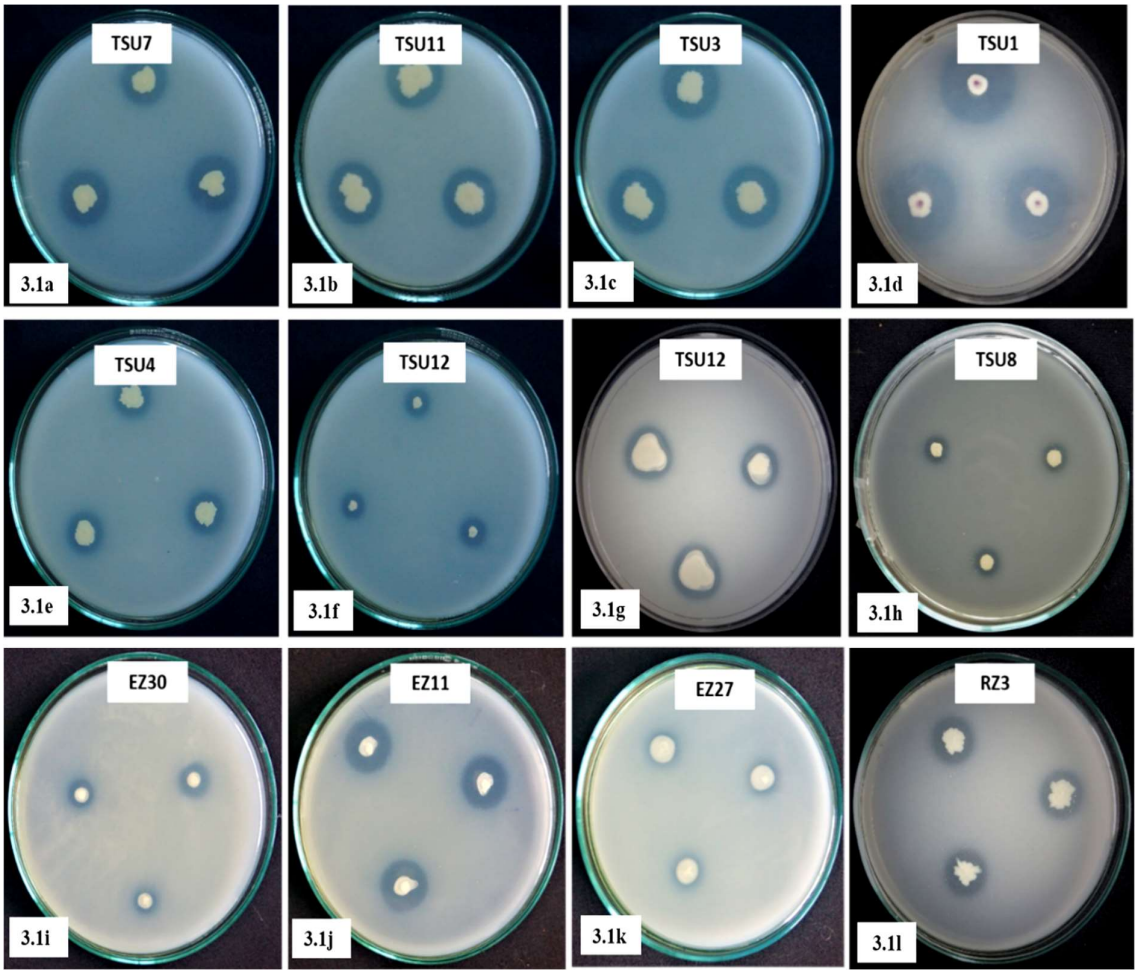

Figure 3.1: Phosphate solubilisation test by the bacterial isolates on NBRIP agar media. Development of clear halo zone around the colony indicates the ability to solubilise phosphate by the isolates.

Table 3.1: Qualitative estimation Phosphate solubilisation by PGPR strains

Sl. No.	Isolates No.	P Solubilisation	PSI± SE*	Sl. No	Isolate No.	P Solubilisation	PSI± SE*	Sl. No.	Isolate No.	P Solubilisation	PSI± SE*
1	LUMB1	+	2.14 ± 0.01	49	LBS4	+	2.78 ± 0.15	97	25E20	+	1.37 ± 0.03
2	LUMB2	+	2.23 ± 0.06	50	LBS31	-	-	98	5E11	+	1.23 ± 0.03
3	LUMB3	+	2.39 ± 0.14	51	LBS15	+	2.38 ± 0.05	99	5E3	+	1.27 ± 0.03
4	LUMB4	+	2.81 ± 0.18	52	LBS19	+	2.19 ± 0.04	100	25EZ6	+	1.63 ± 0.07
5	LUMB5	-	-	53	LBS12	+	2.24 ± 0.02	101	25EZ17	-	-
6	LUMB6	-	-	54	LBS17	-	-	102	RZ5	+	1.97 ± 0.03
7	LUMB7	-	-	55	LBS22	-	-	103	RZ20	+	1.97 ± 0.03
8	LUMB8	+	4.23 ± 0.19	56	5E20	+	2.91 ± 0.24	104	5EZ13	+	1.87 ± 0.03
9	LUMB9	-	-	57	5E8	+	1.27 ± 0.03	105	5R3	+	4.09 ± 0.22
10	LUMB10	-	-	58	25E12	+	1.30 ± 0.06	106	25R1	+	1.17 ± 0.03
11	LUMITI1	+	1.15±0.09	59	5E24	-	-	107	25E3	+	2.55 ± 0.04
12	LUMITI2	+	2.67 ± 0.04	60	5E5	-	-	108	TSU1	+	3.55 ± 0.17
13	LUMITI3	+	2.37 ± 0.09	61	25R23	+	1.27 ± 0.03	109	TSU2(4)	+	2.58 ± 0.07
14	LUMITI4	+	2.51 ± 0.09	62	25E10	-	-	110	TSU3	+	2.75 ± 0.13
15	LUMITI5	+	2.47 ± 0.12	63	5R1	-	-	111	TSU1(5)	-	-
16	LUMITI6	+	2.21±0.01	64	5R5	-	-	112	TSU4	+	2.40 ± 0.12
17	LUMITI7	+	2.49 ± 0.11	65	5E22	+	1.90 ± 0.06	113	TSU8	+	3.17 ± 0.09
18	LUMBB1	+	2.48 ± 0.18	66	5R16	-	-	114	TSU6	+	2.92 ± 0.08
19	LUMBB2	+	2.60 ± 0.20	67	EZ11	+	3.43 ± 0.07	115	TSU7	+	3.12 ± 0.18
20	LUMBB3	+	2.58 ± 0.09	68	E24	+	1.35 ± 0.03	116	TSU11	+	2.64 ± 0.05
21	LUMBB4	+	2.54 ± 0.06	69	25E18	+	4.07 ± 0.03	117	TSU12	+	2.19 ± 0.01

22	LUMBB6	+	2.72 ± 0.03	70	5R15	+	1.23 ± 0.03	118	TSU9	-	-
23	LUMBB7	+	2.83 ± 0.17	71	5R12	+	2.11 ± 0.21	119	TSU2(1)	+	2.31 ± 0.09
24	LUMBB8	-	-	72	25EZ1	+	1.53 ± 0.03	120	TSU2(2)	+	3.23± 0.15
25	LUMBB9	-	-	73	RZ23	+	1.27 ± 0.03	121	TSU3(7)	-	-
26	LBS11	-	-	74	5E9	-	-	122	TSU3(2)	-	-
27	LBS12A	+	2.24 ± 0.02	75	5E28	+	1.97 ± 0.03	123	TSU3(6)	-	-
28	LBS18	-	-	76	25E16	+	1.53 ± 0.03	124	TSU3(4)	-	-
29	LBS25A	+	1.37 ± 0.03	77	RZ7	+	2.57 ± 0.03	125	TUL1	-	-
30	LBS36	+	1.30±0.13	78	25R10	+	1.47 ± 0.03	126	TUL2	+	1.76±0.02
31	LBS25B	-	2.12 ± 0.02	79	RZ27	+	1.57 ± 0.03	127	TUL3	+	1.34±0.08
32	LBS16	+	4.09 ± 0.22	80	25R2	+	1.67 ± 0.03	128	TUL4	-	-
33	L.BS44A	-	-	81	EZ30	+	3.60 ± 0.06	129	TUL5	+	1.89±0.05
34	L.BS44B	-	-	82	5R9	-	-	130	TUL6	+	1.21±0.01
35	L.BS21	+	3.95 ± 0.16	83	5R10	+	2.17 ± 0.03	131	TUL8	+	2.01±0.05
36	L.BS13	+	2.33 ± 0.09	84	25Z13	+	3.43 ± 0.03	132	TUL9	-	-
37	LBS14	+	4.02 ± 0.24	85	25E22	+	2.35 ± 0.02	133	TUL10	-	-
38	LBS29	-	-	86	25E5	+	1.30 ± 0.06	134	TUL11	+	1.25±0.01
39	LBS23	+	2.24 ± 0.01	87	EZ27	+	2.80 ± 0.3	135	TUL12	+	2.37 ± 0.11
40	LBS7	+	1.12 ± 0.01	88	25EZ2	+	1.37 ± 0.03	136	NC11	+	1.25±0.03
41	5NC8	+	1.67±0.05	89	25NC12	-	-				
42	5NC10	+	2.34±0.08	90	25NC9	+	2.11 ± 0.21				
43	5NC14	+	1.20±0.12	91	25NC3	+	2.01 ± 0.01				
44	25NC8	+	1.15±0.16	92	5NC17	+	1.37 ± 0.03				
45	5NC6	-	-	93	25NC12	-	-				
46	5NC13	-	-	94	25NC10	-	-				
47	NC7	+	2.47±0.02	95	5NC1	-	-				
48	NC26	-	-	96	5NC20	+	1.57 ± 0.03				

Note: '+': Indicates present, '- ': Indicates absent, *PSI = Phosphate solubilising index.

* ±SE: Standard error from mean.

Table 3.2: Quantitative estimation of phosphate solubilization and change in *pH* by the selected bacterial isolates

Bacterial isolates	P Solubilisation by the Bacterial Isolates (µg/ml)						*pH* of the medium					
	2nd day ±SE*	4thday ±SE*	6th day ±SE*	8th day ±SE*	10th day ±SE*	12th day ±SE*	2nd day ±SE*	4th day ±SE*	6th day ±SE*	8th day ±SE*	10th day ±SE*	12th day ±SE*
30E13	39.41±1.24	46.31±0.98	67±2.56	104.93±2.05	177.34±3.45	215.28±1.32	5.32±0.98	5.21±5.76	4.89±4.56	4.76±2.01	4.51±1.34	4.38±2.37
RZ27	41.87±0.89	86.25±1.67	107.39±1.56	164.52±3.04	223.17±5.05	107.93±2.17	4.98±0.96	4.70±1.56	4.54±2.12	4.52±2.09	4.46±1.78	4.22±2.09
RZ5	18.72±4.23	18.73±1.78	18.80±1.67	15.29±0.97	15.28±2.54	15.23±0.94	6.34±1.56	6.46±3.01	6.43±0.67	6.49±0.89	6.53±1.32	6.67±1.43
EZ11	36.98±2.65	88.69±1.45	105.93±2.57	140.41±1.24	187.24±3.44	225.62±5.01	4.91±1.34	4.87±0.56	4.65±2.34	4.51±1.43	4.47±1.05	4.31±1.76
30E22	135.97±2.45	177.34±2.34	204.93±1.98	225.62±1.07	291.14±0.67	118.28±0.98	4.45±1.56	4.43±1.34	4.4±2.89	4.38±1.09	4.35±1.56	4.39±2.31
10E13	56.66±4.98	118.72±3.23	146.83±0.98	160.1±1.45	211.48±1.78	235.97±2.98	4.62±0.45	4.56±8.12	4.45±0.45	4.4±0.98	4.33±1.56	4.27±3.33
30E18	43.87±3.23	99.03±1.66	105.48±2.32	125.62±2.56	187.69±6.34	263.55±1.78	4.92±5.67	4.71±3.56	4.69±7.21	4.65±2.45	4.48±0.87	4.43±1.24
10E9	22.17±2.45	84.24±4.04	149.76±2.45	184.24±0.45	273.9±1.67	125.62±0.43	5.35±1.56	4.7±2.56	4.54±3.21	4.46±1.67	4.43±3.56	4.53±2.87
30E1	49.76±0.96	67±1.56	80.79±3.21	108.38±2.56	170.45±3.21	242.86±1.45	5.42±1.67	5.29±3.21	4.91±3.24	4.87±3.67	4.46±2.90	4.27±2.17
10R5	18.72±0.99	19.78±1.94	17.73±1.98	ND	ND	ND	6.29±4.2	5.29±1.88	6.47±1.56	6.7±0.98	6.71±0.67	6.64±1.23
30E24	67±0.67	108.38±1.56	132.52±2.01	180.79±0.99	232.51±1.29	329.07±1.33	4.99±0.78	4.84±0.77	4.75±1.22	4.57±0.91	4.43±2.34	4.20±2.12
30E3	98.03±2.01	170.45±2.02	242.86±0.99	273.9±1.90	339.41±1.78	356.66±0.89	4.67±0.56	4.56±1.23	4.42±1.11	4.34±0.98	4.28±2.03	4.17±2.56
EZ30	67±1.67	101.48±1.05	129.07±1.50	160.1±2.00	187.69±0.98	240.93±2.33	5.04±0.45	4.84±4.89	4.74±0.99	4.59±0.98	4.50±1.00	4.51±1.23
RZ20	42.86±5.90	49.76±2.78	70.45±3.33	104.93±1.89	149.76±5.78	156.98 ±4.44	5.79±0.97	5.26±1.54	4.89±0.78	4.69±1.22	4.49±1.11	4.35±0.98
10R9	132.52±3.67	187.69±4.09	267±1.56	332.52±3.21	360.1±45	332.52±3.21	4.61±1.11	4.56±0.49	4.39±1.78	4.35±0.48	4.29±0.12	4.43±1.21
RZ23	18.72±1.65	29.07±1.45	46.31±2.31	56.66±2.76	63.55±1.90	70.45±1.78	5.95±1.09	5.85±0.99	5.34±0.97	5.32±1.21	5.23±1.56	4.21±0.89
10R16	49.76±1.78	56.66±2.21	80.79±5.76	135.97±4.32	187.69±2.55	246.31±7.09	5.23±1.90	5.01±0.89	4.99±2.44	4.73±1.78	4.53±1.05	4.49±1.99
30E17	80.79±0.09	125.62±0.07	153.2±1.03	145.86±2.89	142.86±3.21	132.52±1.89	4.72±0.09	4.76±0.09	4.74±0.20	4.82±1.99	4.97±1.01	4.99±2.09
30E5	149.76±4.09	208.38±5.23	249.76±3.89	260.1±1.59	280.79±3.33	287.68±3.11	4.51±1.21	4.49±1.20	4.51±0.91	4.49±0.89	4.48±0.92	4.46±0.97
30E6	56.66±2.31	84.24±2.31	108.38±1.89	149.76±1.78	232.52±1.66	298.03±4.32	5.1±0.09	4.8±0.04	4.73±1.04	4.61±2.00	4.37±1.23	4.35±0.98
10E24	56.66±2.09	98.03±2.08	115.28±1.79	141.83±2.81	198.03±3.29	222.17±3.21	4.81±1.06	4.78±2.09	4.72±1.89	4.67±2.31	5.52±1.89	4.41±1.21
30E23	187.69±1.09	201.48±3.20	242.86±2.23	273.9±4.21	267±2.01	265±3.01	4.48±1.12	4.45±0.09	4.42±0.5	4.41±0.7	4.45±1.01	4.43±1.01
30R10	180.79±2.89	239.41±1.12	253.2±3.01	256.66±1.01	301.48±2.34	342.86±1.34	6.11±1.23	5.69±1.54	5.58±0.98	5.61±0.45	5.47±0.66	4.37±1.03
RZ15	153.21±2.34	204.93±5.12	218.72±7.09	218.72±3.89	260.1±4.09	225.62±2.67	5.76±1.09	5.79±1.23	5.83±1.89	6.01±2.09	5.74±1.77	5.76±1.09

LBS25(B)	52.86 ±3.62	121.44±0.47	93.21±3.60	83±4.09	65.25±8.51	62.01± 2.90	4.56±1.08	4.34±2.67	4.44±2.99	5.06±2.90	5.11±1.56	5.13±1.09
LBS14	54.29±2.44	144.31±5.15	83.09±3.37	43.25±1.43	22.99±2.89	9.45±1.90	4.89±1.22	4.39±1.90	4.50±1.20	5.09±1.90	6.25±1.24	6.49±3.23
LBS29	31.34±0.75	117.94±3.65	114.16±25.38	101.00±5.14	90.01±2.45	89.12±3.96	6.99±2.56	5.78±2.31	6.01±2.34	6.78±2.45	7.88±2.76	7.89±1.45
LBS19	42.71±0.60	52.72±7.94	132.56±2.29	69.79±12.37	56.23±4.09	52.56±4.89	6.90±2.97	6.04±2.12	5.76±2.56	5.77±2.13	5.68±1.89	5.88±2.13
LBS16	57.25±3.39	83.75±4.40	154.23±6.82	152.69±4.38	110.01±5.01	98.78±4.10	6.98±1.42	6.67±2.15	5.75±2.17	5.78±2.14	5.99±2.76	6.01±2.13
LBS35	18.95 ±5.81	71.79±4.48	89.84±1.11	91.37±8.82	101.09±2.56	143.56±5.01	6.99±2.14	6.80±5.10	6.01±4.12	5.67±1.09	5.45±3.16	4.67±2.19
LBS4	83.83 ±1.82	208.08±2.98	147.06±21.42	125.79±1.70	98.78±2.56	80.98±3.67	5.89±3.78	4.65±3.09	4.78±4.09	4.80±3.18	5.67±2.89	5.88±5.01
LBS7	16.41±0.94	64.23±11.55	103.31±3.18	145.17±19.34	168.65±3.87	176.56±4.97	6.98±1.89	6.56±2.45	5.70±2.43	5.62±2.89	4.66±3.67	4.38±2.14
LBS12(A)	54.49 ±4.04	76.18±7.41	100.44±4.21	100.18±5.82	90.12±2.89	78.45±1.80	6.67±2.10	6.12±2.18	5.01±2.89	5.11±2.80	5.89±3.67	5.99±3.87
LBS18	17.53 ±2.24	107.89±5.43	54.38±1.42	41.82±0.19	12.45±3.41	7.89±4.12	6.89±3.22	5.77±3.23	5.89±4.07	5.90±1.23	6.09±2.11	6.34±2.33
LBS15	45.16 ±4.74	57.90±3.74	124.92±10.68	126.16±1.21	135.09±1.34	140.56±2.07	6.67±2.17	5.89±3.12	4.76±2.78	4.56±3.14	4.34±2.71	4.21±1.12
LBS44(A)	65.36 ±3.33	102.57±0.98	142.11±6.37	137.64±6.32	134.91±2.78	123.76±5.01	6.67±5.87	5.87±3.18	4.95±2.58	4.99±1.74	5.54±3.67	5.76±4.09
LBS13	88.78±13.32	95.53±5.34	98.57±3.65	94.11±12.40	82.98±6.90	78.90±3.76	6.89±4.02	6.70±2.77	6.43±2.11	6.56±4.05	6.78±9.12	6.90±2.18
LBS16	59.05 ±6.31	82.61±0.56	151.32±7.41	154.87±18.47	161.90±3.70	169.05±3.79	6.78±2.11	6.45±2.67	5.78±4.12	4.99±3.45	4.72±4.19	4.51±2.57
LBS11	49.53 ±8.10	110.52±7.87	111.98±2.86	101.32±3.01	98.78±2.78	87.98±3.17	6.56±2.87	5.78±3.19	5.56±2.18	5.57±2.76	6.01±3.65	6.55±2.18
LBS21	27.17±1.08	27.82±1.13	33.95 ±1.47	36.83±2.28	40.23±2.19	42.89±2.15	6.99±2.67	6.98±1.45	6.56±1.58	6.45±3.05	6.32±2.98	6.12±1.05
LBS23	ND	ND	7.95±0.53	13.63±1.08	26.89±1.56	35.76±1.34	6.99±2.1	6.98±2.45	6.67±4.05	6.64±2.18	6.32±3.13	6.35±2.19
LUMBB7	13.17±0.61	14.47±0.57	14.54±0.45	125.79±1.70	98.78±2.56	ND	5.74±3.78	5.09±3.09	4.66±4.09	5.00±3.18	5.67±2.89	5.88±5.01
TSU1	10.69±0.45	21.94±0.64	51.10±0.20	87.90±4.12	90.34±0.45	84.20±3.01	6.48±0.12	5.01±0.34	4.27±1.34	4.43±1.76	5.78±1.56	5.87±1.58
TSU3	15.94±0.58	15.39±2.98	15.55±0.68	125.79±1.70	98.78±2.56	80.98±3.67	5.09±3.78	4.65±3.09	4.52±4.09	4.36±3.18	5.67±2.89	5.88±5.01
TSU4	13.26±1.15	14.471±0.70	14.62±0.85	16.98±1.23	23.21±0.34	27.47±1.49	5.25±0.34	5.23±2.21	4.57±2.54	4.32±2.13	4.27±0.56	4.12±0.87
TSU8	14.90±2.40	14.93±2.24	14.56±2.06	13.65±1.34	10.90±0.15	ND	5.17±1.56	5.12±2.10	5.37±4.12	5.41±0.87	6.12±1.65	6.20±0.34
10RZ23	18.72±1.65	29.07±1.45	46.31±2.31	56.66±2.76	63.55±1.90	70.45±1.78	5.95±1.09	5.85±0.99	5.34±0.97	5.32±1.21	5.23±1.56	4.21±0.89
RZ7	12.91±0.48	14.59±0.20	13.11±0.31	12.13±0.12	7.09±3.21	ND	5.56±2.67	5.28±0.32	6.36±1.98	6.63±3.15	6.67±1.45	6.78±1.09
EZ27	67±1.67	101.48±1.05	129.07±1.50	160.1±2.00	187.69±0.98	240.93±2.33	5.04±0.45	4.84±4.89	4.74±0.99	4.59±0.98	4.50±1.00	4.51±1.23
10RZ3	13.60±0.94	14.47±0.83	12.55±0.74	10.76±1.34	7.09±1.89	ND	4.64±0.54	4.51±1.34	4.70±2.65	5.74±4.13	6.23±0.67	6.33±0.19
LUMITI4	20.22±4.39	19.76±4.68	19.05±4.14	13.63±1.08	26.89±1.56	ND	5.54±2.1	6.64±2.45	4.53±4.05	5.40±2.18	6.32±3.13	6.35±2.19
LBS23	ND	ND	7.95±0.53	13.63±1.08	26.89±1.56	35.76±1.34	6.99±2.1	6.98±2.45	6.67±4.05	6.64±2.18	6.32±3.13	6.35±2.19
LUMITI1	12.85±0.55	13.44±0.41	12.90±0.38	10.09±1.43	5.01±1.34	0.38±0.53	5.63±0.37	5.06±2.24	5.40±2.88	5.46±1.34	5.67±2.78	6.01±2.14

LUMITI6	14.07±0.43	13.90±0.91	12.84±0.54	10.90±2.12	5.02±2.14	ND	5.36±1.82	5.64±2.17	5.65±1.66	6.28±2.17	6.45±1.23	6.65±2.44
TSU7	14.32±0.36	14.59±0.03	14.99±0.56	15.92±2.12	7.19±2.09	1.13±0.81	4.64±1.29	4.53±1.56	4.48±1.45	4.45±0.19	4.23±1.23	4.39±0.12
TSU11	12.68±0.03	14.24±0.20	13.24±0.30	12.09±1.34	10.34±1.02	ND	4.81±2.21	4.77±1.34	5.48±3.12	6.66±331	6.78±3.04	6.79±2.09
TUL6	42.86±0.89	87.24±1.67	108.38±1.56	163.55±3.04	232.07±2.03	107.93±2.17	4.98±0.96	4.70±1.56	4.54±2.12	4.52±2.09	4.46±1.78	4.22±2.09
LBS4	83.83±1.82	208.08±2.98	147.06±21.42	125.79±1.70	98.78±2.56	80.98±3.67	5.89±3.78	4.65±3.09	4.78±4.09	4.80±3.18	5.67±2.89	5.88±5.01
TSU1(9)	15.06±0.70	15.15±0.98	15.21±0.72	17.98±3.12	14.67±1.87	ND	4.60±1.27	4.58±3.02	4.40±2.12	4.29±3.54	4.44±2.34	5.32±2.98
TUL11	40.86±0.89	86.24±1.66	109.38±1.06	163.63±3.04	221.27±2.05	105.92±2.07	4.98±0.96	4.70±1.56	4.54±2.12	4.52±2.09	4.46±1.78	4.22±2.09
LBS36	14.54±1.00	15.51±1.30	15.67±1.09	14.56±1.67	8.87±1.89	ND	5.62±3.33	5.18±2.15	4.57±1.22	6.05±2.22	6.12±2.55	6.24±1.76
LBS12(A)	54.49±4.04	76.18±7.41	100.44±4.21	100.18±5.82	90.12±2.89	78.45±1.80	6.67±2.10	6.12±2.18	5.01±2.89	5.11±2.80	5.89±3.67	5.99±3.87
LBS16	59.05±6.31	82.61±0.56	151.32±7.41	154.87±18.47	161.90±3.70	169.05±3.79	6.78±2.11	6.45±2.67	5.78±4.12	4.99±3.45	4.72±4.19	4.51±2.57
LBS22	14.16±1.49	15.39±1.33	14.94±1.61	13.09±2.35	10.89±0.23	ND	4.61±4.01	4.58±1.23	4.61±0.91	4.89±2.17	5.78±3.01	6.01±2.89
NC2(5)	16.92±0.28	17.46±1.10	17.72±0.86	18.98±4.13	12.89±1.28	ND	5.07±4.90	5.00±3.56	4.92±1.99	4.86±1.45	4.99±2.09	5.06±3.01
RZ23	14.78±0.36	15.74±0.11	15.64±0.32	14.89±3.02	9.98±3.12	ND	5.79±0.89	5.28±2.77	5.39±3.02	5.53±2.12	5.67±1.87	5.99±2.13
LUMBB2	39.41±1.24	46.31±0.98	68.01±2.56	106.94±2.04	178.35±3.46	218.31±1.33	5.32±0.98	5.21±5.76	4.89±4.56	4.76±2.01	4.5±1.34	4.38±2.37
TSU2(4)	43.86±0.88	88.24±1.66	108.39±1.56	163.56±3.03	222.17±5.05	107.93±2.17	4.97±0.95	4.71±1.56	4.55±2.13	4.51±2.08	4.47±1.78	4.21±2.09
NC7	18.73±4.23	15.29±0.97	18.73±1.78	18.73±1.67	15.28±2.54	15.28±0.94	6.34±1.56	6.46±3.01	6.43±0.67	6.49±0.89	6.53±1.32	6.67±1.43
TSU6	36.98±2.67	87.69±1.43	104.93±2.56	139.41±1.23	184.24±3.45	225.62±5.01	4.91±1.34	4.87±0.56	4.65±2.34	4.51±1.43	4.47±1.05	4.31±1.76
TSU2(1)	135.98±2.45	177.35±2.35	204.95±1.97	225.63±1.08	291.15±0.68	118.29±1.98	4.46±1.57	4.42±1.32	4.41±2.88	4.37±1.07	4.34±1.57	4.38±2.32
TSU12	56.67±4.97	117.71±3.21	142.85±0.98	159.12±1.34	201.49±1.76	235.98±2.99	4.61±0.45	4.55±8.12	4.46±0.45	4.41±0.97	4.34±1.57	4.26±3.31
TSU3(7)	42.87±3.22	98.04±1.66	102.48±2.31	125.63±2.56	187.68±6.35	263.56±1.77	4.91±5.67	4.70±3.56	4.68±7.21	4.64±2.45	4.47±0.87	4.42±1.25

*ND=not detected.

*±SE: Standard error from mean.

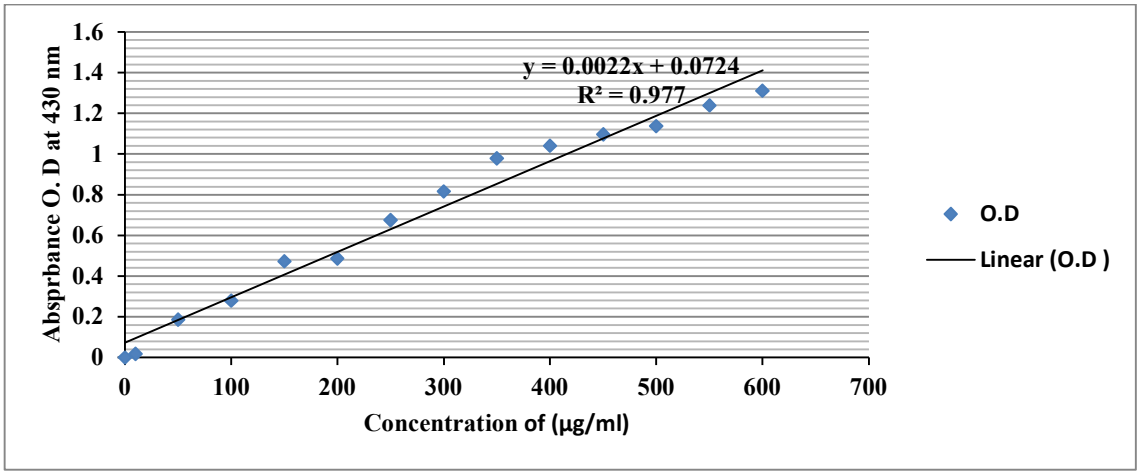

Figure 3.2: Phosphate standard curve

For quantitative analysis, 25 isolates were selected and incubated in liquid media (NBRIP), for 12 days and spectrophotometric reading was taken on 2nd, 4th, 6th, 8th, 10th and 12th day at 430 nm. With the help of standard (**Figure 3.2**), the concentration of phosphate solubilised was calculated and along with that *pH* of the media was also measured every time the phosphate solubilised was calculated to check the production of organic acid. It was observed that as the solubilisation of phosphate increased in the media the *pH* decreased confirming the organic acid production which is required to solubilise the phosphate (**Table 3.2**). The highest solubilisation (µg/ml) was observed by 10RZ9 (360.1±45), followed by 30EZ3 (356.66±0.89 µg/ml), 30RZ10 (342.86±1.34), 30E24 (329.07±1.33), 30E6 (298.03±4.32), TSU2(1) (291.15±0.68), 30E22 (291.14±0.67), 30E5 (287.68±3.11), 30E23 (273.9±4.21), 10E9 (273.9±1.67), TSU3(7) (263.56±1.77), 30E18 (263.55±1.78), RZ15 (260.1±4.09), 10RZ16 (246.31±7.09), 30E1 (242.86±1.45), EZ30 (240.93±2.33), 10E13 (235.97±2.98), TSU12 (235.98±2.99), TUL6 (232.07±2.03), TSU6 (225.62±5.01), EZ11 (225.62±5.01), RZ27 (223.17±5.05), 10E24 (222.17±3.21), TSU2(4), (222.17±5.05), TUL11(221.27±2.05), LUMBB2 (218.31±1.33),

30E13 (215.28±1.32) and LBS4 (208.08±2.98). It was observed that on the 12th day most of the isolates showed highest phosphate solubilisation.

Table 3.3: Qualitative analysis of IAA production by the bacterial isolates

Sl. No.	Isolates No.	AA production	Sl. No.	Isolate No.	IAA production	Sl. No.	Isolate No.	IAA production	Sl. No	Isolate No.	IAA production
1	LUMB1	-	35	LBS21	-	69	RZ7	+	104	TSU2(2)	-
2	LUMB2	+	36	LBS13	-	70	25R10	-	105	TSU3(7)	+
3	LUMB3	+	37	LBS14	+	71	RZ27	-	106	TSU3(2)	-
4	LUMB4	+	38	LBS29	-	72	25R2	-	107	TSU3(6)	+
5	LUMB5	+	39	LBS23	+	73	EZ30	-	108	TSU3(4)	-
6	LUMB6	+	40	LBS7	+	74	5R9	-	109	TUL1	+
7	LUMB7	-	41	LBS4	+	75	5R10	+	110	TUL2	-
8	LUMB8	+	42	LBS31	+	76	25Z13	-	111	TUL3	+
9	LUMB9	+	43	LBS15	+	77	25E22	-	112	TUL4	-
10	LUMB10	-	44	LBS19	+	78	25E5	-	113	TUL5	-
11	LUMITI1	+	45	LBS12	+	79	EZ27	+	114	TUL6	-
12	LUMITI2	-	46	LBS17	+	80	25EZ2	-	115	TUL8	+
13	LUMITI3	+	47	LBS22	+	81	25E20	-	116	TUL9	-
14	LUMITI4	+	48	5E20	-	82	5E11	-	117	TUL10	-
15	LUMITI5	-	49	5E8	+	83	5E3	-	118	TUL11	-
16	LUMITI6	+	50	25E12	+	84	25EZ6	+	119	TUL12	+
17	LUMITI7	+	51	5E24	+	86	RZ5	-	120	NC11	-
18	LUMBB1	-	52	5E5	+	87	RZ20	-	121	5NC8	+
19	LUMBB2	+	53	25R23	+	88	5EZ13	-	122	5NC10	-
20	LUMBB3	+	54	25E10	+	89	5R3	-	123	5NC14	-
21	LUMBB4	+	55	5R1	+	90	25R1	-	124	25NC8	+
22	LUMBB6	-	56	5R5	+	91	25E3	+	125	5NC6	-
23	LUMBB7	-	57	5E22	-	92	TSU1	+	126	5NC13	+
24	LUMBB8	+	58	5R16	+	93	TSU2(4)	+	127	25NC12	-
25	LUMBB9	-	59	EZ11	-	94	TSU3	+	128	25NC9	+
26	LBS11	+	60	E24	+	95	TSU1(5)	+	129	25NC3	-
27	LBS12A	+	61	25E18	-	96	TSU4	+	130	5NC17	+
28	LBS18	-	62	5R15	-	97	TSU8	-	131	25NC12	-
29	LBS25A	+	63	5R12	-	98	TSU6	+	132	25NC10	+
30	LBS36	+	64	25EZ1	+	99	TSU7	+	133	5NC1	-
31	LBS25B	-	65	RZ23	+	100	TSU11	+	134	5NC20	-
32	LBS16	-	66	5E9	+	101	TSU12	+	135	NC26	+
33	LBS44A	+	67	5E28	+	102	TSU9	+	136	NC7	+
34	LBS44B	-	68	25E16	-	103	TSU2(1)	+			

Note: '+': indicates present, '- ': Indicates absent.

Indole Acetic Acid production

Out of 136 isolates, 77 isolates (LUMB2, LUMB3, LUMB4, LUMB5, LUMB6, LUMB8, LUMB9, LUMITI1, LUMITI3, LUMITI4, LUMITI6, LUMITI7, LUMBB2, LUMBB3, LUMBB4, LUMBB8, LBS11, LBS12A, LBS25A, LBS36, LBS44A, LBS14, LBS23, LBS7, EZ27, 5R10, RZ7, 5E28, 5E9, RZ23, 25EZ1, E24, 5R16, 5R5, 5R1, 25E10, 25R23, 5E5, 5E24, 25E12, 5E8, LBS22, LBS17, LBS12, LBS19, LBS15, LBS31, LBS4, 25EZ6, 25E3, TSU1, TSU2(4), TSU3, TSU1(5), TSU4, TSU6, TSU7, TSU11, TSU12, TSU9, TSU2(1), TSU3(7), TSU3(6), TUL1, TUL3, TUL8, TUL12, 5NC8, 25NC8, 5NC13, 25NC9, 5NC17, 25NC10, NC26 and NC7)were able to produce IAA which was indicated by the development of pink colour after missing bacterial supernatant and reagent (**Table 3.3**). For quantitative estimation, 58 isolates were selected and incubated for 12 days. IAA produced was measured on 4^{th}, 6^{th}, 8^{th}, 10^{th}, and 12^{th} day by recording the O.D. at 530 nm. With the help of standard (**Figure 3.3**), the concentration of IAA produced was calculated. Different isolates showed their optimum IAA production on different days (**Figure 3.4**). Some isolates started producing IAA from 4^{th} day while some took more days to start producing IAA in the medium. Seven bacterial isolates (TSU12, TSU11, NC7, TSU7, TSU3(10), LUMBB8, and LBS23) produced the highest IAA under *in vitro* conditions (149.86 µg mL-1, 126.35 µg mL-1, 22.38 µg mL-1, 10.09 µg mL-1, 8.80 µg mL-1, 4.65 µg mL-1 and 4.29 µg mL-1) on 6^{th} day, 4^{th} day, 12^{th} day, 6^{th} day, 4^{th} day, 8^{th} day and 10^{th} day respectively (**Table 3.4**).

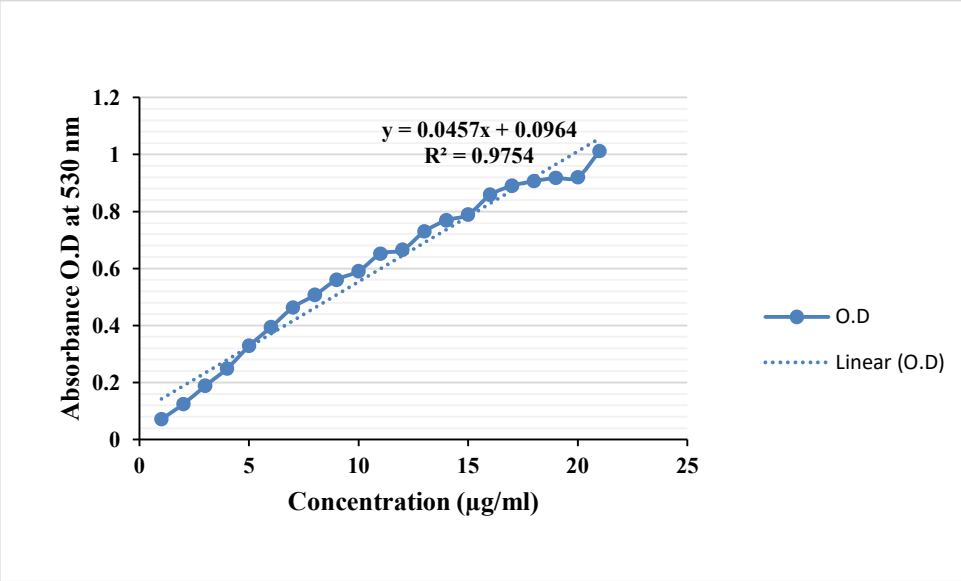

Figure 3.3: IAA standard curve.

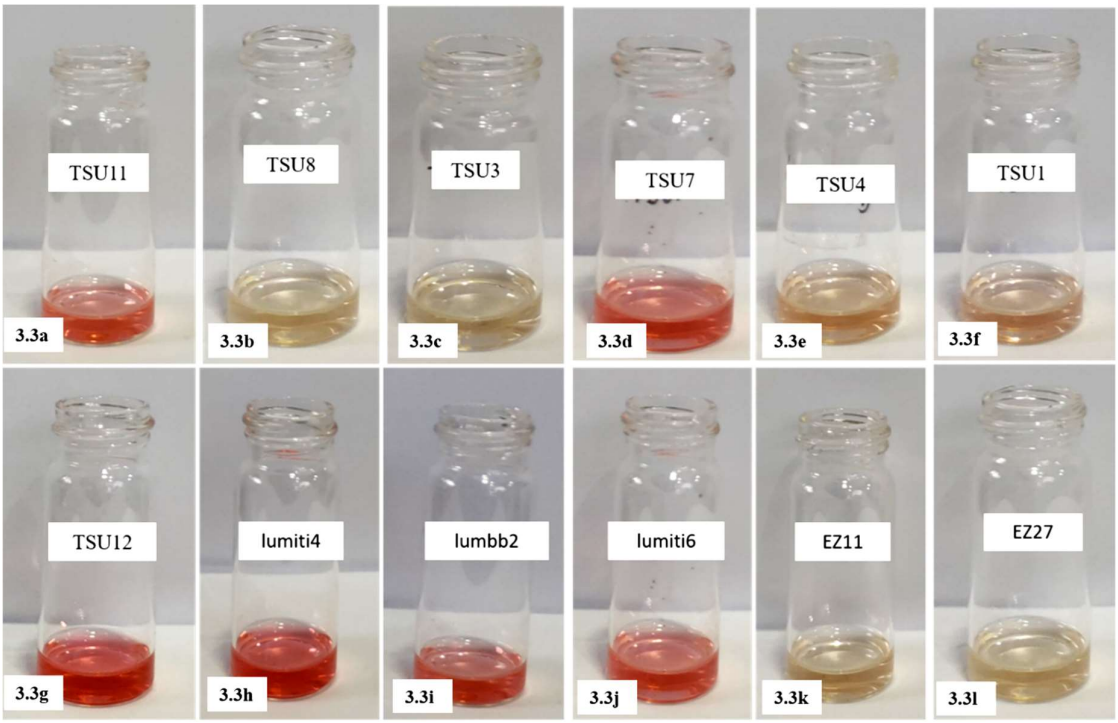

Figure 3.4: IAA production by the bacterial isolates indicated by the development of pink colour after addition of Salkowski reagent. Colour change to dark pink, indication high IAA production while to faint pink, indicated moderate IAA production. No change in Colour, indicating no IAA production.

Table 3.4: IAA production by the bacterial isolates in nutrient broth medium supplemented with 0.1% of L-tryptophan

Sl. No.	Bacterial Isolates	4th Day ±SE*	6th Day ±SE*	8th Day ±SE*	10th Day ±SE*	12th Day ±SE*
1	LBS 16	ND**	ND**	ND**	ND**	ND**
2	LBS14	0.81 ± 0.20	2.63 ± 0.37	2.77 ± 0.04	0.96 ± 0.08	0.04 ± 0.02
3	LBS23	1.97 ± 0.40	2.69 ± 0.12	4.01 ± 0.10	4.29 ± 0.08	ND
4	LBS13	ND**	ND**	ND**	ND**	0.10 ± 0.06
5	LBS15	ND**	0.13 ± 0.16	ND**	ND**	ND**
6	LBS7	ND**	0.41 ± 0.19	ND**	ND**	ND**
7	LBS36	ND**	ND**	ND**	ND**	ND**
8	LBS19	ND**	ND**	ND**	ND**	ND**
9	LBS25B	ND**	ND**	ND**	ND**	ND**
10	LBS11	ND**	ND**	ND**	ND**	ND**
11	LBS12	ND**	ND**	ND**	ND**	ND**
12	LBS4	ND**	ND**	ND**	ND**	ND**
13	LBS25A	1.32 ± 0.07	1.02 ± 0.09	0.63 ± 0.07	ND**	ND**
14	LBS22	ND**	ND**	ND**	ND**	ND**
15	TSU4	ND**	ND**	ND**	ND**	ND**
16	TSU1(1)	ND**	ND**	ND**	ND**	ND**
17	TSU3	ND**	ND**	ND**	ND**	ND**
18	TSU1(9)	ND**	ND**	ND**	ND**	ND**
19	TSU2	ND**	ND**	ND**	ND**	ND**
20	TSU8	ND**	ND**	ND**	ND**	ND**
21	TSU2(6)	ND**	ND**	ND**	ND**	ND**
22	TSU2(5)	ND**	ND**	ND**	ND**	ND**
23	TSU1(5)	ND**	ND**	ND**	ND**	ND**
24	TSU1(6)	1.25±0.86	5.24±0.09	3.89±1.13	2.88±2.14	0.28±2.23
25	TSU11	126.35± 1.70	52.08± 1.63	1.19±0.08	1.27±0.04	2.66±0.05
26	TSU12	114.94± 0.46	149.86±1.75	4.81±0.12	6.87±0.15	10.01±0.07
27	TSU7	ND**	10.09 ± 0.25	ND**	ND**	ND**
28	TSU2(4)	0.14±0.02	1.20±0.09	8.90±0.04	18.56±0.67	19.25±0.29
29	TSU2(2)	ND**	ND**	ND**	ND**	ND**
30	TSU2(1)	ND**	1.09±0.13	1.30±0.19	1.55±0.15	3.82±0.10
31	TSU3(1)	1.45±0.89	6.14±0.09	4.89±1.23	2.78±2.13	0.26±2.13
32	TSU3(7)	3.99±0.13	1.40±0.02	1.19±0.06	0.58±0.04	ND**
33	TSU3(10)	8.80±0.17	7.93±0.19	0.94±0.04	0.87±0.12	ND**
34	TSU3(4)	ND**	ND**	ND**	ND**	ND**
35	TSU3(6)	0.13±0.06	0.70±0.06	0.96±0.03	1.34±0.01	1.23±0.12
36	TSU3(5)	ND**	ND**	ND**	ND**	ND**
37	TSU3(2)	ND**	ND**	ND**	ND**	ND**
38	NC2(5)	ND**	ND**	1.74 ± 0.06	0.26 ± 0.02	0.11 ± 0.05
39	NC2(4)	ND**	ND**	ND**	ND**	ND**
40	10NC5	ND**	ND**	ND**	ND**	ND**
41	10NC8	ND**	ND**	ND**	ND**	ND**
42	NC7	0.68±0.04	3.38±0.05	9.61±0.11	13.28±0.07	22.38±0.18
43	10NC21	ND**	ND**	ND**	ND**	ND**
44	LUMB5	ND**	0.44±0.03	0.34 ± 0.08	0.65±0.07	1.00±0.10
45	LUMB6	ND**	ND**	ND**	ND**	ND**
46	LUMB2	ND**	ND**	ND**	ND**	ND**

47	LUMB8	ND**	ND**	ND**	ND**	ND**
48	LUMB9	ND**	ND**	ND**	ND**	ND**
49	LUMBB1	ND**	ND**	ND**	ND**	ND**
50	LUMBB2	ND**	0.60 ± 0.03	2.98 ± 0.09	3.11 ± 0.06	4.32 ± 0.09
51	LUMBB3	ND**	ND**	0.08 ± 0.02	0.23 ± 0.04	2.12 ± 0.14
52	LUMBB4	ND**	0.60 ± 0.01	ND**	ND**	ND**
53	LUMBB6	ND**	ND**	ND**	ND**	ND**
54	LUMBB7	ND**	ND**	ND**	ND**	ND**
55	LUMBB8	2.63±0.09	2.99±0.01	4.65±0.15	3.48±0.11	2.34±0.12
56	LUMITI2	ND**	ND**	ND**	ND**	ND**
57	LUMITI4	ND**	1.17±0.20	2.94±0.13	3.40±0.26	1.00± .02
58	LUMITI5	ND**	ND**	ND**	ND**	ND**

*±SE: Standard error from mean.

**ND = Not detected.

Siderophore production

Ninety bacterial isolates were able to produce siderophore when tested on CAS agar medium which was indicated by the formation of orange halo zone around the bacterial colonies (**Table 3.5, Figure 3.5**). Forty-five isolates were selected for further quantitative analysis in liquid media. In liquid media Percent siderophore unit (PSU) was found to be highest for Isolates EZ30 (197.138), followed by 10E28 (110.350), 30E18 (48.995), EZ24 (46.132), 10R3 (45.327), 10R15 (40.594), 10E18 (40.392), 10E3 (40.392), 10E9 (39.618), 20E13 (39.170), 10R9 (37.514), 10R10 (37.066), EZ11(35.596), 30E6 (33.663), 30E10 (34.622), RZ23 (33.292), 30E1 (31.435), EZ27 (31.202), 10E11 (31.172), 10R7 (30.810), 10R12 (30.785), 10R16 (29.594), 10E5 (29.594), 30E17 (29.408), 10E13 (29.377), 10R1 (28.603), 30R10 (28.418), 10R15 (28.032), 10E22(27.985), RZ5 (27.165), 10R5 (26.639), 30E2 (25.107), 10E24 (23.746), 30R1 (23.669), 30E20 (22.075),5R16 (20.745), 30E22 (19.569), 10E20 (14.00) and 30E23 (3.944) (**Table 3.6**).

Table 3.5: Qualitative analysis of siderophore production by the bacterial isolates

Sl. No.	Isolates No.	Siderophore Production	Sl. No.	Isolate No.	Siderophore Production	Sl. No.	Isolate No.	Siderophore Production	Sl. No.	Isolate No.	Siderophore Production
1	LUMB1	-	39	LBS25B	+	77	25E18	+	115	TSU7	+
2	LUMB2	-	40	LBS16	+	78	5R15	+	116	TSU11	-
3	LUMB3	+	41	LBS44A	+	79	5R12	+	117	TSU12	+
4	LUMB4	+	42	LBS44B	+	80	25EZ1	-	118	TSU9	+
5	LUMB5	+	43	LBS21	+	81	RZ23	+	119	TSU2(1)	+
6	LUMB6	-	44	LBS13	+	82	5E9	-	120	TSU2(2)	+
7	LUMB7	-	45	LBS14	-	83	5E28	+	121	TSU3(7)	-
8	LUMB8	+	46	LBS29	+	84	25E16	+	122	TSU3(2)	-
9	LUMB9	+	47	LBS23	+	85	RZ7	-	123	TSU3(6)	+
10	LUMB10	+	48	LBS7	-	86	25R10	+	124	TSU3(4)	+
11	LUMITI1	+	49	LBS4	+	87	RZ27	-	125	TUL1	+
12	LUMITI2	-	50	LBS31	+	88	25R2	-	126	TUL2	+
13	LUMITI3	+	51	LBS15	+	89	EZ30	+	127	TUL3	+
14	LUMITI4	+	52	LBS19	+	90	5R9	-	128	TUL4	+
15	LUMITI5	+	53	LBS12	-	91	5R10	-	129	TUL5	-
16	LUMITI6	+	54	LBS17	+	92	25Z13	-	130	TUL6	+
17	LUMITI7	+	55	LBS22	+	93	25E22	-	131	TUL8	+
18	LUMBB1	+	56	5E20	-	94	25E5	+	132	TUL9	-
19	LUMBB2	+	57	5E8	-	95	EZ27	+	133	TUL10	-
20	LUMBB3	+	58	25E12	+	96	25EZ2	-	134	TUL11	+
21	LUMBB4	+	59	5E24	+	97	25E20	-	135	TUL12	+
22	LUMBB6	+	60	5E5	-	98	5E11	-	136	NC11	+
23	LUMBB7	+	61	25R23	-	99	5E3	-			
24	LUMBB8	+	62	25E10	-	100	25EZ6	-			
25	LUMBB9	+	63	5R1	+	101	25EZ17	-			
26	LBS11	+	64	5R5	-	102	RZ5	+			
27	LBS12A	+	65	5E22	-	103	RZ20	+			
28	LBS18	-	66	5R16	+	104	5EZ13	-			
29	LBS25A	+	67	EZ11	+	105	5R3	+			
30	LBS36	+	68	E24	-	106	25R1	+			
31	5NC8	+	69	25NC12	+	107	25E3	+			
32	5NC10	+	70	25NC9	+	108	TSU1	+			
33	5NC14	-	71	25NC3	-	109	TSU2(4)	-			
34	25NC8	+	72	5NC17	+	110	TSU3	-			
35	5NC6	-	73	25NC12	+	111	TSU1(5)	+			
36	5NC13	-	74	25NC10	+	112	TSU4	+			
37	NC26	+	75	5NC1	+	113	TSU8	+			
38	NC7	+	76	5NC20	+	114	TSU6	-			

Note: '+': Indicates present, '-': Indicates absent.

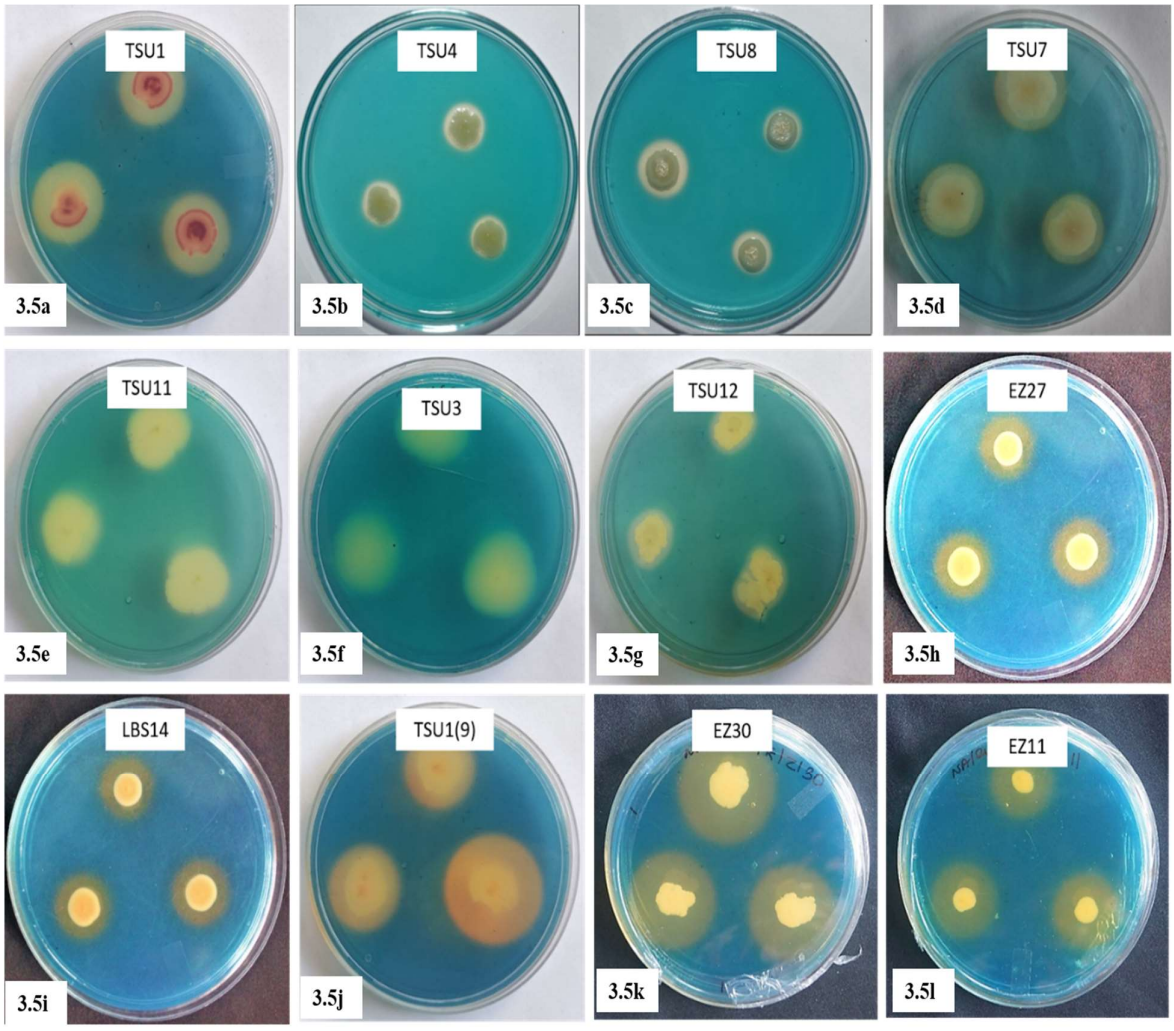

Figure 3.5: Siderophore production test by the bacterial isolates indicated by development of orange halo zone around the bacterial colony.

Table 3.6: Percent siderophore unit of the bacterial isolates from rhizospheric soil of *Musa* sp.

Sl. No.	Bacterial Isolates	On 2nd Day ±SE*	On 4th Day ±SE*	On 6th Day ±SE*	On 10th Day ±SE*	On 12th Day ±SE*
1	10E9	27.768±1.45	37.762±2.67	39.618±2.88	32.285±3.09	26.825±1.05
2	10R16	24.876±2.21	26.840±2.87	29.594±1.09	29.517±2.31	24.241±2.09
3	10E24	ND	23.746±3.23	23.622±2.34	21.689±1.22	16.460±1.11
4	30E17	12.221±1.01	19.863±1.23	25.835±3.21	29.408±4.00	23.900±1.34
5	10E5	ND	ND	ND	29.594±2.34	26.283±1.33
6	10E22	8.941±0.99	12.81±0.99	20.668±3.11	27.985±2.17	21.627±2.07
7	10R15	ND	ND	10.303±1.02	28.032±2.05	ND
8	30E23	ND	ND	ND	3.944±0.04	ND
9	30E22	ND	ND	ND	19.569±1.02	ND
10	EZ27	ND	ND	ND	31.202±1.34	ND
11	30R10	7.487±1.01	7.533±0.12	24.783±2.77	26.608±2.05	28.418±1.67
12	30E12	ND	ND	ND	ND	ND
13	10R1	15.794±1.22	18.131±1.06	28.603±1.66	28.155±2.11	21.008±2.01
14	30E10	24.148±2.32	34.622±1.99	34.482±3.21	28.665±3.01	25.278±2.01
15	10E11	ND	ND	29.037±2.99	31.172±3.21	27.335±2.23
16	10R10	21.704±3.01	32.502±2.45	36.926±3.12	37.066±3.56	31.914±2.99
17	30E6	28.047±2.05	32.549±2.22	33.663±3.97	30.676±2.67	31.512±3.00
18	30E20	ND	ND	15.00±1.99	22.075±2.11	4.655±0.89
19	10R3	6.141±1.12	28.975±3.01	13.273±1.24	45.327±1.56	24.659±2.21
20	30R1	ND	ND	ND	23.669±2.22	23.498±1.89
21	30E5	NA	ND	ND	ND	ND
22	RZ23	24.319±2.03	33.292±3.44	31.357±2.09	25.757±1.89	27.042±3.07
23	10R12	1.113±1.09	14.789±2.22	30.785±1.56	26.252±2.43	24.845±1.90
24	30E2	ND	5.152±1.09	22.834±2.13	25.107±2.12	15.532±1.01
25	30E13	ND	15.485±1.34	39.170±2.45	24.024±2.01	10.194±1.78
26	10E13	ND	12.699±1.12	29.377±2.56	25.788±2.02	27.506±2.45
27	10E20	12.638±1.76	6.095±2.18	5.321±2.11	14.00±2.18	4.594±3.12
28	RZ5	14.680±1.07	27.165±1.99	20.930±2.70	23.003±2.09	12.051±1.02
29	10R15	25.138±2.45	38.628±2.99	40.594±1.09	34.653±3.87	21.318±2.55
30	10R9	16.553±2.12	33.368±2.09	37.514±2.44	26.252±1.67	31.790±2.09
31	EZ11	18.193±1.39	35.596±2.24	28.077±3.76	27.211±2.99	24.581±3.90
32	EZ30	17.929±2.23	37.159±2.67	197.138±2.01	18.347±2.02	24.179±3.04
33	EZ24	13.521±1.90	20.080±1.89	15.872±1.09	46.132±2.90	4.129±2.89
34	RZ27	ND	ND	ND	ND	ND
35	10R16	20.745±2.03	13.459±2.09	16.784±2.22	14.742±1.89	12.530±1.94
36	10E28	30.336±2.66	13.690±2.90	110.350±3.67	4.470±1.87	ND
37	30E18	12.530±2.70	48.995±2.80	29.764±3.76	29.486±4.89	18.347±2.44
38	10E3	31.759±3.90	40.392±4.87	13.040±2.90	25.974±2.66	27.289±2.88
39	10E18	30.955±2.90	40.392±1.90	34.405±2.65	35.829±3.11	36.726±2.49
40	10R7	28.186±2.90	30.810±2.85	29.099±3.12	13.613±1.09	11.448±1.67
41	30E1	31.435±2.88	30.816±2.09	29.037±1.56	23.699±2.89	17.821±2.11
42	30E16	ND	ND	ND	ND	ND
43	10R5	17.790±3.89	26.639±3.76	26.314±3.90	26.469±3.45	26.233±2.87

44	10E8	ND	ND	ND	ND	ND
45	30R23	ND	ND	ND	ND	ND

ND= Not detected; Note: ±SE: Standard error from mean.

Ammonia production test

The studied isolates exhibited varying levels of ammonia production in the medium (**Figure 3.6**). Isolates TSU8, LUMB2, 5NC8, 5NC6, LUMB9, LUMITI6, LBS36, LBS16, LBS44A, 25E22, EZ27, 5R9, RZ27, RZ7, 5E9, 5R12, 5R15, 5R3, TSU4, TSU6, TSU3(4), TSU3(2), TSU3(6), TSU3(7), TUL12, NC11, 5NC6 and 5NC13 found to be highest ammonia production (+++) isolates indicated by the brownish colouring of broth in presence of Nessler's reagent, followed closely (++) with an orange colour development by isolates LUMB8, LUMB10, LUMITI2, LUMITI3, LUMITI4, LUMITI7, LUMBB3, LUMBB4, LUMBB6, LUMBB7, LBS12A, LBS18, LBS25A, LBS25B, LBS21, LBS23, LBS4, LBS31, LBS15, LBS19, LBS12, LBS17, 5E20, 5E24, 25E10, 5R1, E24, 25E18, 25EZ1, 5E28, 5R10, 25E20, 5E11, 5E3, 25EZ6, RZ20, 5EZ13, 25R1, 25E3, TSU2(4), TSU3, TSU1(5), TSU11, TSU12, TSU2(1), TSU2(2), TUL2, TUL4, 25NC12, 25NC9, 25NC10, 5NC1 and NC7. The remaining isolates produced relatively lower ammonia indicated by a weaker colour response (+) (**Table 3.7**).

Salinity test

Soil salinity exerts an impact on plants growth by creating hyper osmotic and hypertonic stress conditions. Halo tolerant PGPR plays vital role in plants adaptation to the challenging situations by inducing systemic tolerance. The isolates RZ27, TUL6, and TUL11 exhibited highest tolerance (up to 14% NaCl), followed by EZ11, RZ20, TSU4, and TUL12 (12%). While, isolatesLUMITI3, LUMITI4, LUMITI6, LBS36, LBS16, LBS13, 25E17 and RZ5 could tolerate up to 10% salt and LBS11, LBS29, RZ23, 5R3, TSU3(2), and 5NC17 tolerated up to 8%. Other isolates LUMB7, LUMB9, LUMBB4, LUMBB8 , LBS18 , LBS44B, LBS4, LBS17, LBS22, 5E24 , 25E10 , 5R1 , 25E18 , 5R15 , 5E28 , 25E16 , RZ7, 5R10, 25Z13, 25E22, 25E20, 5E11, 5E3 , 25EZ6 , TSU2(4),

TSU3, TSU12, 5NC8, 5NC6, 5NC13, 25NC12 , 5NC20 and NC26 could tolerate till 6% NaCl level. Rest of the isolates could tolerate only till 2-4% (**Table 3.8, Figure 3.7**).

Table 3.7: Ammonia production test by the bacterial isolates

Sl. No.	Isolates No.	NH_3 Production	Sl. No.	Isolates No.	NH_3 Production	Sl. No.	Isolates No.	NH_3 Production
1	LUMB1	+	47	LBS22	+	93	TSU2(4)	++
2	LUMB2	+++	48	5E20	++	94	TSU3	++
3	LUMB3	+	49	5E8	+	95	TSU1(5)	++
4	LUMB4	+	50	25E12	+	96	TSU4	+++
5	LUMB5	+	51	5E24	++	97	TSU8	+++
6	LUMB6	+	52	5E5	+	98	TSU6	+++
7	LUMB7	+	53	25R23	+	99	TSU7	+
8	LUMB8	++	54	25E10	++	100	TSU11	++
9	LUMB9	+++	55	5R1	++	101	TSU12	++
10	LUMB10	++	56	5R5	+	102	TSU9	+
11	LUMITI1	+	57	5E22	+	103	TSU2(1)	++
12	LUMITI2	++	58	5R16	+	104	TSU2(2)	++
13	LUMITI3	++	59	EZ11	+	105	TSU3(7)	+++
14	LUMITI4	++	60	E24	++	106	TSU3(2)	+++
15	LUMITI5	+	61	25E18	++	107	TSU3(6)	+++
16	LUMITI6	+++	62	5R15	+++	108	TSU3(4)	+++
17	LUMITI7	++	63	5R12	+++	109	TUL1	+
18	LUMBB1	+	64	25EZ1	++	110	TUL2	++
19	LUMBB2	+	65	RZ23	+	111	TUL3	+
20	LUMBB3	++	66	5E9	+++	112	TUL4	++
21	LUMBB4	++	67	5E28	++	113	TUL5	+
22	LUMBB6	++	68	25E16	+	114	TUL6	++
23	LUMBB7	++	69	RZ7	+++	115	TUL8	+
24	LUMBB8	+	70	25R10	++	116	TUL9	+
25	LUMBB9	+	71	RZ27	+++	117	TUL10	+
26	LBS11	+	72	25R2	+	118	TUL11	++
27	LBS12A	++	73	EZ30	+	119	TUL12	+++
28	LBS18	++	74	5R9	+++	120	NC11	+++
29	LBS25A	++	75	5R10	++	121	5NC8	+++
30	LBS36	+++	76	25Z13	+	122	5NC10	+
31	LBS25B	++	77	25E22	+++	123	5NC14	+
32	LBS16	+++	78	25E5	++	124	25NC8	+
33	LBS44A	+++	79	EZ27	+++	125	5NC6	+++
34	LBS44B	+	80	25EZ2	++	126	5NC13	+++
35	LBS21	++	81	25E20	++	127	25NC12	++
36	LBS13	+	82	5E11	++	128	25NC9	++
37	LBS14	+	83	5E3	++	129	25NC3	+
38	LBS29	+	84	25EZ6	++	130	5NC17	+
39	LBS23	++	85	25EZ17	+	131	25NC12	++
40	LBS7	+	86	RZ5	+	132	25NC10	++
42	LBS4	++	87	RZ20	++	133	5NC1	++
42	LBS31	++	88	5EZ13	++	134	5NC20	+
43	LBS15	++	89	5R3	+++	135	NC26	+
44	LBS19	++	90	25R1	++	136	NC7	++
45	LBS12	++	91	25E3	++			
46	LBS17	++	92	TSU1	+			

Note: '+++': Indicates high ammonia production; '++': Indicates moderate ammonia production and '+': Indicates low ammonia production.

Table 3.8: NaCl tolerance test of bacterial isolates on nutrient agar medium

Sl. No.	Bacteria Isolates	NaCl Concentration (%)						
		2	4	6	8	10	12	14
1	LUMB1	+	-	-	-	-	-	-
2	LUMB2	-	-	-	-	-	-	-
3	LUMB3	-	-	-	-	-	-	-
4	LUMB4	+	+	-	-	-	-	-
5	LUMB5	+	-	-	-	-	-	-
6	LUMB6	+	-	-	-	-	-	-
7	LUMB7	+	+	+	-	-	-	-
8	LUMB8	+	+	-	-	-	-	-
9	LUMB9	+	+	+	-	-	-	-
10	LUMB10	+	-	-	-	-	-	-
11	LUMITI1	-	-	-	-	-	-	-
12	LUMITI2	+	+	-	-	-	-	-
13	LUMITI3	+	+	+	+	+	-	-
14	LUMITI4	+	+	+	+	+	-	-
15	LUMITI5	-	-	-	-	-	-	-
16	LUMITI6	+	+	+	+	+		-
17	LUMITI7	-	-	-	-	-	-	-
18	LUMBB1	+	-	-	-	-	-	-
19	LUMBB2	-	-	-	-	-	-	-
20	LUMBB3	+	+	-	-	-	-	-
21	LUMBB4	+	+	+	-	-	-	-
22	LUMBB6	+	+	-	-	-	-	-
23	LUMBB7	+	+	-	-	-	-	-
24	LUMBB8	+	+	+	-	-	-	-
25	LUMBB9	+	+	-	-	-	-	-
26	LBS11	+	+	+	+	-	-	-
27	LBS12A	+	-	-	-	-	-	-
28	LBS18	+	+	+	-	-	-	-
29	LBS25A	+	-	-	-	-	-	-
30	LBS36	+	+	+	+	+	-	-
31	LBS25B	+	+	+	-	-	-	-
32	LBS16	+	+	+	+	+	-	-
33	LBS44A	-	-	-	-	-	-	-
34	LBS44B	+	+	+	-	-	-	-
35	LBS21	+	-	-	-	-	-	-
36	LBS13	+	+	+	+	+	-	-
37	LBS14	+	+	-	-	-	-	-
38	LBS29	+	+	+	+	-	-	-
39	LBS23	+	+	-	-	-	-	-
40	LBS7	+	-	-	-	-	-	-

41	LBS4	+	+	+	-	-	-	-
42	LBS31	+	+	-	-	-	-	-
43	LBS15	+	-	-	-	-	-	-
44	LBS19	+	+	-	-	-	-	-
45	LBS12	+	-	-	-	-	-	-
46	LBS17	+	+	+	-	-	-	-
47	LBS22	+	+	+	-	-	-	-
48	5E20	+	-	-	-	-	-	-
49	5E8	+	-	-	-	-	-	-
50	25E12	+	-	-	-	-	-	-
51	5E24	+	+	+	-	-	-	-
52	5E5	+	-	-	-	-	-	-
53	25R23	+	-	-	-	-	-	-
54	25E10	+	+	+	-	-	-	-
55	5R1	+	+	+	-	-	-	-
56	5R5	+	-	-	-	-	-	-
57	5E22	+	-	-	-	-	-	-
58	5R16	+	-	-	-	-	-	-
59	EZ11	+	+	+	+	+	+	-
60	E24	+	-	-	-	-	-	-
61	25E18	+	+	+	-	-	-	-
62	5R15	+	+	+	-	-	-	-
63	5R12	+	-	-	-	-	-	-
64	25EZ1	+	-	-	-	-	-	-
65	RZ23	+	+	+	+	-	-	-
66	5E9	+	-	-	-	-	-	-
67	5E28	+	+	+	-	-	-	-
68	25E16	+	+	+	-	-	-	-
69	RZ7	+	+	+	-	-	-	-
70	25R10	+	-	-	-	-	-	-
71	RZ27	+	+	+	+	+	+	+
72	25R2	+	-	-	-	-	-	-
73	EZ30	+	+	-	-	-	-	-
74	5R9	+	-	-	-	-	-	-
75	5R10	+	+	+	-	-	-	-
76	25Z13	+	+	+	-	-	-	-
77	25E22	+	+	+	-	-	-	-
78	25E5	+	+	-	-	-	-	-
79	EZ27	+	+	-	-	-	-	-
80	25EZ2	+	-	-	-	-	-	-
81	25E20	+	+	+	-	-	-	-
82	5E11	+	+	+	-	-	-	-
83	5E3	+	+	+	-	-	-	-
84	25EZ6	+	+	+	-	-	-	-
85	25E17	+	+	+	+	+	-	-
86	RZ5	+	+	+	+	+	-	-
87	RZ20	+	+	+	+	+	+	-
88	5EZ13	+	+	-	-	-	-	-
89	5R3	+	+	+	+	-	-	-
90	25R1	+	+	-	-	-	-	-
91	25E3	+	+	-	-	-	-	-

92	TSU1	+	+	-	-	-	-	-
93	TSU2(4)	+	+	+	-	-	-	-
94	TSU3	+	+	+	-	-	-	-
95	TSU1(5)	+	-	-	-	-	-	-
96	TSU4	+	+	+	+	+	+	-
97	TSU8	+	+	+	+	+	-	-
98	TSU6	+	+	+	+	+	-	-
99	TSU7	+	-	-	-	-	-	-
100	TSU11	+	+	-	-	-	-	-
101	TSU12	+	+	+	-	-	-	-
102	TSU9	+	+	+	+	+	-	-
103	TSU2(1)	+	+	+	+	+	-	-
104	TSU2(2)	+	+	+	+	+	-	-
105	TSU3(7)	+	+	+	+	+	-	-
106	TSU3(2)	+	+	+	+	-	-	-
107	TSU3(6)	+	+	-	-	-	-	-
108	TSU3(4)	+	+	+	+	+	-	-
109	TUL1	+	+	-	-	-	-	-
110	TUL2	+	+	-	-	-	-	-
111	TUL3	+	+	-	-	-	-	-
112	TUL4	+	+	-	-	-	-	-
113	TUL5	+	+	-	-	-	-	-
114	TUL6	+	+	+	+	+	+	+
115	TUL8	+	+	-	-	-	-	-
116	TUL9	+	+	-	-	-	-	-
117	TUL10	+	+	+	+	+	-	-
118	TUL11	+	+	+	+	+	+	+
119	TUL12	+	+	+	+	+	+	-
120	NC11	+	-	-	-	-	-	-
121	5NC8	+	+	+	-	-	-	-
122	5NC10	+	-	-	-	-	-	-
123	5NC14	+	-	-	-	-	-	-
124	25NC8	+	-	-	-	-	-	-
125	5NC6	+	+	+	-	-	-	-
126	5NC13	+	+	+	-	-	-	-
127	25NC12	+	-	-	-	-	-	-
128	25NC9	+	+	+	-	-	-	-
129	25NC3	+	-	-	-	-	-	-
130	5NC17	+	+	+	+	-	-	-
131	25NC12	+	+	+	-	-	-	-
132	25NC10	+	-	-	-	-	-	-
133	5NC1	+	-	-	-	-	-	-
134	5NC20	+	+	+	-	-	-	-
135	NC26	+	+	+	-	-	-	-
136	NC7	+	-	-	-	-	-	-

‘+’ indicates positive result, ‘- ‘indicates negative results.

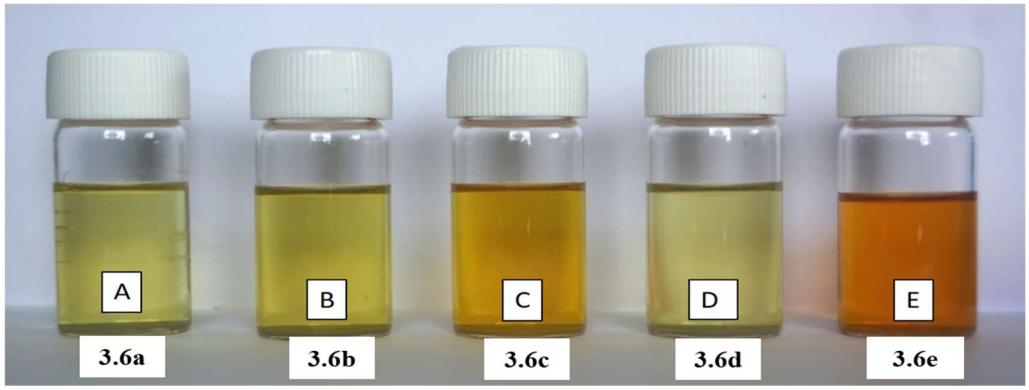

Figure 3.6: Ammonia production by the bacterial isolates where high amount of ammonia production was indicated by development of dark brown colour, while moderate amount of ammonia production by development of orange colour and low amount of ammonia production was indicated by yellow colour development of low amount of ammonia production.

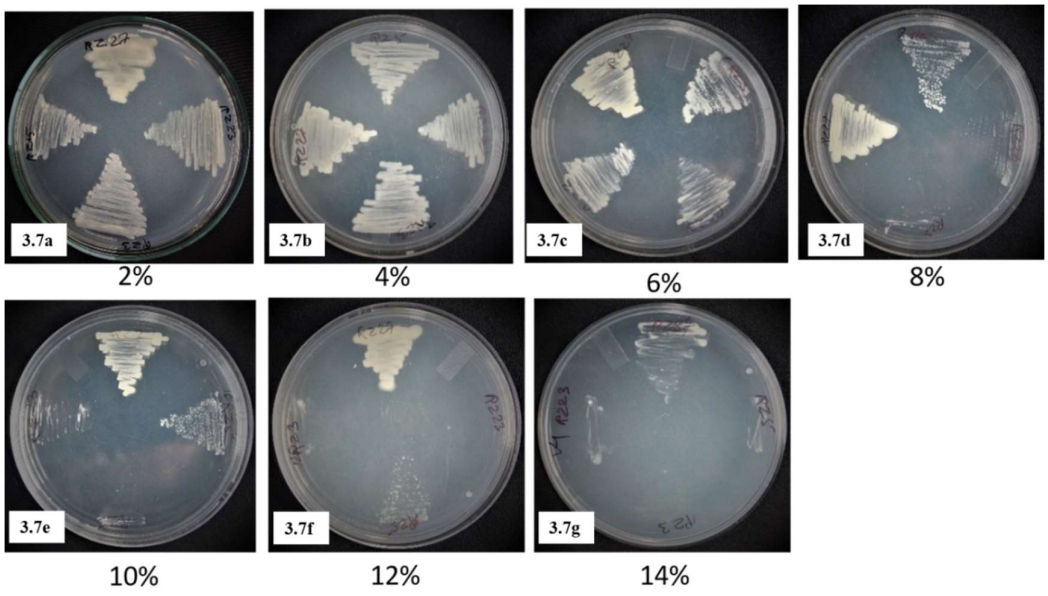

Figure 3.7: Salt tolerance test by the bacterial isolates: The bacterial isolates were screened for salt tolerance by inoculating them on nutrient agar plates containing different concentrations of NaCl (0-14% with an increment of 2%). Pure isolates were streaked on media plates and incubated for 5 days at 28±2°C and observed for growth.

Heavy metal tolerance test

When tested for heavy metal tolerance, different isolates showed varied tolerance to different heavy metals (µg/ml). Isolate TSU7 and TSU11 had the highest tolerance for Cu (770µg/ml), followed by TSU1 (740µg/ml); while, for Cr, it was LUMITI2, LUMBB4, and LUMBB7 (10010µg/ml), followed by LBS23 and LSB4 (8060µg/ml). In case of Zn, highest tolerance was shown by LUMBB2, LUMBB9, and LUMBB7 (10010µg/ml), followed by TSU1 and TSU7 (640µg/ml). Whereas, for Cd highest tolerance was shown by TSU7 (570µg/ml), followed by TSU1 (540µg/ml) and LUMB1 (530µg/ml). For Ni, highest tolerance was displayed by LUMITI3 and LUMBB2 (560µg/ml), followed by LBS23, LBS4 and TSU3 (460µg/ml). Whereas, the highest tolerance was exhibited by LUMB10 (270µg/ml) followed by LUMBB9 (180µg/ml) and for arsenic highest was shown by LBS23, LBS4 and TSU3 (850µg/ml), followed by LUMB10 (700µg/ml) (**Table 3.9, Figure 3.8**).

Molecular characterization of PGPR isolates

Growth promoting test followed by sequencing analysis of 1.5 kb fragment of 16S *rRNA* gene sequencing of 34 isolates was analysed by nucleotide Blast analysis (**Table 3.10**). The sequence of isolates LUMBB7, TSU1, TSU3, TSU4, TSU8, 30E11, 10R23, 10R7, RZ27, EZ27, RZ5, 10R20,10R3, EZ30, LUMITI4, LBS23, LUMITI1, LUMBB2, NC7, LUMITI6, TSU6, TSU2(1), TSU12, TSU3(7), TSU7, TSU11, TUL6, LBS4, TSU1(9), TUL12, TUL11, LBS36, LBS12A and LBS16 showed similarity with *Bacillus cereus* (97.44%), *Serratia marcescens* (99.22%), *Bacillus cereus* (97.23%), *Bacillus safensis* (95.02%), *Bacillus pumilus* (96.95%), *Pseudomonas putida* (98.12%),

Agrobacterium larrymoorei (98.69%), *Bacillus safensis* (97.53%), *Burkholderia cepacian* (97.20%), *Kosakonia arachidis* (99.62%), *Cupriavidus necator* (99.78%), *Pseudomonas putida* (99.57%), *Pseudomonas orientalis* (100%), *Pseudomonas monteilii* (99.75%), *Pseudomonas gessardii* (95.58%), *Pseudomonas gessardii* (94.06%), *Bacillus* sp.(97.34%), *Chryseobacterium cucumeris* (99.06%), *Cedecea neteri* (100%), *Bacillus subtilis* (100%), *Proteus terrae* (100%), *Proteus terrae* (100%), *Lelliottia amnigena* (99.31%), *Providencia rettgeri* (93.81%), *Pseudomonas koreensis* (98.97%), *Klebsiella variicola* (99.66%), *Burkholderia cepacian* (99.85%), *Bacillus cereus* (99.77%), *Proteus terrae* (99.85%), *Bacillus safensis* (96.58%), *Burkholderia cepacian* (99.21%), *Alcaligenes faecalis* (99.70%), *Pseudomonas fluorescens* (99.54%), and *Pseudomonas* sp. (90.55%) respectively. Based on the sequences obtained a phylogenetic tree was constructed using MegaX software to show the evolutionary history between a set of species or taxa during a specific time and major transitions in the evolution (**Figure 3.9**).

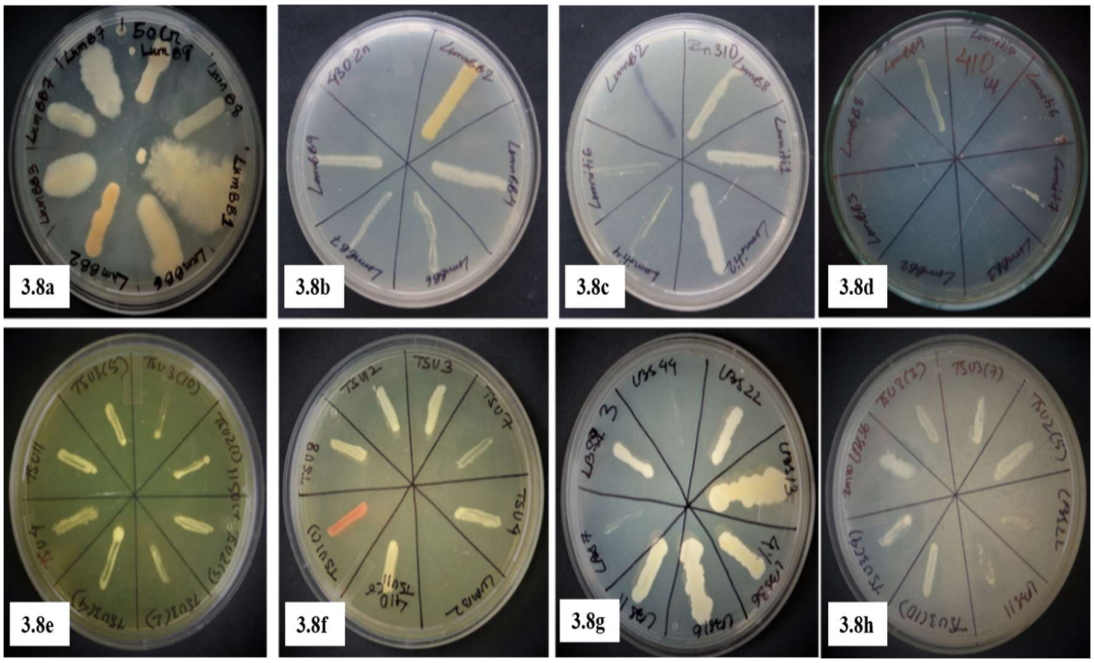

Figure 3.8: Heavy metal tolerance test by bacterial isolates for $Cd^{2+}(CdCl_2.H_2O)$, $Cu^{2+}(CuSO_4)$, $Ni^{2+}(NISO_4.H_2O)$, $As^{3+}(NaAsO_2)$, $Sb^{3+}(K_2(SbO)_2C_8H_4O_{40}, 3H_2O)$, $Zn^{2+}(ZnSO_4,7H_2O)$, and $Cr^{2+}(K_2CrO_4)$ was done by streaking the isolates on agar medium supplemented with different concentration of heavy metals (30-10,080µg/ml) with an increment of 30µg/ml in the medium.

Table 3.9: Minimum inhibitory concentration of the various heavy metals on growth of bacterial isolates

Sl. No.	Bacteria Isolates	Heavy Metal Tolerance(µg/ml)						
		Cu	Cr	Zn	Cd	Ni	An	As
1	LUMB1	270	880	90	530	>30	>30	30
2	LUMB2	240	80	530	390	320	80	30
3	LUMB3	180	80	90	140	>30	>30	60
4	LUMB4	30	80	90	30	>30	>30	60
5	LUMB5	150	1730	90	270	380	>30	270
6	LUMB6	150	8000	150	30	380	>30	450
7	LUMB7	30	1030	180	300	60	>30	60
8	LUMB8	300	260	490	460	410	>30	60
9	LUMB9	60	1090	60	80	350	>30	60
10	LUMB10	300	80	60	460	320	270	700
11	LUMITI1	180	1840	460	30	380	>30	60
12	LUMITI2	210	10010	490	270	380	>30	30
13	LUMITI3	300	760	180	460	560	>30	30
14	LUMITI4	120	80	280	30	260	>30	30
15	LUMITI5	300	730	30	110	>30	>30	30
16	LUMITI6	180	260	250	110	380	>30	60
17	LUMITI7	240	80	180	30	320	>30	30
18	LUMBB1	>30	760	60	110	>30	>30	30
19	LUMBB2	90	80	760	110	560	>30	30
20	LUMBB3	>30	8000	180	270	380	>30	>30
21	LUMBB4	210	10010	530	270	380	>30	>30
22	LUMBB6	210	730	490	270	380	>30	60
23	LUMBB7	150	10010	760	270	380	30	60
24	LUMBB8	120	80	120	30	380	>30	60
25	LUMBB9	240	80	760	490	440	180	450
26	LBS16	120	80	280	30	260	>30	30
27	LBS23	370	8060	530	370	460	≥30	850
28	LBS4	370	8060	530	370	460	≥30	850
29	TSU1	740	1030	640	540	430	≥30	110
30	TSU3	370	8060	530	370	460	≥30	850
31	TSU4	270	1400	470	140	260	≥30	≥30
32	TSU8	230	1160	530	260	260	≥30	≥30
33	TSU7	770	540	640	570	430	≥30	≥30
34	TSU11	770	1740	530	400	370	≥30	≥30
35	TSU12	540	1030	530	400	310	≥30	≥30

Table 3.10: Molecular identification of the PGPR isolates using 16S *rRNA* sequencing

Sl. No.	Isolate No.	Bacterial Species Name	% Identity	Query Cover	E-value	GenBank Accession No.
1	LUMBB7	*Bacillus cereus*	97.44	92%	0.0	OQ547229
2	TSU1	*Serratia marcescens*	99.22	99%	0.0	OQ726408
3	TSU3	*Bacillus cereus*	97.23	96%	0.0	OQ547230
4	TSU4	*Bacillus safensis*	95.02	90%	0.0	OQ547231
5	TSU8	*Bacillus pumilus*	96.95	93%	0.0	OQ547232
6	M5	*Pseudomonas putida*	98.12	99%	0.0	ON495940
7	M7	*Agrobacterium larrymoorei*	98.69	100%	0.0	OL662933
8	M1	*Bacillus safensis*	97.53	97%	0.0	OL662929
9	M6	*Burkholderia cepacian*	97.20	99%	0.0	OL662932
10	M14	*Kosakonia arachidis*	99.62	100%	0.0	OL662986
11	M5	*Cupriavidus necator*	99.78	100%	0.0	OL662931
12	M8	*Pseudomonas putida*	99.57	100%	0.0	OL662935
13	M9	*Pseudomonas orientalis*	100	100%	0.0	OL662936
14	M11	*Pseudomonas monteilii*	99.75	100%	0.0	OL662939
15	LUMITI4	*Pseudomonas gessardii*	95.58	99%	0.0	OQ772292
16	LBS23	*Pseudomonas gessardii*	94.06	99%	0.0	OQ772326
17	LUMITI1	*Bacillus* sp.	97.34	92%	0.0	OQ773420
18	LUMBB2	*Chryseobacterium cucumeris*	99.06	96%	0.0	OQ947076
19	NC7	*Cedecea neteri*	100	96%	0.0	OQ947075
20	LUMITI6	*Bacillus subtilis*	100	97%	0.0	OQ947079
21	TSU6	*Proteus terrae*	100	95%	0.0	OQ947085
22	TSU2(1)	*Proteus terrae*	100	96%	0.0	OQ947090
23	TSU12	*Lelliottia amnigena*	99.31	96%	0.0	OQ947097
24	TSU3(7)	*Providencia rettgeri*	93.81	98%	0.0	OQ947074
25	TSU7	*Pseudomonas koreensis*	98.97	94%	0.0	OQ947088
26	TSU11	*Klebsiella variicola*	99.66	96%	0.0	OQ947091
27	TUL6	*Burkholderia cepacia*	99.85	98%	0.0	OR373102
28	LBS4	*Bacillus cereus*	99.77	99%	0.0	OR373114
29	TSU1(9)	*Proteus terrae*	99.85	97%	0.0	OR373123
30	TUL12	*Bacillus safensis*	96. 58	99%	0.0	OR373874
31	TUL11	*Burkholderia cepacia*	99. 21	99%	0.0	OR373879
32	LBS36	*Alcaligenes faecalis*	99.70	98%	0.0	OR373961
33	LBS12A	*Pseudomonas fluorescens*	99.54	95%	0.0	OR374026
34	LBS16	*Pseudomonas* sp.	90.55	97%	0.0	OR374038

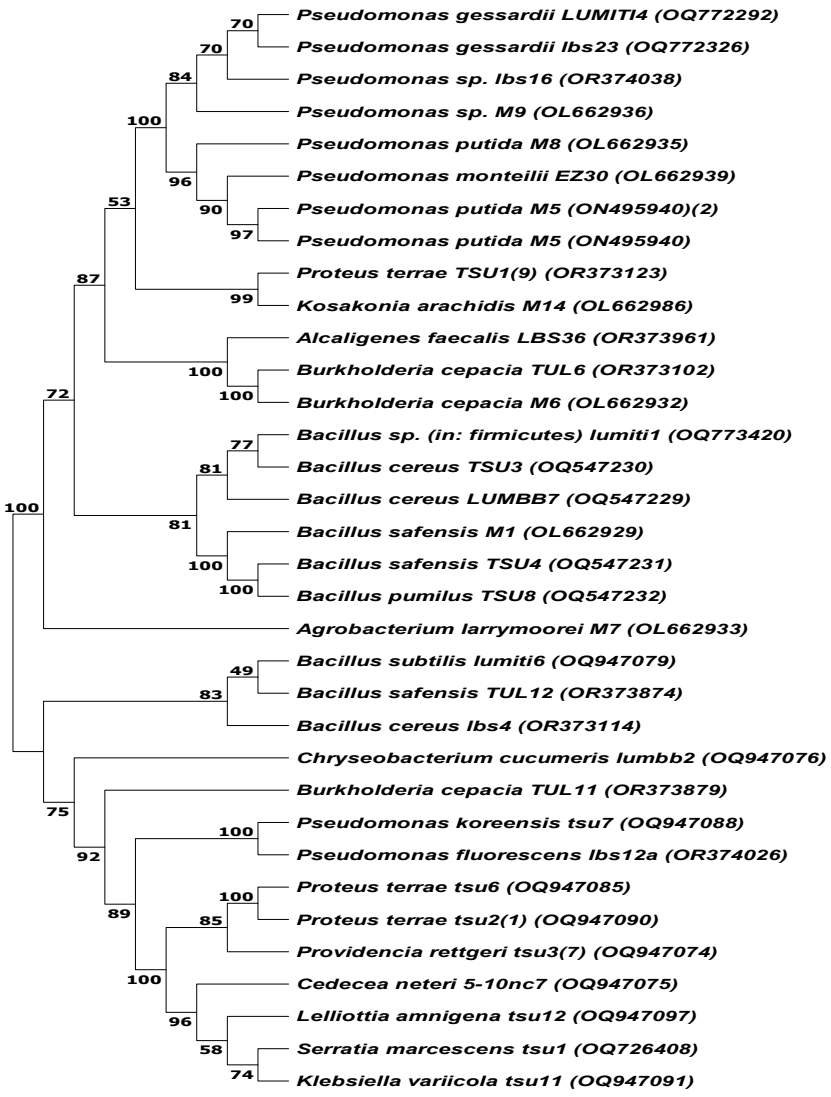

Figure 3.9: The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model [1]. The tree with the highest log likelihood (-12983.78) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura 3 parameter model, and then selecting the topology with superior log likelihood value. This analysis involved 34 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1269 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [2].

Discussion

In the last few decades, the application of PGPR for sustainable farming has increased considerably in different regions of the world. PGPR have various applications such as increasing nutrient availability and inhibiting the growth of pathogens resulting in enhanced growth and yields of economically important crops and has been reported continuously **(**Kloepper et al., 1980; Vessey, 2003). The nutrient status of the cultivated soil is a factor that contributes to optimum seasonal crop yield. Studies have shown that certain PGPR have a high potential to increase the soil nutrients of crop fields. Chemical fertilizers are applied by the farmers to fulfil the nutrient supply of crop plants (Tatung and Deb, 2021). However, a large portion of soluble inorganic form of NPK (Nitrogen, Phosphorus and Potassium) in the chemical fertilizers applied to agricultural soils gets immobilized quickly after application and thus is inaccessible to the crop's plants. Further, leaching of these additives magnifies the residual load of the synthetic fertilizer in the soil and run-down water. In our investigation total 136bacterial isolates were screened for the PGP traits such as phosphate solubilisation, siderophore production, IAA production, ammonia production, salinity and heavy metal tolerance.

The phosphate released by the PSB serves to immobilize and stabilize the free heavy metal ions within the soil. Additionally, PSB has the capacity to uptake, accumulate, and form complexes with heavy metal ions through cellular mechanisms, as well as through the secretion of organic compounds (Cheng et al., 2023; Wang et al., 2022b). Here we isolated the bacterial strains from *Musa* rhizospheric soil. The qualitative as well as quantitative analysis of phosphate solubilisation activity of the selected isolates was expressed in the present study. Out of 136 isolates, 94 isolates were

able to display the capability to solubilise phosphates when cultured on National Botanical Research Institute's phosphate agar medium (NBRIP) as evidenced by the formation of a distinct halo zone surrounding the bacterial colony (**Table 3.4**).The bacterial isolate LUMB8 (4.23±0.19), LBS16 (4.09±0.22), LBS14 (4.02±0.24), 25E18 (4.07±0.03) and 5R3 (4.09±0.22), obtained in the present study, showed the maximum phosphate solubilisation index (PSI) followed by other isolates L.BS21 (3.95±0.16), EZ11 (3.43±0.07), EZ30 (3.60±0.06), 25E13 (3.43±0.03), TSU1 (3.55±0.17), TSU8 (3.17±0.09), TSU7 (3.12±0.18) and TSU2(2) (3.23±0.15). Other isolates which showed good phosphate solubilising ability on agar plates were the isolatesLUMB1, LUMB2, LUMB3, LUMB4, LUMITI1, LUMITI12, LUMITI3, LUMITI4, LUMITI5, LUMITI6, LUMITI7, LUMBB1, LUMBB2, LUMBB3, LUMBB4, LUMBB6, LUMBB7, LBS12A, LBS25B L.BS13, LBS23, 5NC10, NC7, LBS4, LBS15, LBS19, LBS12, 5E20, 5R12, RZ7, 5R10, 25E22, EZ27, 25NC9, 25NC3, 25E3, TSU2(4), TSU3, TSU4, TSU6, TSU11, TSU12, TSU2(1), TUL8, TUL12, NC11, TUL11, TUL6, TUL5, TUL3, TUL2, 25R1, 5EZ13, RZ20, RZ5, 25EZ6, 5E3, 5E11, 25E20, 5E8, 25R23, 5E22, E24, 5R15, 25EZ1, RZ23, 5E28, 25E16, 25R10, RZ27, 25R2, 25E5, 25EZ2, 5NC17, 5NC20, 25NC8, 5NC14, 5NC8, LBS7, LBS36, LBS25A and LUMITI1.

Selected isolates were further studied for their quantitative estimation of their phosphate solubility during period of 12 days incubation in NBRIP broth (**Table 3.5**). The estimation of soluble phosphate in the culture media was carried using standard graph with different concentrations of KH_2PO_4 ranged between 0- 600µg/ml. The slope of the curve i.e., y= 0.0022x + 0.0724 with 0.98 R 2 value (**Figure 3.1**). The solubilisation of tricalcium phosphate varied significantly and depends upon the phosphate solubilisation capacity of the different isolates (Gupta et al., 2022b). It was

observed that all isolates were well efficient in phosphate solubilisation and the higher activity observed during 12th day of incubation for most of the isolates. The phosphate solubilising activity was ranged (µg/ml) from0.38±0.53 (LUMITI1) to 360.1±45 (10RZ9) **(Table 3.5).** Ten highest solubilisation was observed by 10RZ9 (360.1±45), followed by 30EZ3 (356.66±0.89), 30RZ10 (342.86±1.34), 30E24 (329.07±1.33), 30E6 (298.03±4.32), TSU2(1) (291.15±0.68), 30E22 (291.14±0.67), 30E5 (287.68±3.11), 30E23 (273.9±4.21) and 10E9 (273.9±1.67).

It is well well-known that decline in pH of liquid NBRIP medium in the presence of PSB plays important role in solubilisation of insoluble tricalcium phosphate (Chen et al., 2006). Maximum reduction in *pH* (4.12±0.87), observed in the present study, was by TSU4 isolate that also enhanced solubilisation of insoluble tricalcium phosphate of liquid medium. Similarly, the study of Pande et al. (2017) and Ghosh et al. (2017), showed the acceleration of mineral solubilisation by increasing acidification of the medium. Phosphate solubilization in the broth medium was found to be inversely related to pH as was observed by Chen et al. (2006) and Amri et al. (2023). There was a direct relationship between the phosphate solubilisation and *pH* of the medium. Higher the P solubilisation there was reduction on *pH* level. The PSB produce various types of organic acids, namely, acetic acid, adipic acid, butyric acid, fumaric acid, glyconic acid, malonic acid, malic acid, lactic acid, oxalic acid, succinic acid that result in lowering of *pH* of the liquid medium and consequent increase in phosphorus concentration (Rodriguez et al., 1999; Chen et al., 2006; Liang et al., 2020; Kpomblekou and Tabatabai, 1994). For example, in one study by Liu et al., (2014), it was reported that *Acinetobacter pittii, E. coli* and *E. cloacae* produce gluconic acid which in turn reduced the pH and increased P solubilisation.

Many bacteria produce plant growth regulators or phytohormones. Production of phytohormones by bacteria alters the level of endogenous plant hormones and therefore, affects plant growth and development (Gray, 2004). Among the various kinds of phytohormones, the most abundant and important plant growth regulator produced by bacteria is IAA. Several studies have shown that bacterial isolates could enhance synthesis of IAA only in the presence of tryptophan (Spaepen et al., 2007). Similar observations were record by Zahir et al. (2010), in *Rhizobium phaseoli* strain. In the present study, of the 136 isolates, 77 isolates were able to produce IAA in the presence of L-tryptophan. It has been reported earlier also that production of IAA can vary amongst different bacterial species and is also controlled by the condition of culture, growth stage, and availability of substrates (Spaepen et al., 2007). The standard graph has drawn for concentrations of IAA ranged from 0.00-200µg/ml, has used for the estimation of IAA in the culture media; the slope of the curve, that is; $y = 0.0457x + 0.0964$ and the R^2 value was 0.9754 **(Figure 3.2**). From the selected isolates which were further analysed quantitatively, seven bacterial isolates (TSU12, TSU11, NC7, TSU7, TSU3(10), LUMBB8 and LBS23) produced the highest IAA (µg/ml) (149.86, 126.35, 22.38, 10.09, 8.80, 4.65 and 4.29 on 6^{th} day, 4^{th} day, 12^{th} day, 6^{th} day, 4^{th} day, 8^{th} day and 10^{th} day respectively (**Table 3.7**). It is suggested that the decrease in IAA production along with incubation period might be due to release of IAA degrading enzymes such as indole acetic acid oxidase and peroxidase (Arora et al., 2015). The efficiency of IAA production and optimal conditions for its maximum accumulation were varied between bacteria to bacteria. However, production of IAA *in vitro* depends on various factors such as hours of incubation, temperature, *pH* and amount of the tryptophan added in the media (Tatung and Deb, 2023).

When there is a shortage of iron, microorganisms make siderophores, which are tiny organic molecules that increase the uptake of iron by the microorganism and is one of the mechanisms by which PGPR acts as biocontrol agents' and inhibits the growth of phytopathogens (Hu et al., 2011). Siderophores can help organisms to uptake iron in form of siderophore-Fe^{3+} complexes and then in the cell cytosol, iron is reduced and released in ferrous form (Sun et al., 2022). In the present study, ninety bacterial isolates were found to be able to produce siderophore when inoculated on CAS agar media which was indicated by the formation of orange halo zone around the bacterial colonies (**Table 3. 8).** Positive isolates formed an orange halo zone around the colonies as siderophores produced by the bacterial isolates remove Fe from the Fe-CAS complex which is blue as described by Alexander et al. (1991). Forty-five isolates were selected for further quantitative analysis in liquid medium. In liquid medium, 10 isolates with highest percent siderophore unit (PSU) EZ30 (197.138), followed by 10E28 (110.350), 30E18 (48.995), EZ24 (46.132), 10R3 (45.327), 10R15 (40.594), 10E18 (40.392), 10E3 (40.392), 10E9 (39.618) and 20E13 (39.170) (**Table 3.9**). Though siderophores are specific ferric iron chelators they can also bind to other metals such as divalent heavy metals and actinides because of potentially high metal-siderophore stability constants (Deb and Tatung, 2024).

PGPR also produced several active enzymes under drought, heavy metals and salts stress (Kumar et al., 2019). PGPR have been employed in the phytoremediation of the heavy contaminated soil in many studies. For example, *P. monteilii* when inoculated on the plants growing in extreme saline and drought condition shown to improve growth, increased in seedling growth (root length, shoot length, dry weight, and fresh weight) (Zhang et al., 2019). Furthermore, *P. monteilii* and *Pseudomonas plecoglossicida* when tested have shown had high MIC values for Cr (VI) making it a good candidate for

biofertilizer in Cr contaminated areas (Dharniet al., 2014). In this study, the bacterial isolates have shown a great deal of tolerance towards several heavy metals and variable levels of salinity (2-14%) tolerance. El-Akhdar et al. (2020), in their study have also reported that inoculation of *Lelliottia amnigena* and *Bacillus halotolerans* have alleviate the stress of salt on *Triticum aestivum*. However, according to their test results, the level of tolerance is higher in solid media than in liquid media. Similar report was found by Bhojiya and Joshi (2016), where *P. putida* exhibited high MTC for many heavy metals (Zn, Cd, Co, Ni, Cu, and Pb) on agar plates than in liquid media. Soil with high heavy metal content also had a high organic content, which can probably explain the maintenance of the microbial community diversity due to lack of competition (Rathaur et al., 2012). Certain *P. putida* strains harbour accessory genes that confer specific biodegradative properties and because these microorganisms can thrive on the roots of plants, they can be exploited to remove pollutants via rhizoremediation (Molina et al., 2020). The mechanisms PGPB employed to promote the phytoremediation process involve improvement of plant metal tolerance and increased plant growth, as well as alteration of metal accumulation in plants (Kong and Glick, 2017). Disposal of the phytoremediator treatment includes heat treatment, extraction treatment, microbial treatment, compression landfill and synthesis of nano particle (Liu and Tran, 2021). Several studies have demonstrated the importance of bacterial inoculation for plant growth and heavy metal accumulation in heavy metal contaminated environments (Nath et al., 2014; Wu et al., 2019; Sharma et al., 2020). Heavy metals such as Zn, Cu, Ni and Cr are essential micronutrients for microbes, plants and animals at lower concentrations. While, at an elevated level, these heavy metals are known to be a major toxicant for all form of living organisms (Manoj et al., 2020). The escalation of metal pollutants like lead

(Pb), cadmium (Cd), chromium (Cr), mercury (Hg) and arsenic (As) over time has inflicted severe distress upon the agricultural sector. This has resulted in a notable reduction in both plant growth and crop yield (Tirry et al., 2021). The PGPR can promote phytoremediation by neutralizing the harmful effects caused by heavy metal pollution, boosting tolerance of the plant, increasing the accumulation of the heavy metal in the plant tissue, and by secreting antioxidants enzymes and siderophores (Wrobel et al., 2023). When tested for heavy metal tolerance, different isolates showed varied tolerance to different heavy metals. Isolate TSU7 and TSU11 had the highest tolerance for Cu (770µg/ml), followed by TSU1 (740µg/ml), for Cr it was LUMITI2, LUMBB4 and LUMBB7 (10010µg/ml), followed by LBS23 and LSB4 (8060µg/ml). In case of Zn highest tolerance was shown by LUMBB2, LUMBB9 and LUMBB7 (10010µg/ml), followed by TSU1 and TSU7 (640µg/ml). Whereas, for Cd highest tolerance (µg/ml) was shown by TSU7 (570µg/ml), followed by TSU1 (540µg/ml) and LUMB1 (530µg/ml). For Ni highest tolerance was displayed by LUMITI3 and LUMBB2 (560µg/ml), followed by LBS23, LBS4, and TSU3 (460µg/ml). Whereas, for An, highest tolerance was presented by LUMB10 (270µg/ml) followed by LUMBB9 (180µg/ml) and for arsenic highest was shown by LBS23, LBS4 and TSU3 (850µg/ml), followed by LUMB10 (700µg/ml) (**Table 3.12**). This indicates the potential of the studies isolates to be used for heavy metal bioremediation. Molina et al. (2020) also presented that a comparable account in their research, proposing that particular strain of *P. putida* harbour accessory genes with distinct biodegradative capabilities. These genes could potentially be harnessed for the purpose of pollutants removal through the process of rhizoremediation. Similarly, the collaboration between *M. sativa* and *Pseudomonas* sp. has demonstrated an effective biological mechanism for remediating soils contaminated with chromium (Cr)

(Tirry et al., 2021). PGPB employ various mechanisms to promote the phytoremediation process, including boosting plant metal tolerance and alternating metal accumulation within plants. These actions collectively contribute to the successful and effective phytoremediation of contaminated environments (Kong and Glick, 2017). Uptake of heavy metals by the PGPR can be achieved by various mechanism including biosorption, bioaccumulation, bio-precipitation, and secretion of extracellular polymeric substance (EPS) (Wrobel et al., 2023). However, the effectiveness of bioremediation relies on the dynamic interplay among soil microbes, plants and the heavy metals present. Any slight alternation in the physicochemical and biological properties of rhizosphere soils by biotic and abiotic stress can significantly impact the plant-microbe interaction. Furthermore, isolation and characterization of suitable PGPR is a time-consuming process (Deb et al., 2020).

Ammonia in volatile form is important for the control of soil-borne diseases (Bhattacharyya and Jha, 2012). It enhances growth of plants by providing accessible form of nitrogen to the plant (Kandjimi et al., 2015) and increases plant growth as a result of their potential to fix free nitrogen to ammonia, allowing more ammonia to be available for plant development and growth (Hayat et al., 2010). In plants, ammonia serves as a source of nitrogen and aids in the metabolic processes that produce amino acids. As a result, PGPR that produces ammonia promotes plant growth and biomass production (Alali et al., 2021). In the present study, all PGPR isolates had the ability to produce ammonia however in different concentration. Some isolates produced more ammonia than others as indicated by the intensity of the colour developed after the addition of Nessler's reagent. In the present study, 26 isolates (e.g., LUMB9, LUMB2, 5NC8, 5NC6, 5NC13, LUMITI6, LBS36, LBS44A) produced high amount of ammonia; while, 60 (e.g. LUMB8, LUMB10, LBS4, LBS31, LBS19) isolates produced moderate amount of

ammonia and 50 (e.g. LUMITI1, LUMITI5, LUMBB1, LUMBB2, LUMBB8, LBS13, LBS14, LBS7) isolates produced low amount of ammonia (**Table 3.10**). Similarly, many researchers identified PGPR isolates that have ammonia production with other PGP traits.

Dealing with soil salinization is vital as it leads to a reduction in production potential of up to 46 million ha per year and causes the annual loss in agricultural productivity estimated to be of US$ 31 million according to the Food and Agricultural Organization**.** It has become necessary to utilize such salt- affected soil for farming during increasing food demand. One possible way to get rid of it is to explore new bioagents or bioinoculants (Egamberdiyeva et al., 2019). Salinity stress contributes to the production of reactive oxygen species, including hydrogen peroxide, superoxide ions, and singlet oxygen. This stress condition leads to a reduction in the activity of plant defensive enzymes, disrupts sodium balance, impairs iron uptake, and affects the levels of phenols and various trace elements (Sharma et al., 2021). Salinity beyond admissible level also affects different physiological parameters like seed germination, photosynthesis, transpiration, stomatal conductance, leaf water potential and turgor pressure etc. and all these collectively affect the growth and development of plants (Arif et al., 2020). Salt stress also induces nutrient deficiencies in plants, leading to stunted growth and development (Gul et al., 2023). In the arid soils, the crop productivity is reduced due to elevated salinity levels and iron deficiency (Sultana et al., 2021). It has been previously reported that different bacterial strains showed varying adeptness to concentrations of NaCl and majority of rhizobacterial isolates grew optimally at 0.5 % NaCl (Shahab and Ahmed, 2008). In the present study, RZ27, TUL6 and TUL11 exhibited highest tolerance, withstanding up to 14% NaCl. This was followed by isolates EZ11, RZ20, TSU4 and TUL12, which displayed a 12% tolerance level. Isolates LUMITI3, LUMITI4, LUMITI6, LBS36, LBS16, LBS13, 25E17 and RZ5 demonstrated a 10% tolerance, and LBS11, LBS29, RZ23, 5R3, TSU3(2) and 5NC17 exhibited the lowest level of tolerance at 8%

NaCl. Other isolates such as LUMB7, LUMB9, LUMBB4, LUMBB8 , LBS18 , LBS44B, LBS4, LBS17, LBS22, 5E24 , 25E10 , 5R1 , 25E18 , 5R15 , 5E28 , 25E16 , RZ7, 5R10, 25Z13, 25E22, 25E20, 5E11, 5E3 , 25EZ6 , TSU2(4) , TSU3 , TSU12, 5NC8, 5NC6, 5NC13, 25NC12 , 5NC20 and NC26 could tolerate till 6% NaCl level. Rest of the isolates could tolerate only till 2-4% (**Table 3.11**). The tolerance by the PGPR can aid in plants adaptation of the salinity stress. For example, *Bacillus aryabhattai*, a halo tolerant siderophore producing rhizobacteria could produce a significant amount of siderophore (43%) even under 200 mM saline conditions making it a good candidate for being used as biofertilizer in high salinity regions (Sultana et al., 2021). Salinity stress by PGPR has been studied by Grossi et al. (2020), on *Solanum tuberosum* L. cv. Desirée. Their study has shown that plantlets inoculated with bacterial isolate *Methylobacterium* sp. 2A have shown increased lateral root, a greater number of leaves. Inoculation of canola plant with halo tolerant PGPR *Enterobacter* sp. S16-3 and *Pseudomonas* sp. C16-2O plant biomass and biochemical traits under salinity stress (Neshat et al., 2022). PGPR are classified according to their mechanisms, but in the context of salt stress the analysis of their influence on the plant's response has shown that their promoting activity is never due to a single mechanism (Giannelli et al., 2023).

There are several approaches available for bacteria identification that includes morphology and biochemical characterization but these techniques are known as challenging and time-consuming. These approaches are not always effective in explicitly identifying the microorganism to the level of species, or even more rarely to the level of the strain (Franco-Duarte et al., 2019). Many closely related species isolated in PGP studies cannot be distinguished using conventional methods. Misidentification occurs often in traditional methods because of several unknown phenotypic features that often emerge due to culture conditions (Cherkaoui et al., 2010). On the other hand, 16S *rRNA*

technique offers an effective and efficient way of identification of any community of bacteria up to species level. The 16S *rRNA* genes are highly conserved that exist universally in all bacterial genome (Klappenbach et al., 2000). For all bacteria the procedure for molecular identification is identical. It needs only DNA extraction, amplification of 16S *rRNA* and sequencing (Reller et al., 2007). Besides, the use of 16S *rRNA* sequences of different bacteria is a more effective approach for identifying bacteria compared to traditional identification approaches, since the chances of misidentification are significantly minimized (Boivin-Jahns et al., 1995). The BLAST search results of 16S *rRNA* gene sequences of our isolates on comparison with sequences of similar bacteria deposited in the NCBI GenBank database did not indicate full identification with a single target bacteria but with specific genus or species, suggesting that the 16S rRNA gene sequence, though frequently used could act as a genetic marker for the classification of bacteria. A total of 34 morphologically different strains were selected for identification through 16S *rRNA* gene sequencing from the larger collection of isolates based on higher PGP efficiency and number of PGP traits. The PGPR strains isolated in the present study was subjected to molecular characterization using 16S *rRNA* sequences and identified as *Serratia marcescens* (TSU1), *Bacillus cereus* (TSU3), *Klebsiella variicola* (TSU11), *Lelliottia amnigena* (TSU12), *Pseudomonas koreensis* (TSU7), *Bacillus pumilus* (TSU8), and *Bacillus safensis* (TSU4), LUMBB7 (*Bacillus cereus*), M5 (*Pseudomonas putida*), M7 (*Agrobacterium larrymoorei*), M1 (*Bacillus safensis*), M6 (*Burkholderia cepacian*), M14 (*Kosakonia arachidis*), M5 (*Cupriavidus necator*), M8 (*Pseudomonas putida*), M9 (*Pseudomonas orientalis*), M11 (*Pseudomonas monteilii*), LUMITI4 (*Pseudomonas gessardii*), LBS23 (*Pseudomonas gessardii*), LUMITI1 (*Bacillus* sp.), LUMBB2 (*Chryseobacterium cucumeris*), NC7 (*Cedecea neteri*), LUMITI6 (*Bacillus subtilis*),

LUMITI6 (*Bacillus subtilis*), TSU6 (*Proteus terrae*), TSU2(1) (*Proteus terrae*), TSU3(7) (*Providencia rettgeri*), TUL6 (*Burkholderia cepacia*), LBS4 (*Bacillus cereus*), TSU1(9) (*Proteus terrae*), TUL12 (*Bacillus safensis*), TUL11 (*Burkholderia cepacia*), LBS36 (*Alcaligenes faecalis*), LBS12A (*Pseudomonas fluorescens*), and LBS16 (*Pseudomonas* sp.) to which they exhibited 99.22, 97.23, 99.66, 99.31, 98.97, 96.95, 95.02, 97.44, 98.12, 98.69, 97.53, 97.20, 99.62, 99.78, 99.57, 100, 99.75, 95.58, 94.06, 97.34, 99.06, 100, 100, 100, 100, 100, 93.81, 99.85, 99.77, 99.85, 96.5 ,99.21, 99.70, 99.54 and 90.55%homology respectively and a phylogenetic tree was generated using MegaX software. In this study an attempt was made to collect PGPR isolates with plant growth promoting activities such as IAA production, siderophore production, phosphate solubilisation, ammonia production, salinity tolerance, and heavy metal tolerance to be used as biofertilizer in the sustainable agriculture system. *Musa* rhizospheric soil collected from the sites highlighted the promising potential of PGPR as sustainable and effective solution for improving plant growth and reducing heavy metal contamination in the environment.

Conclusion

The findings of the study presented that *Musa* rhizospheric soil harbours PGPR bacteria, possessing Phosphate solubilizing ability, IAA and siderophore production, heavy metal and salinity tolerance. The most dominant genera of the resident bacterial population were *Bacillus* and *Pseudomonas*. The other isolates belong to the following genera *Burkholderia*, *Proteus*, *Alcaligenes*, *Serratia, Agrobacterium, Kosakonia, Cupriavidus, Chryseobacterium, Cedecea, Lelliottia, Klebsiella,* and *Providencia.* All the bacterial strains were found to be very diverse in terms of plant growth promoting activities. Overall, the selected organisms show high potential to be used to design biofertilizers. Also, expanding the knowledge about the microbial genetics taking part in

resistance of heavy metal is required to be studied to develop bacterial candidates for multifunctional PGPR - based formulations. By understanding the mechanisms involved in mobilization and transfer of heavy metals, future strategies can be developed and use in the bioremediation process. With enough evidence in the recent past there is no doubt that PGPR helps the plants for better growth and development. However, for genetic improvement to be accomplished, a rock-hard understanding of the physiological and biochemical changes in plants induced by PGPR and PGR is essential. With genetic engineering scientist have targeted the genes involved in growth promoting activities and has modified the increase the activities in lesser time.

Chapter – 4

Effect of Inoculation of PGPR Strains on Growth Parameters of Different Crop Plants

Introduction

As the world's population continues to grow, it is essential to enhance agricultural productivity to meet the increasing demand for food and alleviate poverty (Shah et al., 2021). Traditional agricultural methods widely use fertilizers to boost crop yields, but these practices have significant environmental drawbacks, including nutrient losses, decreased soil fertility, and water and air pollution (Chaudhary et al., 2021). The overuse of chemical fertilizers and pesticides has created substantial environmental issues, adversely affecting human health (Tatung and Deb, 2021). However, the use of biofertilizers containing plant growth promoting rhizobacteria (PGPR) can reduce the negative impacts of these harmful agrochemicals, if not eliminate the need for them entirely (Sedri et al., 2022; Tatung and Deb, 2023). Despite extensive research by universities and private sectors in India over the

past two decades, which has identified numerous beneficial microbes, there have been no ground-breaking discoveries or successful commercial application in the biofertilizers field (Keswani et al., 2019). Severalfactors contribute to the lack of progress in the field of biofertilizers, including poor acceptance among farmers, issues with proper carrier, challenges in formulating effective microbial consortia, field level constraints, and the higher cost compared to commercially available synthetic fertilizers (Basu et al., 2021). Biofertilizers and biopesticides are vital for promoting sustainable agriculture, making the generation of demand among farmers a crucial step for their advancement (Singh et al., 2016).

The emergence of microbial inoculants technology offers promise for improving agricultural productivity by leveraging the benefits of beneficial microorganisms for crop nutrition and protection (Tatung and Deb, 2023). For instances, Delshadi et al. (2017), suggested that the use of PGPR, either individually or in combination, improved the germination of *B. tomentellus*. Similarly, Kang et al. (2020), reported that endophytic *Klebisiella Pneumoniae* exhibited significant potential as rich source of herbicidal metabolites, inhibiting weed plant growth and offering an alternative to chemical herbicides. In another study by Amogou et al. (2021), demonstrated that applying *Pseudomonas syringae* to maize with a reduced dose of 50% NPK + urea resulted in higher fresh and dry aerial biomass, indicating the potential to reduce or even eliminate synthetic fertilizers through the use of PGPR. Furthermore, Dinesh et al., (2013), reported that applying PGPR strains *Burkholderia cepacian*, *Klebsiella* sp., *Serratia marcescens*, and *Enterobacter* sp., alone or in combination with varying degrees of NPK fertilizer on *Zingiber officinale* Rosc., led to a 24% increase in mean DOC levels in treatment with PGPR+NPK compared to

control. In another study, Sedri et al. (2022) evaluated four commercial brands of PGPR (fla-wheat (F), Nitroxin® (N1), Nitrokara® (N2) and Barvar-2® (B)) and new PGPR *Pseudomonas* bacteria. They found that integrating these with chemical fertilizers increased the grain yield of treated plants by 28, 28, 37 and 33% respectively, compared to non-inoculated control. Additionally, grain protein content increased by 0.54%, 0.88% and 0.34% with the integrated application of F, N1 and N2 PGPR plus 50% of need-based chemical fertilizers, respectively. Therefore, incorporating PGPR can be a promising alternative to reduce the application of synthetic fertilizers.

Phosphorus is vital nutrient for all life form on earth, with soils worldwide containing 400-1000 mg/kg of total phosphorus. However, only 1.0-2.50 % of this phosphorus is available to plants, significantly impacting plant growth (Pan and Cai, 2023). Utilizing bacterial strains with phosphate solubilizing abilities has demonstrated improved plant growth and development (Tatung and Deb, 2023). For example, *Pseudomonas moraviensis*, *Bacillus safensis*, and *Falsibacillus pallidus*, which efficiently solubilize phosphorus and produce indole-3-acetic acid (IAA) in sandy fluvo-aquic soil, significantly increased *Triticum aestivum* yield by up to 14.42% compared to control treatments in phosphate fertilizer -utilized farmland (Wang et al., 2022e). In another study, PSB strains *Acinetobacter pittii*, Escherichia coli, and *Enterobacter cloacae* significantly enhanced plant height, shoot and root dry weight, and nutrient uptake in *A. catechu* seedlings (Liu et al., 2014). The primary drivers of phosphorus solubilization in the rhizosphere include inorganic and organic compounds such as mucilage, organic acids, phosphatases and specific signaling substances (proton release, chelation, and ligand exchange) (Elhaissoufi et al., 2021). Among these the production of organic acids is the most well-recognized and common in rhizobacteria. The

organic acids secreted by PSB act through various modes, including chelating mineral ions bound to precipitated inorganic phosphate and dissolving phosphorus by lowering the *pH* (Brito et al., 2020).

Plant hormones (phytohormones) regulate or influence a variety of cellular and physiological process, including cell division, cell enlargement, bud dormancy, flowering, fruit ripening, seed dormancy, seed germination and leaf abscission (Lwin et al., 2012). PGPR produce phytohormones such as Indole-3- acetic acid (IAA), gibberellic acid, cytokinin, and ethylene (Tatung and Deb, 2021, 2024). The ability to produce IAA may enable bacteria to detoxify excess tryptophan/tryptophan analogues that are harmful to the bacterial cell (Prasad et al., 2019). The amino acid tryptophan, found in plant root exudates, is a precursor that modulates the level of IAA biosynthesis (Kamilova, 2009). Starting with tryptophan, at least five different pathways for the synthesis of IAA have been described: indole-3-acetamide pathway: Indole-3-Acteamide Pathway, Indole-3-Pyruvate Pathway, Tryptophan Pathway, IAA Conjugation and Degradation pathways. These pathways are similar to those found in plants, although some intermediates may differ (Patten and Glick, 1996; Woodward and Bartel, 2005; Spaepen et al., 2007). Exogenous application of L-TRP to soils has been shown to stimulate synthesis of auxins, positively influencing plant growth and development. However, the information about the mechanisms by which PGPR promote plant growth remains quite limited (Kundan et al., 2015; Goswami et al., 2016).

Iron is required by all forms of life, however, is not accessible to all in the soluble form (Deb and Tatung, 2024). It serves as a catalyst in enzymatic processes, oxygen metabolism, electron transfer and DNA and RNA synthesis. Iron is also crucial for biofilm formation, as it regulates surface motility and stabilizes the polysaccharide matrix (Ahmed,

2014). Most organisms need iron for various metabolic and informational cellular pathways (Tatung and Deb, 2024). Over 100 enzymes in primary and secondary metabolism contain iron cofactors such as iron-sulphur cluster or heme groups. The Fe (II)/Fe (III) redox pair is ideal for catalyzing a widerange of redox reactions and meditating electron transfer chains (Miethke and Marahiel, 2007). Soil microbes and certain plants, known as strategy II plants; secrete siderophores into therhizosphere to scavenge iron (Dimkpa, 2016). Siderophore are low molecular weight compounds (500-1500 Da) that bind strongly to iron (Manck et al., 2022). There are more than 500 different types of siderophores, with 270 structurally characterized (Ahmed, 2014). Siderophore production is inversely correlated with environmental iron levels, decreasing as Fe^{3+} concentration increases (Wang et al., 2022c). Additionally, siderophore act as bio-remediators by altering the oxidation states of heavy metals such as Cd, Cu, Ni, Pb, Zn, Th, U, and Pu, reducing their toxicity (Singh et al., 2022). For example, two siderophillic strains, *Brucella* sp. and *Pseudomonas brassicae*, isolated from rhizospheric soil, significantly increased various growth indicators of *Vigna radiata* seedlings as single strain or in combination. Under both no-iron and high-iron stress, the inoculation treatment promoted growth in both *Vigna radiata* and *Lolium multiflorum* (Sun et al., 2022). In another study, *Enterobacter quasihormaechei* isolated from Fe-deficient spinach plants significantly enhanced the nutrient content and colonization in spinach roots and shoots under Fe-sufficient and Fe-deficient conditions compared to uninoculated controls (Misra et al., 2023).

The crop productivity declines due to various biotic and abiotic stresses. Biotic factors include stresses caused by phytopathogens and pest, such as fungi, nematodes, viruses and insects. Major abiotic factors include stresses like drought, salinity, heavy metals,

flooding, and extreme temperatures (Kumar et al., 2019). Heavy metals are naturally occurring elements with high atomic numbers, weight and densities (about 5% on earth's crust) (Manoj et al., 2020). While modern techniques can remediate metal-polluted soil and water, physicochemical approach are costly and time consuming (Dharni et al., 2014). To address this issue, a cost effective, co-friendly, and sustainable alternative would be harnessing the power of microbes and plants. This approach effectively removes heavy metals from soil, sediments, and water throughmechanisms such as bioaccumulation, bio-mineralization, biosorption and biotransformation, which microorganisms have developed to thrive in a heavy metal-rich environment (Ayangbenro and Babalola, 2017; Tatung and Deb, 2023). Compared to traditional physical and chemical remediation methods, this biological method is not only cost-effective and simpler but also environmentally friendly (Liu and Tran, 2021). Plant growth promoting rhizobacteria (PGPR) are highly effective in immobilizing heavy metals and reducing their translocation in plants through precipitation, complex formation and adsorption (Khanna et al., 2019). For example, the germination rate of maize in the Cu-contaminated soil inoculated with *Sphingomonas* sp. PbM2 (38.9%) was seven times higher than in non-inoculated soil (5.56%), while the germination rate in the Cu-contaminated soil inoculated with strain *Novosphingobium* sp. CuT1 (22.2%) was four times higher. In the Pb-contaminated soil, the germination rates of maize in the soil inoculated with PbM2 and CuT1 were 72.2% and 69.4%, respectively, compared to 44.44% in non-inoculated soil (Lee et al., 2024).

Soil salinity is another significant abiotic stress affecting global agricultural productivity, with an estimated 50% of arable land predicted to become salinized by 2050 (Giannelli et al., 2023). The accumulation of excess soluble salts in cultivable land directly

impacts crop yield, as high salt uptake inhibits diverse physiological and metabolic processes in plants, even threatening their survival (Egamberdieva et al., 2019). The co-inoculation of halo tolerant bacteria such as *Azospirillum*, *Agrobacterium*, *Pseudomonas* and various other gram-positive *Bacillus* species is an environment-friendly approach to reclaiming salinity-affected lands and maximizing biomass production (Kashyap et al., 2019, Haroon et al., 2021). For example, inoculating maize seeds with *Acinetobacter jonsonii* resulted in a significant increase in urease (1.58-fold), acid (1.38-fold), and alkaline phosphatase (3.04-fold) and dehydrogenase (72%) activities compared to uninoculated control. The inoculation also improved the soil enzyme activities and soil biological health, corrected nutritional imbalances, and enhanced nutrient acquisition by the plant under salt stress (Shabaan et al., 2022). Similarly, *Enterobacter cloacae* PM23 inoculation in maize increased radical scavenging capacity, relative water content, soluble sugars, proteins, total phenolic, and flavonoid content compared to control plants. Additionally, higher levels of antioxidant enzymes and osmoprotectants (free amino acids, glycine, betaine and proline) were observed in *E. cloacae* inoculated plants (Ali et al., 2022a).

Considering the above-mentioned facts, in this chapter, study was undertaken to elucidate the concept on the enhancement of plant growth parameters of two selected crop plants by the application of consortium to study the effect of PGPR strains with the following objectives:

- Selection of the bacterial isolates for inoculation on different test plants.
- Inoculation of the PGPR strains as singly and as consortium and assessment of the role of PGPR on plant growth.

Material and Methods

Selection of test plants

PGPR isolates with various growth promoting traits were selected and inoculated on different plants such as *B. juncea* (L.) Czern**.**, *H. annuus* L., *P. vulgaris* L. and *C. arietinum* L. to see their effect on the plant growth and development, either individually and or as a consortium.

Bacterial strains as PGPR

Bacterial strains exhibiting plant growth promoting traits were selected for the development of biofertilizer consortia or inoculated individually. The isolates used included *Pseudomonas putida* (EZ11), *Kosakonia arachidis* (EZ27), *Pseudomonas monteilii* (EZ30), *Serratia marcescens* (TSU1), *Bacillus cereus* (TSU3), *Klebsiella variicola* (TSU11), *Lelliottia amnigena* (TSU12), *Psedomonas koreensis*(TSU12), *Bacillus pumilus* (TSU8) and *Bacillus safensis* (TSU4), *Bacillus subtilis* (LUMITI6), *Chryseobacterium cucumeris* (LUMBB2)*, Bacillus cereus* (LUMBB7) and *Pseudomonas gessardii* (LUMITI4*), Cedecea neteri* (NC7), *Pseudomonas orientalis* (RZ3), *Agrobacterium larrymoorei* (RZ23)**,** *Burkholderia cepacian* (RZ27), *Pseudomonas taiwanensis* (RZ5). Selected strains were either applied singly or developed into consortia, and different combinations of the strains were inoculated into different crop plants.

Seed sterilization and bacterization

Seeds were surface-sterilized with 2% sodium hypochlorite (NaOCl) (v/v) for 2 min, followed by 70% ethanol for 1 min, and washed thoroughly 7 times with sterilized deionized water. From the last washings, 100 µL of the aliquot was checked for the presence of bacteria and there was no bacterial growth, indicating the complete surface sterilization of

the seeds. Sterilized seeds were then immersed in each bacterial suspension for 3½ hrs in shaking conditions at room temperature (28±2ºC). After bacterizations, seeds were then sown in pots containing mixture of soil and sand in 1:1 ratio.

Pot experiment

Sterilized seeds were then immersed in each bacterial suspension for 3½ h in shaking conditions at 28 ±2ºC. After bacterization, seeds were sown in pots containing mixture of soil and sand at 1:1 ratio. Soil and sand used for the experiment was sterilized by autoclaving at 1.05 $kgcm^{-2}s^{-1}$ for 15 min three times consecutively and then put in plastic pots with three replicates of each treatment. The plants were harvested after 30 days of plantation and all the plantlets were uprooted and vegetative parameters such as shoot length, root length, root and shoot fresh biomass and dry biomass were measured. These parameters were compared with PGPR treated and non-treated plantlets under controlled greenhouse conditions. Non-inoculated seeds sown were considered as control. Plants were watered everyday with autoclaved tap water. Different set of pot experiments were conducted on different plants which are as follows:

Inoculation of PGPR strains as individual inoculums on *Cicer arietinum* L.

Three PGPR strains were selected and inoculated on to plant singly. The isolates selected were *K. arachidis* (EZ27), *P. monteilii* (EZ30) and *P. putida* (EZ11). The effects of the investigated rhizobacterial isolate on plants growth of model plant *C. arietinum* L. in pot experiment. For each treatment, 30 seeds were sown in each pot and experiments were repeated thrice. Seedlings were harvested after 30 days of sowing and morphological characteristics were determined. For the purpose, vegetative characteristics including shoot length, root length, root and shoot fresh biomass and dry biomass were measured after

uprooting all the plantlets. Under the control condition, these metrics were compared with PGPR treated and untreated plantlets. Booster shots were administered two times a week and plantlets were watered regularly with autoclaved sterilized water.

Inoculation of *Brassica juncea* (L.) Czern. and *Helianthus annuus* L. with PGPR consortia under 150 mg/ml of Cd/Cu contamination in soil

For the second pot experiment, two plants species were considered as model plant *viz*., *B. juncea* (L.) Czern. and *H. annuus* L. A control and three different treatments were set up in a fully randomized design. Following treatments were composed for the experiment: 'C' or control treatment (No heavy metal and PGPR in the potting mix), P (PGPR + No heavy metal in the potting mix), H (No PGPR + Heavy metal in the potting mix (150 mg/ml of Cd/Cu)) and P+H (PGPR + Heavy metal in the potting mix (150 mg/ml each of Cd/Cu)). The potting mixture consists of a 1:1 ratio of soil and sand, with the soil collected from the Nagaland University campus. The soil-sand blend was autoclaved at 121 psi for 15 min and subsequently placed into plastic pots. In each pot, 10 seeds soaked in bacterial suspension were sown and allowed to grow for a span of 30 days. In the pot containing *H. annuus* L., the total plantlets were trimmed to 3 per pots, while for *B. juncea* 10 plants were maintained per pot. Booster shots of the PGPR consortia were given twice a week. After 30 days of plantation, the influence of bacterial inoculation in the presence of heavy metal in the soil was assessed. This involved uprooting the plants and conducting a comparative analysis of growth metrics such as root and shoot length, as well as fresh and dry weights for shoots and roots.

Inoculation of PGPR strains as single inoculants onto *Brassica juncea* (L.) Czern.

In the third pot trial, four PGPR strains (*B. cereus* (LUMBB7), *P. gessardii*

(LUMITI4), *C. cucumeris* (LUMBB2), *B. subtilis* (LUMITI6) were selected and combined to form a PGPR consortium and inoculated onto *B. juncea* (L.) Czern. Two treatments were established: one treatment involved the application of the PGPR consortium, while the other served as the control without any PGPR application. The experiment was conducted in pots, with each pot containing ten bacterized seeds. To ensure a sterile environment, the potting mix, consisting of a 1:1 ratio of soil and sands, was autoclaved at 1.05 $kgcm^{-2}s^{-1}$ for 15 min. The PGPR mixture was administrated to the plants twice a week as a booster does to enhance their growth potential. After 30 days of plantation, the plants were uprooted, and various growth parameters such as shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight were measured.

Inoculation of PGPR strains onto *Phaseolus vulgaris* L. as single strains

For the fourth trial, the impact of the isolated rhizobacterial isolates on plants growth was studied on the plant *P. vulgaris* L. A fully randomized design was employed, comprising a control and four different treatments. The treatments included: C control treatment (No PGPR), RZ3 (*P. orientalis*), RZ5 (*P. taiwanensis*), RZ23 (*A. larrymoorei*) and RZ27 (*B. cepacian*). The potting mixture consists of a 1:1 ratio soil and sand, with soil collected from the Nagaland university campus. This soil and sand mixture were sterilized by autoclaving at 121 psi for 15 minutes before being placed in plastic pots. In each pot, 10 bacterized seeds were sown, later trimmed to a 3 plantlets per pot. Control pots were planted with non-inoculated seeds. Plants were watered regularly with autoclaved tap water and booster shots were given twice a week. After 30 days of plantation, the impact of the inoculation was assayed by uprooting plants and conducting a comparative analysis of growth parameters, including root and shoot length, fresh weight, and dried weight of both shoot and roots was

conducted. These parameters were compared among PGPR treated and non-treated plantlets under control conditions.

Statistical Analysis

The SPSS software was used for statistical analysis of the experimental data. All the reported results are the mean of the three replicates and deviations were calculated as the standard error of the mean (SEM). For assessing the importance treatment effect was done following one-way ANOVA and Least Significance Test (LSD) at the 0.05 level of confidence was used to compare means in cases where the F values were significant. When the *p* value was ≤ 0.05, differences were deemed significant.

Results

In the current study, PGPR strains were isolated from rhizosphere of *Musa* plants growing in the jungle of Nagaland. Various species of PGPR were identified following screening for growth promoting traits. After the molecular characterization, inoculation was performed on different crop plants and multiple experiments were set up. Crops plants after growing for 30 days were uprooted and plant growth parameters such as shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight were measured as presented in **Table 4.1**.

In the first set of experiment all three isolates promoted the plant growth compared to control treatment. Each bacterial isolate stimulated one or more plant experiment growth parameters. Shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight were the growth parameters that were tested (**Figure 4.1 and Figure 4.2**). The plant inoculated with isolate *K. arachidis* was shown to have the highest shoot length; shoot fresh weight, root length and root dry weight. *Cicer arietinum* L. when

inoculated with *P. monteilii*, *K. arachidis* and *P. putida*, the length of the shoot rose from 7.17cm (under control conditions) to 17.27cm, 17.90cm, and 13.10cm, respectively. Similarly, *K. arachidis* supported higher root length (18.87cm) followed by *P. putida* (16.43cm) and *P. monteilii* (12.00cm) against 10.63cm under controlled condition. Shoot fresh weight was highest with *K. arachidis* (1250 mg) followed by *P. monteilii* (1150 mg) and *P. putida* (840 mg) against 500 mg under controlled growth. Shoot dry weight was highest in *P. monteilii* (240 mg) followed by *K. arachidis* (200 mg) and *P. putida* (170 mg). Root fresh weight was highest in *P. putida* (1110 mg) followed by *P. monteilii* (850 mg) and *K. arachidis* (730 mg). Finally, root dry weight was improved by inoculation with *K. arachidis*, *P. monteilii* and *P. putida* from 100 mg in control to 190 mg, 180 and150 mg respectively (**Table 4.1**).

In the second set of experiment, After 30 days of plantation, to determine the impact of the inoculation in the presence of heavy metals in the soil, plants were uprooted and a comparative analysis of growth metrics including root and shoot length, fresh weight, and dried weight of both shoot and roots was conducted **(Figure 4.3 and Figure 4.4**). *B. cereus* (TSU3), *B. safensis* (TSU4), *B. pumilus* (TSU8), *K. variicola* (TSU11), *L. amnigena* (TSU12), *P. koreensis* (TSU7), *S. Marcescens* (TSU1). *B. juncea* and *H. annuus* plants were selected because of their high bioremediation potential. The inoculation potential of all the selected isolates showed a significant increase in most of the growth parameters of under controlled experiments. In both the plants species, shoot and root length, root fresh weight, and root dry weight was highest in P treatment (only PGPR consortia) ($p \leq 0.05$). In *B. juncea* shoot fresh and dry weight was highest in P+H treatment ($p \leq 0.05$). Whereas, in *H. annuus* L., shoot fresh weight, dry weight was highest in P treatment ($p \leq 0.05$). In *B. juncea*,

shoot length was increased from 7.46 in control (C) to 6.27 cm in heavy metal (H) treatment, 8.89cm (P+H) and 10.13cm (P). Root length was highest in P (7.03cm) followed by C (4.63cm), P+H (3.74cm) and H (3.60cm). Shoot fresh weight was highest in P+H (891.67mg) followed by P (868.67mg), C (519.67mg) and H (417.67mg). Shoot dry weight was highest in P+H (137.67mg) followed by P (130.67mg), C (75mg) and H (60.33mg). root fresh increased from 26cm in H to 32.33mg in C, 37.33mg in P+H and 62.33mg in P. Root dry was highest in P (44.33cm) followed by P+H (20.33cm), C (19.33mg) and H (13.33mg).In *H. annuus,* shoot length (34.87cm), root length (11.84cm), shoot fresh weight (8426.33mg), root fresh weight (1081.33 mg), shoot dry weight (1976mg) and root dry weight (460mg) were highest in treatment P. After P shoot length was enhanced in P+H (24.33) followed by C (24.17cm) and H (21 cm). Root length was second highest in P+H (10.33 cm) followed by H (10.03 cm) and C (6.53 cm). Shoot fresh weight was second highest in P+H (5524.33mg) followed by C (4529.67mg) and H (3492mg). Shoot dry weight was second highest in P+H (1604mg) followed by C (1091.67mg) and H (596.67mg). root fresh weight was second highest in C (863.67mg), H (832 mg) and P+H (759.33mg) and root dry weight was second highest in P+H (259.33mg) followed by C (218mg) and H (171.33mg) (**Table 4.1**).

Figure 4.1: Pattern of growth and development of *Cicer arietinum* L. inoculated with: A) *Kosakonia arachidis*, B) *Pseudomonas monteilii*, C) *Pseudomonas putida*; D) Control. Evaluation parameters: Shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight.

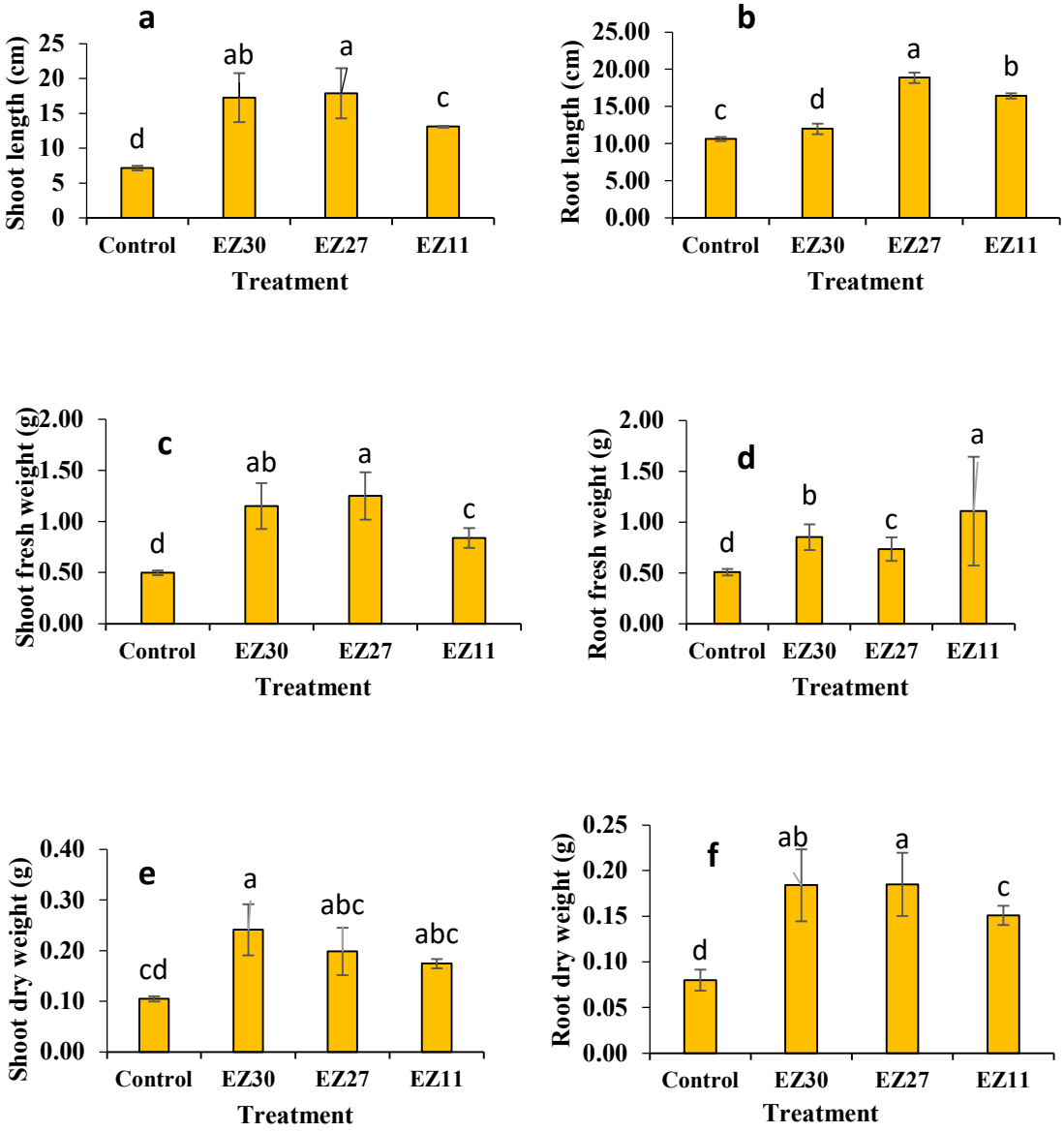

Figure 4.2: Effect of PGPR consortia on growth parameters of *Cicer arietinum* L. such as **a**. Shoot length, **b**. Root length, **c**. Shoot fresh weight, **d**. Root fresh weight, **e**. Shoot dry weight and **f**. Root dry weight.

Table 4.1: Effect of inoculation of PGPR isolates on plants growth parameters

Test Plants	Treatments	Root Length (cm)*± SE**	Shoot Length (cm)*± SE**	Root Fresh Weight(mg)* ± SE**	Shoot Fresh Weight (mg)* ± SE**	Root Dry Weight (mg)*± SE**	Shoot Dry Weight (mg)*± SE**
***Brassica juncea* L.**	P	7.03±1.41^{a}	10.13±0.35^{ab}	62.33±6.23^{a}	868.67±6.84^{a}	44.33±1.86^{a}	130.67±8.14^{ab}
	P+H (150 µg/ml each of Cd and Cu in1:1 ratio)	3.74±1.05^{b}	8.89±0.97^{abc}	37.33±5.21^{bcd}	891.67±7.84^{b}	20.33±2.40^{bc}	137.67±4.70^{ab}
	C	4.63±0.37^{c}	7.46±0.28^{bcd}	32.33±4.98^{bcd}	519.67±4.63^{c}	19.33±2.03^{cd}	75±2.65^{c}
	H (150 µg/ml each of Cd and Cu in1:1 ratio)	3.60±0.70^{d}	6.27±0.32^{cd}	26±3.21^{bcd}	417.67±1.45^{d}	13.33±2.03^{cd}	60.33±2.60^{d}
***Helianthus annuus* L.**	P	11.84±0.98^{abc}	34.87±1.62^{a}	1081.33±9.39^{a}	8426.33±5.93^{a}	460±1.73^{a}	1976±7.02^{a}
	P+H (150 µg/ml each of Cd and Cu in1:1 ratio)	10.33±1.45^{abcd}	24.33±1.45^{bcd}	759.33±7.22^{d}	5524.33±6.98^{b}	259.33±5.21^{b}	1604±3.21^{b}
	C	6.53±0.66^{abcd}	24.17±1.30^{bcd}	863.67±6.33^{b}	4529.67±2.91^{c}	218±2.52^{c}	1091.67±3.71^{c}
	H (150 µg/ml each of Cd and Cu in1:1 ratio)	10.03±1.41^{bcd}	21±2.08^{bcd}	832.±5.77^{c}	3492±2.52^{d}	171.33±2.40^{d}	596.67±3.93^{d}
***Cicer arietinum* L.**	C	10.63 ± 0.27^{d}	7.17 ± 0.34^{d}	510 ± 0.03^{d}	500 ± 0.02^{d}	80 ± 0.01^{d}	100 ± 0.01^{cd}
	EZ30	12.00 ± 0.71^{c}	17.27 ± 3.53^{ab}	850 ± 0.13^{b}	1150 ± 0.22^{ab}	180 ±0.04^{ab}	240 ± 0.05^{a}
	EZ27	18.87 ± 0.69^{a}	17.90 ± 3.59^{a}	730 ± 0.12^{c}	1250 ± 0.23^{a}	190 ± 0.03^{a}	200 ±0.05^{abc}
	EZ11	16.43 ± 0.36^{b}	13.10 ± 0.15^{c}	1110 ± 0.54^{a}	840 ± 0.10^{c}	150 ± 0.01^{c}	170 ± 0.01^{abc}

***Brassica juncea* L.**	PGPR consortia (LUMITI6, LUMBB2, LUMBB7)	14.87±2.05[a]	14.6±0.46[a]	168.33±34.11[a]	1881.00±198.44[a]	108.67±28.67[a]	189.00±21.36[a]
	C	13.17±1.48[a]	12.6±0.36[b]	157.67± 16.37[a]	978.67± 122.22[b]	92.00± 9.00[a]	109.67± 21.50[a]
***Phaseolus vulgaris* L.**	RZ3	40.67 ± 5.21[a]	65.33 ± 3.28[a]	6480 ± 1.30[a]	9700 ± 0.60[a]	780 ± 0.13[a]	1930 ± 0.59[a]
	RZ23	35.67 ± 3.84[ab]	52.33 ± 6.23[ab]	2590 ± 1.01[b]	6570 ± 0.60[ab]	490 ± 0.21[ab]	1440 ± 0.57[ab]
	RZ27	24.00 ± 5.03[abc]	39.00± 5.69[bc]	1900 ± 0.33[bc]	5450 ± 0.33[abc]	280 ± 0.17[abc]	820 ± 0.06[abc]
	RZ5	20.33 ±5.78[abcd]	31.50 ± 1.09[cd]	1140 ± 0.49[bcd]	5230 ± 1.67[abcd]	150 ± 0.01[abcd]	400 ± 0.12[abcd]
	C	12.67 ± 0.33[bcd]	14.00 ± 0.58[d]	850 ± 0.21[bcd]	1460 ± 0.08[cd]	370 ± 0.31[abcd]	360 ± 0.29[abcd]

*Control (C)= No PGPR + No heavy metal, P= PGPR (*Bacillus cereus* (TSU3), *Bacillus safensis* (TSU4), *Bacillus pumilus* (TSU8), *Klebsiella variicola* (TSU11), *Lelliottia amnigena* (TSU12), *Pseudomonas koreensis* (TSU7), *Serratia Marcescens* (TSU1)), P+H = PGPR + Heavy metal, and H= Heavy metal.

**±SE: Standard error from mean; ***Means followed by the same letter are not significantly different at $p \leq 0.05$.

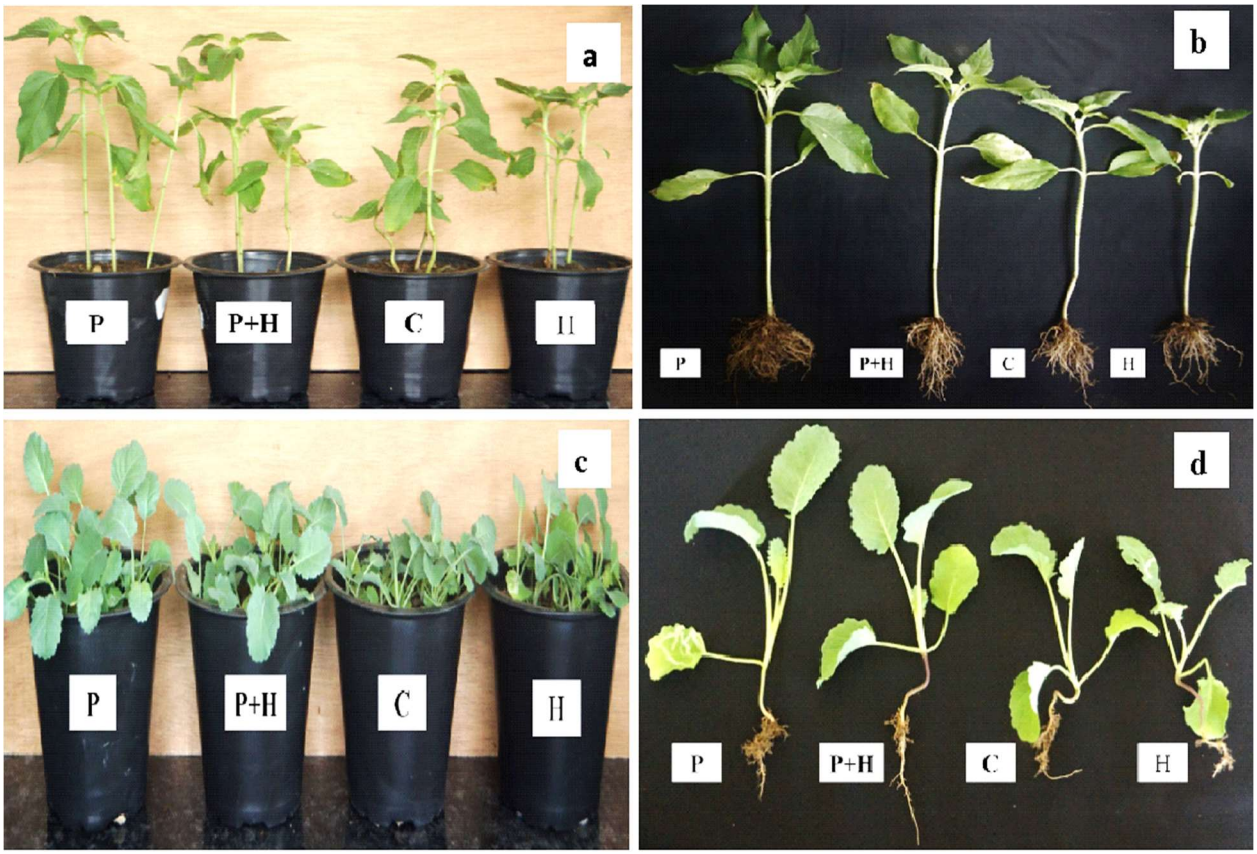

Figure 4.3: Effects of PGPR consortia inoculation on sunflower (*Helianthus annuus* L.) (a and b) and mustard (*Brassica juncea* (L.) Czern.) (c and d) growth parameters such as shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight under different treatment. Treatments: Control= No PGPR + No heavy metal, P= PGPR, P+H = PGPR + Heavy metal and H= Heavy metal.

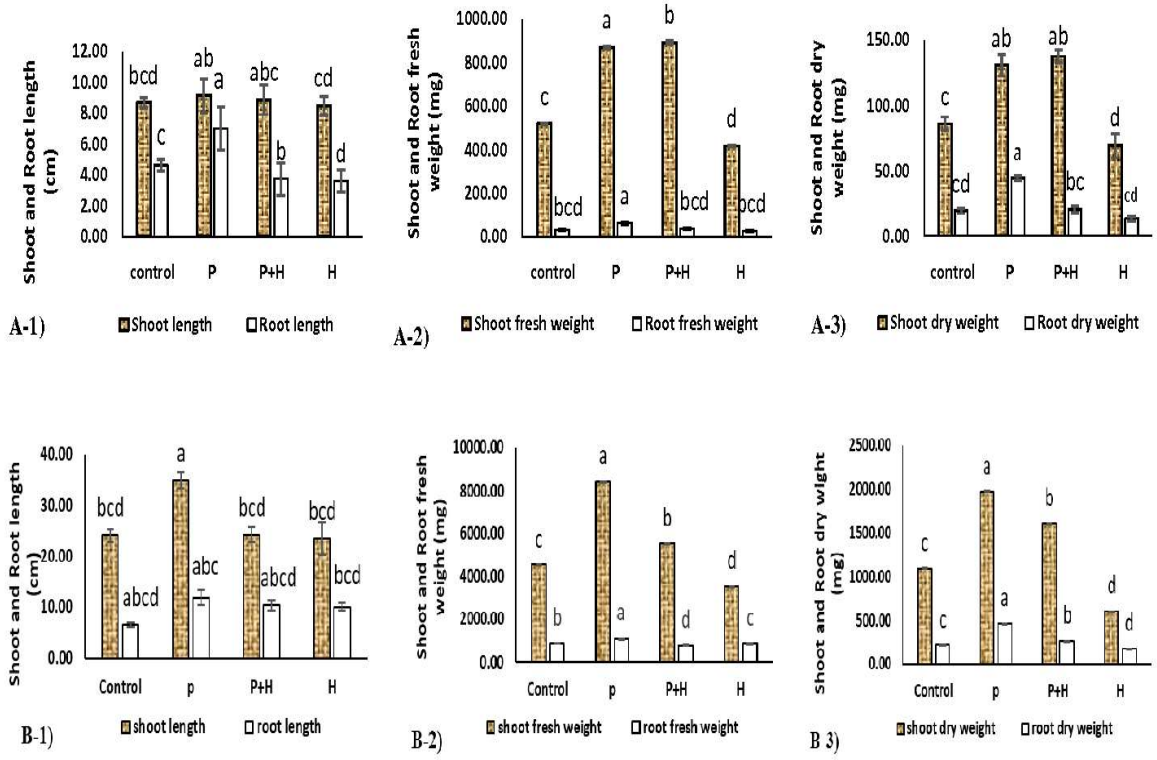

Figure 4.4: Effect of PGPR inoculation on growth parameters of *Brassica juncea* (L.) Czern (A-1 – A-3). A-1. Shoot and root length, A-2. Shoot and root fresh weight, A-3. Shoot and root dry weight; and *Helianthus annuus* L. (B-1 – B-3). B-1. Shoot and root length, B-2. Shoot and root fresh weight, B-3. Shoot and root dry weight. (Treatments: Control= No PGPR + No heavy metal, P= PGPR, P+H = PGPR + Heavy metal and H= Heavy metal).

In the third experiment, *B. juncea* was inoculated by consortium of LUMITI6 (*B. subtilis*), LUMBB2 (*C. cucumeris*), LUMBB7 (*B. cereus*). After inoculation of the *B. juncea* with PGPR consortia growth parameters were measured (**Figure 4.5. and 4.6**). It was recorded that shoot length increased from 12.6 cm in control (C) to 14.6 in PGPR treatment. Similarly, a significant increase in the shoot fresh weight was also observed ($p \leq 0.05$) from 978.67 mg in control to 1881.00mg in PGPR treatment. Root length increase from 13.17 cm in control to 14.87 cm in PGPR treatment. Root fresh weight and dry weight (157.67 and 92.00mg respectively) also increased in PGPR treatment (168.33 and 108.67 mg respectively) ($p \geq 0.05$). Shoot dry weight increased from 109.67mg to 189.00mg from control to PGPR treatment indicating significant increase only in shoot length and shoot fresh weight (**Table 4.1**).

In the fourth pot trial, Plantlets were uprooted after 30 days of plantation and growth parameters were measured. The inoculation potential of all the four selected isolates with *P. vulgaris* L. showed a significant increase in all growth parameters of under controlled experiments. An un-inoculated negative control was used (**Figure 4.7 and Figure 4.8**). All four isolates promoted the plant growth over the control treatment. Each bacterial isolate promoted one or more growth parameters of the experimental plants. The growth parameters tested were shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight. All the growth parameters were highest in the treatment RZ3 (*P.orientalis*) with Shoot length (65.33 cm) ($p \leq 0.05$), root length (40.67 cm) ($p \leq 0.05$), shoot fresh weight (9700mg) ($p \leq 0.05$), root fresh weight (6480 mg) ($p \leq 0.05$), shoot dry weight (1930mg) and root dry weight (780mg) over control treatment which had shoot length (14.00 cm), root length (12.67 cm), shoot fresh weight (1460mg), root fresh weight (850mg), shoot dry weight (360mg) and root dry weight (370mg). RZ23 (*A. larrymoorei*) showed shoot length (52.33 cm) ($p \leq 0.05$), root

length (35.67 cm), shoot fresh weight (6570mg) ($p \leq 0.05$), root fresh weight (2590mg), shoot dry weight ((1440mg) and root dry weight (490mg). RZ27 (*B. cepacian*) showed shoot length (39.00 cm) ($p \leq 0.05$), root length (24.00 cm), shoot fresh weight (5450mg) ($p \leq 0.05$), root fresh weight (1900mg), shoot dry weight (820mg) and root dry weight (280mg). RZ5 (*P. taiwanensis*) showed shoot length (31.50 cm), root length (920.33 cm), shoot fresh weight (5230mg), root fresh weight (1140mg), shoot dry weight (400mg) and root dry weight (150 mg) (**Table 4.1**).

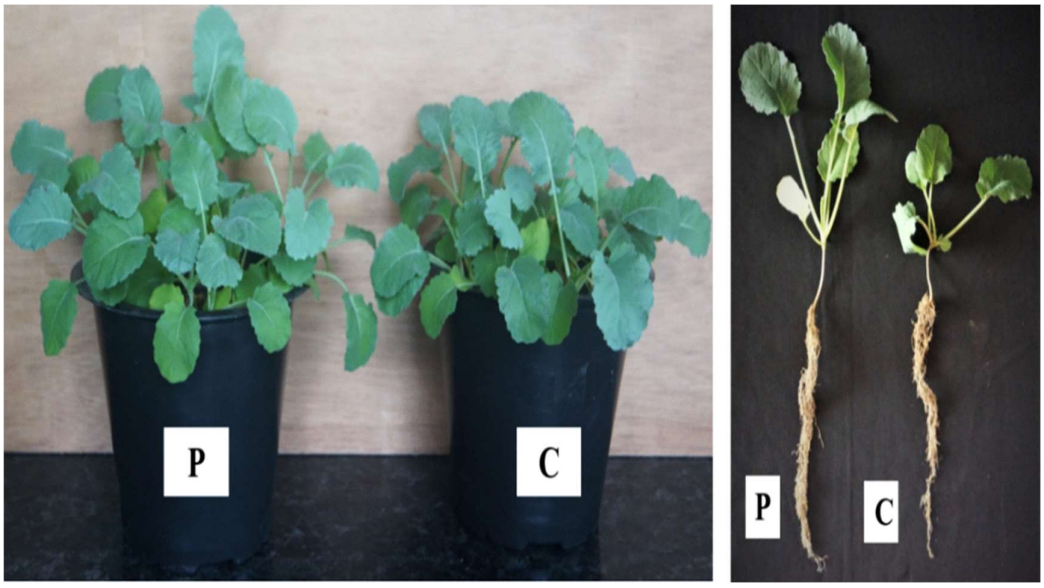

Figure 4.5: Effect of PGPR consortia ((LUMITI6 (*Bacillus subtilis*), LUMBB2 (*Chryseobacterium cucumeris*), LUMBB7 (*Bacillus cereus*)) on growth parameters of *Brassica juncea* (L.) Czern *growth* parameters such as shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight. Note: P: PGPR consortia and C: Control.

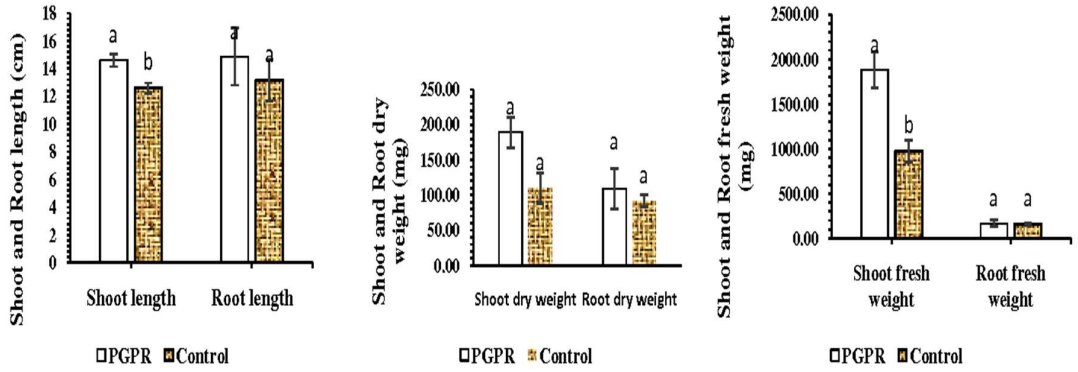

Figure 4.6: Effect of PGPR consortia on growth parameters of *Brassica juncea* (L.) Czern growth parameters such as shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight.

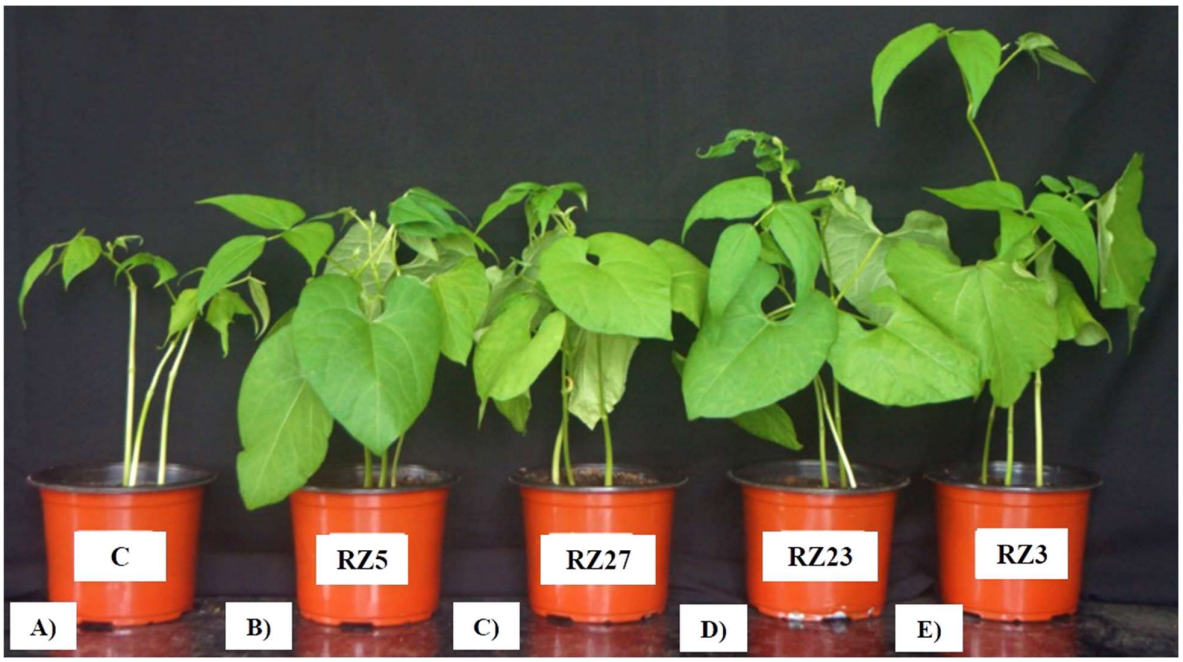

Figure 4.7: Effect of PGPR inoculation on *Phaseolus vulgaris* L. growth (root length, shoot length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight). PGPR isolates: A) C (control), B) RZ5 (*Pseudomonas taiwanensis*), C) RZ27 (*Burkholderia cepacian*), D) RZ23 (*Agrobacterium larrymoorei*) and E) RZ3 (*Pseudomonas orientalis*).

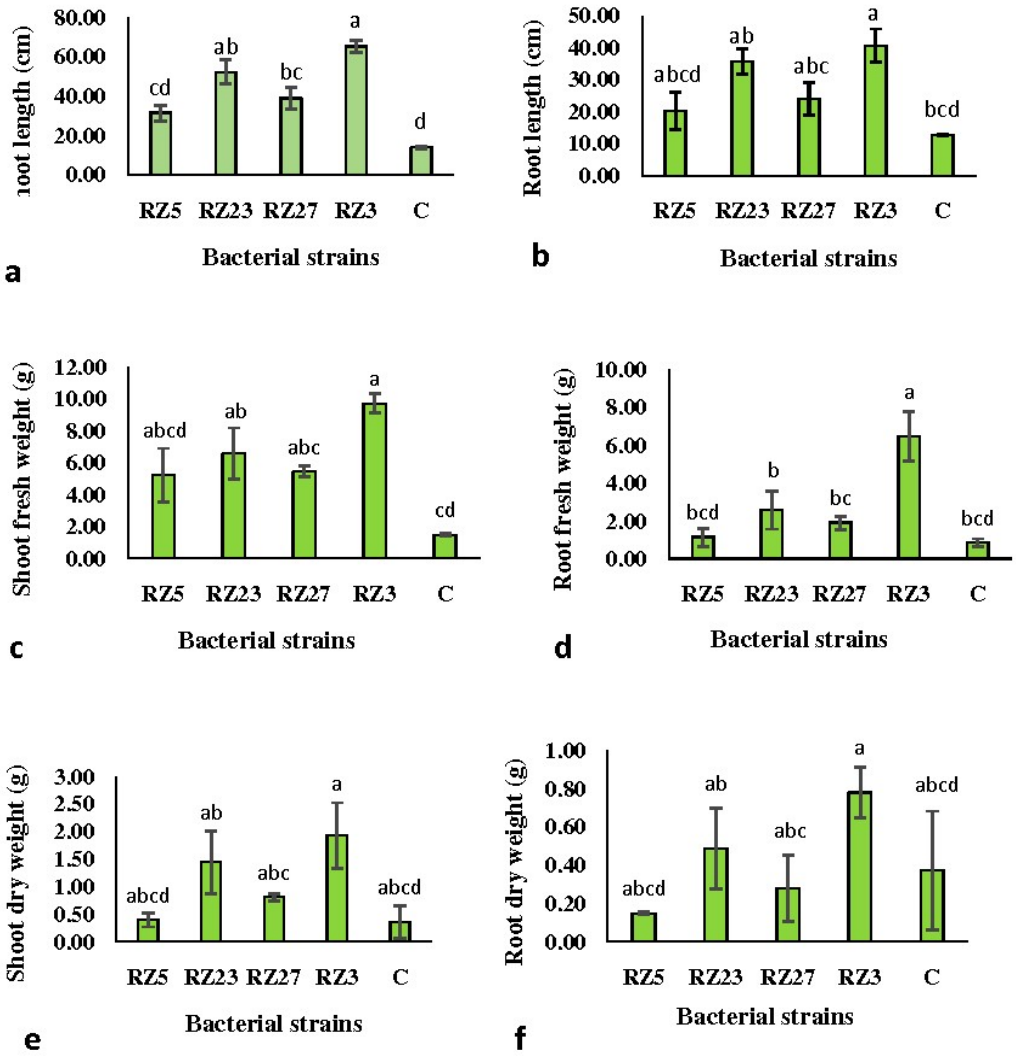

Figure 4.8: Effect of PGPR consortia on growth performance of *Phaseolus vulgaris* L. Growth parameters are: a. Shoot length, b.) Root length, c. Shoot fresh weight, d. Root fresh weight, e. Shoot dry weight and f. Root dry weight.

Discussion

The use of PGPR is increasingly adopted in sustainable agriculture to enhance crop yield, stress tolerance and disease resistance (Tatung and Deb, 2021). Rhizobacterial isolates can affect plant growth and development in neutral, detrimental, or beneficial ways (Kloepper et al., 1989). The application of synthetic fertilizers, pesticides and other agrochemicals in agricultural practices has led to insects and disease developing resistance to these chemicals (Roberts and Reigart, 2013; Desai et al., 2016) and has caused several negative environmental impacts (Chaudhary et al., 2021). Moreover, this indiscriminate use of these chemicals has polluted soil, water, and ecosystem and has had toxic effects on the users (Md Meftaul et al., 2020; Singh and Craswell, 2021). Consequently, the use of biocontrol agents, biofertilizers, biopesticides, and other products derived from microorganisms and other natural substances has become crucial in modern agriculture in recent years (Tatung and Deb, 2021). While, these bioagents might not completely replace chemical fertilizers, increasing awareness among consumer and producer about organic farming and the environment can be highly beneficial for the future (Desai et al., 2016). One direct consequence of the agro-economy's new perspective on the role of microorganisms in sustainable agriculture is the reduced reliance on chemical pesticides and fertilizers. Therefore, utilizing microbe-based formulations tailored to a specific pathogen offers an effective and environmentally friendly solution for disease management (Mawar et al., 2021). Promising PGPR isolates that performed well under laboratory conditions are expected to yield good results in more realistic settings such as pot cultures or field trials (Alali et al., 2021). Numerous studies have reported the capability of PGPR inoculants to enhance plant growth, biochemical and yield parameters and disease protection (Vejan et al., 2016).

In the present study, various experiments were conducted to assess the efficiency

of PGPR isolates, both individually and in consortium, on plant growth and development. First pot experiments were conducted to evaluate the inoculation potential of the three selected isolates (*P. monteilii*, *P. putida,* and *K. arachidis*) on *C. arietinum* L. These experiments demonstrated a significant increase in all growth parameters under greenhouse environment. An un-inoculated negative control was used. Inoculations with these three isolates significantly enhanced the growth parameter such as shoot and root length ($p \leq 0.05$), root and shoot fresh weight and dry weight, except for EZ27 and EZ11 where there was no significant difference in shoot dry weight. Overall, the three selected PGPR strains showed potential biofertilizer traits (phosphate solubilizing, IAA producing, siderophore production, salinity, and cadmium tolerant) to be used in the agricultural fields promoting sustainable practices (Tatung and Deb, 2021). Several studies have shown the use of bacterial strains as biofertilizers in various crops such as corn (Kavamura et al., 2013), wheat (Haroon et al., 2021), sunflower (Kryuchkova et al., 2014), tomato (Gowtham et al., 2020), maize (Shabaan et al., 2022), banana (Gamez et al., 2019), and groundnut (Goswami et al., 2014) to enhance growth and vigour. For instance, *A. hypogaea* treated with PGPR showed higher pod yield, haulm yield, and nodule dry weight compared to the control (Dey et al., 2004). Many studies have demonstrated that combining multiple *Pseudomonas* strains and/or other microbes is more beneficial advantages than using single-strain inoculants (Balthazar et al., 2022). In one study, *Pseudomonas* species significantly increased growth, yield, oil contents and NP uptake in *H. annuus* compared to the control (Majeed et al., 2018). Additionally, *Vigna unguiculate* seedlings grown in the presence of 50µg/ml of AuNPs and *P. monteilii* showed increased growth (Panichikkal et al., 2019). Furthermore, Singh et al., (2021) reported that the activity of nitrogen assimilation enzymes, chitinase and endo-glucanase, as well as the content of phytohormones, significantly increased following the inoculation

of *K. arachidis* in sugarcane. *Kosakonia* sp. also enhanced the growth of *A. hypogaea* L. through its N-fixing and P-solubilizing capabilities, which significantly increased the carbohydrate (129.15 ± 2.03 mg/g) and total protein (189.35±1.76 mg/g) compared to the control (Narayanan et al., 2022). In a study by AlAli et al. (2021), the mixed inoculation of grapevines with *Pseudomonas* sp., *Enterobacter* sp. and *Achromobacter xylosoxidans* was expected to mitigate growth inhibition caused by drought stress, thereby increasing the plant's tolerance to drought and saline alkaline stress. Another study suggested that *P. monteilii* could survive in soils contaminated with PNP dosage between 90 and 155 mg/kg (Zhang et al., 2019). With the use of PGPR products in agricultural practices, the number of synthetic agrochemicals can be reduced to a large extent (Tatung and Deb, 2021). In this study the inoculation of PGPR singly increased the plant growth parameters.

Heavy metals such as cadmium, lead, and mercury are elements that have biological toxicity (Tatung and Deb, 2024). These metals can enter the soil through both natural and anthropogenic sources, including mining activities. PGPR have been demonstrated to enhance plant growth in soil contaminated with heavy metals. They achieve this by neutralizing the harmful effects of heavy metal pollution, boosting plant tolerance, increasing the accumulation of the heavy metal in the plant tissues, and secreting antioxidants enzymes and siderophores (Wrobel et al., 2023). However, the effectiveness of bioremediation depends on the dynamic interplay among soil microbes, plants, and the present heavy metals (Deb et al., 2020). For example, association of *Medicago sativa* with *Pseudomonas* sp. has shown to be an efficient biological system for the bioremediation soils of contaminated with Cr (VI) (Tirry et al., 2021). Molina et al. (2020) proposed that a particular isolate of *P. putida* possesses accessory genes with degradative capabilities, which can potentially be harnessed for pollutants removal

through the process of rhizoremediation. In the second set of pot trial, selected PGPR strains were inoculated in the two plants sp. (*B. juncea* and *H. annuus*) under heavy metal contamination (Cd/Cu 150 µg/ml) and demonstrated their efficiency in enhancing plant growth. Amongst the four treatments, the one with only PGPR consortia presented the best growth results (shoot and root length, shoot and root fresh weight and shoot and root dry weight), followed by the one with PGPR + plus heavy metal contamination significantly ($p \leq 0.05$). The PGPR consortia was composed of the isolates, *S. marcescens* (TSU1), *B. cereus* (TSU3), *B. safensis* (TSU4), *B. pumilus* (TSU8), *P. koreensis* (TSU7), *K. variicola* (TSU11) and *B. safensis* (TSU12). This consortium improved growth performance significantly in both fresh and dry weight basis. In *B. juncea*, enhancement in root length and weight were recorded by 53 and 93% respectively compared to the control, while, fresh weight of shoot was improved by 72% in soil adjunct with PGPR and 67% with PGPR under heavy metal contamination condition. Similar trend was also observed on dry weight basis. Whereas, PGPR consortia treated *H. annuus* registered increased shoot and root lengths both fresh and dry weight basis. The findings that *K. variicola* aids in the plant growth and development is supported by the study conducted by Guerrieri et al. (2021), where tomato seedlings showed increased root length density and diameters class length parameters, indicating high IAA due to inoculation with *K. variicola*. Similar findings were reported by Shreya et al. (2020), where *B. cereus* and *B. subtilis,* along with few other PGPR strains, enhanced plant growth and Cr Phyto-stabilization in *C. arietinum* under Cr contamination. *B. cereus* is one of the most studied PGPR for its plant growth-promoting and stress-tolerating properties. For instance, seed germination and plant growth parameters, along with biochemical properties of *Brassica nigra,* were not affected by chromium contamination due to inoculation of *B. cereus* (Akhtar et al., 2021). Cadmium induced toxicity in seedlings, characterized by reduced

growth (root length, shoot length, fresh weight) and photosynthetic pigments (chlorophyll, carotenoid, and xanthophyll), was mitigated by the inoculation of *P. aeruginosa* and *B. gladioli* (Khanna et al., 2019).

In the third set of experiment, a consortium of four isolates *P. gessardii* (LUMITI4), *B. subtilis* (LUMITI6), *C. cucumeris* (LUMBB2), *B. cereus* (LUMBB7), were applied to *B. juncea* plants to observe its impact on growth parameters. The consortium significantly increased shoot length from 12.6 cm in control to 14.6 in PGPR treatment. A significant increase in shoot fresh weight was also observed from 978.67 mg in control to 1881.00 mg in PGPR treatment. Root length increase from 13.17cm in control to 14.87cm in PGPR treatment. Root fresh weight and dry weight (157.67 and 92.00mg respectively) also increased in PGPR treatment (168.33 and 108.67mg respectively). Shoot dry weight increased from 109.67mg to 189.00mg from control to PGPR treatment. Findings of the present study indicating the significant increase only in shoot length and shoot fresh weight, underscoring the potential of the consortium's isolates in fostering plant growth, development and biomass enhancement. Similar beneficial effects have been noted in other studies as well. For instance, the inoculation of tomato seeds with *Chryseobacterium* sp. led to taller plants (Leontidou et al., 2020). In another study, Kumar et al. (2023) demonstrated that inoculation of *C. cucumeris* enhanced seeds germination, and hairy root growth in *C. arietinum* and *V. radiata,* facilitating healthy seed radicle and plumule elongation. Furthermore, *P. gessardii* inoculation into *Helianthus annuus* L. was found to alleviate lead induced toxicity, improving growth and physiological attributes in contamination environment, as compared to plants grown in the un-inoculated lead contaminated treatments (Saleem et al., 2018). Additionally, the introduction of *B. subtilis* into tomato plant demonstrated improved growth and suppression of early blight caused by *Alternaria solani* (Rasool et

al., 2021). *Chryseobacterium cucumeris* is a highly potential PGPR with stress tolerant, cold adaptation and DNA repair genes, enabling its survival in high-altitude environment (Kumar et al., 2023). *C.cucumeris* also showed antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Listeria monocytogenes* and *Staphylococcus aureus* (Iqbal et al., 2021). PGPR *B. subtilis* have demonstrated antifungal activity against *Fusarium oxysporum* and *Macrophomina phaseolina* in chickpea plant (Patil et al*.,* 2014). Ahmad et al. (2019) reported that inoculation with PGPR *B. aryabhattai* S10 and *B. subtilis* ZM63 improved the growth of mung bean, resulting in better early development and increased fresh and dry weights of both roots and shoots compared to controls. Similar plant growth promoting effects of *Pseudomonas* BHU B13-398 and *Bacillus* BHU M strain on mung beans (Kumari et al., 2018). These findings collectively emphasize the potential of these PGPRs in augmenting plant growth, nutrient availability, and stress tolerance, offering a sustainable and effective strategy for enhancing crop production and remediating soils contaminated with heavy metals.

In the fourth pot experiment, the isolates *B. cepacian* (R27), *A. larrymoorei* (R23), *P. taiwanensis* (RZ5) and *P. orientalis* (RZ3) were selected and inoculated singly onto *Phaseolus vulgaris* L. In the current study, selected PGPR strains, demonstrated their effectiveness in enhancing the plant growth. Amongst the 5 treatments evaluated the treatment with *P. orientalis* exhibited the most significant growth improvements in terms of shoot and root length, as well as shoots and root fresh weight. This was followed by treatments with *A. larrymoorei*, *B. cepacian*, and *P. taiwanensis* all of which outperformed the control treatment. *A. larrymoorei* is a relatively new species in the genus *Agrobacterium* which was discovered in 1991 (Bouzar et al., 1995). It was found to cause tumour in the pruned branches of *Ficus benjamina* (Bouzar and Jones, 2001) and differs from other *Agrobacteria* both genetically and biochemically (Molinaro et al.,

2003). Later, an alternative name, *Rhizobium larrymoorei,* was proposed for it based on Rule 34a of the International Code of Nomenclature of Bacteria (Prokaryotes) (Young, 2004). In our study, it was found that *A. larrymoorei* possessed plant growth promoting traits such as phosphate solubilization, siderophore and IAA production indicating its potential to promote plant growth. In a study by Wang et al. (2020), a consortium of *Rhizobium larrymoorei* along with other PGPR strains (*Agrobacterium tumefaciens*, *Bacillus* sp., *K. pneumoniae*, *Pseudomonas* sp. and *K.variicola*) increased the alkali-hydrolyzed nitrogen, available phosphorus and available potassium in the soil by 49.46, 99.51 and 19.38%, respectively, and enhanced the N, P, and K content of wheat. *Burkholderia cepacian* has also been shown to support significantly maize plant growth upon inoculation under greenhouse condition by solubilizing inorganic tricalcium phosphate (You et al., 2020). Another study demonstrated that *B. cepacian,* promoted the rice growth (two cultivars), increasing grain yield and plant biomass under limiting nitrogen condition (Li et al., 2022). Inoculation of *P. orientalis* and *Chaetomium cupreum* on *Eucalyptus globulus* plants significantly increased plant growth and mitigated the toxic effects of copper (Ortiz et al., 2019). Another study showed that the inoculation with *Pantoea agglomerans*, *Rahnella aquatilis* and *P. orientalis*, enhanced grain yield and K uptake compared to the control treatment (without K fertilizer). Values were higher when KSB inoculums were used with half the dose of K chemical fertilizer (47.5 Kg/ha). Bacterial inoculums also increased K use efficiency in plants (Yaghoubi et al., 2018). Mishra et al. (2023) reported enhanced antioxidant and nutritional properties of wheatgrass (*T. aestivum* L.) when treated with *P. taiwanensis* along with a reduced dose of mineral fertilizers (N, P, and K) in saline soil. In another study, inoculating wheat with *P. taiwanensis* reduced Cd concentration in soil, increased *pH*, and enhanced adsorption to root surfaces, which improved wheat dry weights by 11-36%. Inoculation decreased

Cd content in roots and above ground tissues by 78-94%, lowered Cd bio-concentration and translocation factors significantly, and increased root surface-adsorbed Cd by 99-121% (Cheng et al., 2021). *P. taiwanensis* is a broad-host-range entomopathogenic bacterium that exhibits insecticidal activity against agricultural pests *Plutella xylostella*, *Spodoptera exigua*, *Spodoptera litura*, *Trichoplu siani* and *Drosophila melanogaster.* The TccC protein, a component of the toxin complex (Tc), plays a crucial role in this insecticidal activity (Chen et al., 2014). Inoculating *Anthurium andreanum* with *Pseudomonas taiwanensis* reduced diseases resistance to *Xanthomonas axonopodis* pv. *dieffenbachiae* (Xad) but increased the production of defence-related enzymes, proteins, and phenols, along with greater distribution of secondary metabolites and phenolic compounds. Additionally, root colonization assay showed a significant increase in bacterial presence in root tissue (Dhanya et al., 2020). These findings collectively highlight the potential of these PGPR strains isolates from Musa rhizosphere as promising bioinoculants. Amongst the 5 treatments, the one with *P. orientalis* presented the best growth (shoot and root length, shoot and root fresh weight and shoot and root dry weight) followed by the one with *A. larrymoorei*, *B. cepacian* and *P. taiwanensis* over control treatment.

Conclusions

Considering the whole study, it can be concluded that, selected isolates *Pseudomonas putida* (EZ11), *Kosakonia arachidis* (EZ27), *Pseudomonas monteilii* (EZ30), *Serratia marcescens* (TSU1), *Bacillus cereus* (TSU3), *Klebsiella variicola* (TSU11), *Lelliottia amnigena* (TSU12), *Pseudomonas koreeensis* (TSU7), *Bacillus pumilus*(TSU8) and *Bacillus safensis* (TSU4), *Bacillus subtilis* (LUMITT6), *Chryseobacterium cucumeris* (LUMBB2)*, Bacillus cereus* (LUMBB7) and *Pseudomonas gessardii* (LUMITI4*), Cedecea neteri* (NC7), *Pseudomonas orientalis* (RZ3),

Agrobacterium larrymoorei (RZ23), *Burkholderia cepacian* (RZ27), *Pseudomonas taiwanensis* (RZ5), demonstrate significant potential as PGPR. Their diverse growths promoting characteristics contribute positively to plant growth and productivity, particularly when used in combination. When applied together, these PGPR strains show enhanced efficacy as evident in the improved growth of *B. juncea* and *H. annuus* in heavy metal (150 mg/ml Cd/Cu) contaminated soil underscoring their potential for phytoremediation. In conclusion, these isolates hold promise as inoculants for ameliorating metal toxicity in plants grown in contaminated soil, with further exploration needed to understand soil physio-chemical properties and plant-bacteria interaction. Moreover, expanding our knowledge of microbial genetics involved in heavy metal resistance will facilitate the development of multifunctional PGPR - based formulations with broader applications in sustainable agriculture and environmental remediation.

Chapter – 5

Isolation and Microscopic Characterization of Fungal Isolates from *Musa* Rhizospheric Soil

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Introduction

The Rhizosphere, a biologically active zone, is critical for plant-microbe interactions, influencing nutrient cycling, plant growth and plants diseases resistance (Hossain et al., 2017). Among the rhizosphere's microbiota, fungi hold prominent yet less explored position compared to bacteria (Pattnaik and Busi, 2019). The rhizosphere hosts a taxonomically structured fungal community that plays a vital role in nutrient absorption and provides resistance against pathogens and other abiotic stresses related with their host plants (Chang et al., 2021). Factors such as plant species identity, phylogenetic relatedness and plant traits all affect the rhizosphere fungal community composition (Sweeney et al., 2020). Plants roots produce a variety of organic compounds, including sugars, organic acids and vitamins and These compounds are then used by fungi as nutrients or signals (Mehmood et al., 2019). Determinants of soil fungal community

assembly vary across fungal associations, reflecting their different ecological functions in temperate forest ecosystems (Liang et al., 2023). The fungal community structure in the rhizospheric soil differs from that of the bulk soil of the same plant, also the fungal community dominance in healthy plant rhizospheric soil varies from that of an infected plant rhizospheric soil (Jamil et al., 2023). For example, the relative abundances of *Aspergillus* and *Penicillium* were 3.30 and 3.01%, respectively, in the disease-free soil samples, and 0.34 and 0.52%, respectively; in the *Fusarium* wilt-diseased soil samples (Zhou et al., 2019).

According to Hannula et al. (2017), fungi are generally more abundant and active in natural ecosystems compared to intensively managed systems, which are largely dominated by bacteria. Their study observed that in recently abandoned fields, most of the root-derived ^{13}C was absorbed by bacteria, whereas in long-term abandoned fields, the majority of the root-derived ^{13}C was found in fungal biomass. Additionally, the composition of the active functional fungal community shifted fromfast-growing, pathogenic species to beneficial, slower-growing fungal species.

Plant growth promoting fungi (PGPF) are soil dwelling, non-pathogenic, saprophytic, filamentous soil borne fungi and they provides potential benefits to many plants by serving in disease protection and promoting plant growth (Liu and Zang, 2015; Begum et al., 2019). PGPF suppress plant pathogen in the rhizosphere through the production of hydrolytic enzymes and plant hormone and mineral solubilization (Lewis and Papavizas, 1991). In Many studies plant growth promoting ability of PGPF have been reported. For instances, EI-Maraghy et al. (2020b), mentioned that non-pathogenic *Fusarium* species possess effective plant growth promoting fungi. In another study, it is suggested that *Aspergillus niger* MPF-8 when subjected to field application acted as biofertilizer, thus helping the plant in uptake of nutrient (Bhattacharya et al., 2015). This

indicates the potential of PGPF helps in reducing the use of chemical fertilizer (Tarroum et al., 2022).

There is an evidence of occurrence of rhizospheric phosphate solubilizing microorganism which play and significant role in soil phosphorus dynamic and availability of phosphorus to plant (Richardson and Simpson, 2011). Phosphate solubilizing fungi can be used as biological fertilizer, but screening process is necessary to obtain effective isolate (Bashan et al., 2014). Genus *Penicillium*, *Aspergillus*, *Fusarium* and *Sclerotium* are some of the phosphate solubilizing fungi (PSF). Under the low iron condition, certain microbes and plants produce siderophores, which are low molecular weight high-affinity chelating agents which solubilize ferric ion and transport it to the cell (Hu et al., 2011; Bakthavatchalu et al., 2012). Siderophore can chelate ferric ion with affinity, allowing its solubilization and extraction from most mineral or organic complexes (Wandersman and Delepalaire, 2004). The plant hormone indole-3-acetic acid (IAA) plays a role in the communication between host plant and microbes, including plant-associated microorganisms and endophytes but also plant pathogens. IAA together with other phytohormones is responsible for plant growth and development (Jahn et al., 2021). IAA produced by fungi can induce lateral root formation and root hair development, thus enhancing nutrient absorption capacity of the plants. IAA in different plant-fungal interaction can also lead to changes in the basal defense mechanisms of the plant; can defeat pathogenic strains and disease progression by enhancing the plant's immune response (Mehmood et al., 2019). Several PGPF also have some heavy metal (HM) tolerance mechanisms, such as metal sequestration or accumulation, precipitation, intracellular compartmentalization of metals into fungal cell walls, mineral weathering, bio-absorption, and volatilization (Nandyet al., 2020). Talukdar et al. (2020) noted more than 70% removal percentage for Cr (VI) with the fungi *Aspergillus flavus* (FS4) and

Aspergillus fumigatus (FS6) and 74% removal of Cd (II) by the fungal isolate *Aspergillus fumigatus* (FS9).

Considering the above-mentioned facts, in this chapter, study was undertaken to isolate different culturable fungal strains from *Musa* (*M. itinerans*, *M. balbisiana*, *M. flaviflora*, and *M. velutina*) rhizospheric soil sample collected from three different districts of Nagaland and studiedtheir microscopic characterization with the following objectives:

Objectives

- Collection of *Musa* rhizospheric soil sample from wild *Musa* (*M. itinerans*, *M. balbisiana*, *M. flaviflora* and *M. velutina*) plants growing in the forests of Nagaland and their physicochemical analysis.
- Isolation of the fungal isolates using serial dilution and spread plate technique and morphological characterization of the fungal isolates.
- Lactophenol cotton blue staining of the purified fungal isolates.

Material and Methods

Rhizospheric soil sample collection and isolation of fungal isolates from the *Musa* rhizospheric soil sample

For the isolation of rhizospheric fungi soil sample were collected from the *Musa* rhizosphere growing in three different districts of Nagaland. The soil was dugout out from 5-30 cm depth with intact roots. After the roots were taken it was shaken a little bit to get rid of bulk soil attached and the soil adhering to the roots was then collected in a sterile polythene bag. The sample collected was used immediately or kept in a refrigerator at 4°C for further study.

Serial dilution of soil samples

The isolation of fungal strains was conducted by following a standard microbiological procedure using the serial dilution technique (Koch, 1883). For serial dilution technique, one gram of soil was taken which was mixed with 9ml sterilized distilled water and then 1ml of suspension from that test tube was taken and suspended in a next test tube labelled 10^{-1}; again 1 ml of solution was taken from test tube labelled as 10^{-1} and transferred to the second test tube labelled 10^{-2}, it was then mixed well with the help of pipette. Next 1ml of suspension was transferred to10^{-3} and then 10-^{4} to 10^{-5}. This was continued for up to 10^{-6}.

After dilutions were prepared, 100µl of aliquot from each sample was transferred onto Rose Bengal Chloramphenicol Agar (RBCA) (Composition gL^{-1}: mycological peptone, 5g; glucose, 10g; potassium dihydrogen phosphate, 1g; magnesium sulphate, 0.5g; rose Bengal, 0.05g; chloramphenicol, 0.1g; agar, 15.0g) and Potato Dextrose Agar (PDA)(Composition gL^{-1}: 20 g dextrose, 4g potato extract, 15g agar) media and using asterile glass L rod, the inoculums was spread on to the plate cautiously. Plates were sealed with Parafilm and incubated at 28±2ºC for 1 week. Pouring of sterilized media into Petri plates, solidifying and then inoculation of diluted soil suspension onto the plates was done in aseptic condition inside the laminar air flow chamber.

Isolation of the fungal isolates

Once the mixed culture plate was obtained from different dilutions, culture plate with clear fungal colonies with non-overlapping culture was selected for further purification. Based on the colony morphology fungal isolates were selected and purified in potato dextrose agar plates (PDA) as well as on Rose Bengal Chloramphenicol Agar medium. Purified isolates were then kept in the freezer at -4ºC for further studies. PDA slants and 80% glycerol of purified fungal isolates were then prepared and preserved for future use.

Morphological characterization of fungal isolates

Morphology of each colony was examined on Potato Dextrose Agar and Rose Bengal agar media plates after 96 h of incubation and different colony characteristics such as spore colour, colony colour, form and surface were critically examined and recorded.

Cotton blue staining

For microscopic identification of the fungal isolates, conidia, conidiophores, arrangement of spore and vegetative feature were examined with microscope. The fungal cell wall is composed of chitin of which the components of lactophenol cotton blue solution stains for identification. Lactophenol cotton blue was used as a mounting solution as well as staining agent. The glass slide was cleaned with the with 70% ethanol and with the help of a dropper few drops dye was placed on the glass slide. Usingscissor, a piece of transparent tape was cut and gently placed onto the fungal colony to get small number of spores and fungal hyphae on the tape. The tape with attached hyphae and spore was then placed on to the lactophenol cotton blue drop on glass slide. The stain was then covered carefully with a cover slip without making air bubble to the stain. Using tissue excess stain was absorbed and stain was examined microscopically at 40X, to observe for fungal spores and hyphal structures**.**

Statistical Analysis

The SPSS software was used for statistical analysis of the experimental data. All the reported results are the mean of the three replicates and deviations were calculated as the standard error of the mean (SEM).

Results

Rhizospheric soil sample collection and Isolation of fungal isolates

After collecting *Musa* rhizospheric soil samples from three different districts of Nagaland serial dilution and spread plate technique was used to get the culturable fungal isolates (**Figure 5.1**). Mixed culture plates were obtained incubating for 1 week on PDA

and RBA medium (**Figure 5.2**) A total of 43 fungal pure isolates were obtained from the mixed culture. Once the cultures were purified, isolates were labelled and preserved in 80% glycerol stock at -80ºC.Soil sample were analysed for physicochemical properties such as *pH*, temperature, moisture content, available nitrogen, available phosphorus, available potassium, organic carbon and soil electrical conductivity (**Table 5.1)**

Colony morphology of fungal isolates

Morphological characteristics of fungal isolatecolonies showed a great diversity (**Figure 5.3A and 5.3B**) have been documented in **Table 5.2.** Colony colours of isolates were alsovaried with twenty isolates having white colour colony (VEL6, T8, AG9, AG3, MI1A, FD2B, FD4VI, TSU3A, MI2B, FD2X, FD3II, VEL1, T1, FD3V, T3, T4, NC3, AG2, AG5, FD2A, RZ7, MI2C and FD2D), one with purplish pink (AG4), one olive green (FD5XI), four brown isolates (MI1D, TSU3C, TSU2A and MI1E), three black colony (MI1F, TSU3B, and MI3F), eightgreen cultures (FD1B, FD4D, FD5VII, AG6, FD3III, FD4A, AG1 and AG8) , one light purple (FLALUM4), one blue colony (FD4XIII), oneoff-white (MI1B) (**Figure 5.4, Table 5.2**).

Three isolates had white colour spore colour (AG2, FD3II and VEL6), Nineteen had green colour spores (T8, AG9, AG3, MI1A, FD2B, FD1B, FD4D, FD4VI, MI2B, FD2X, FD3III, VEL1, FD3V, AG8, T4, NC3, AG5, MI2C and FD2D), seven had brown spore colour (AG4, MI1D, TSU3A, TSU3C, TSU2A, RZ7 and MI1E) , five had olive green colour spores (FD5XI, FD5VII, AG6, FLALUM4 and FD2A), three isolates had black spores (MI1F, TSU3B and MI3F), three isolates had yellowish spores (T1, T3 and AG1), one had orange colour spores (FD4A), one isolate with light blue spores (FD4XIII), and one with off white (MI1B) (**Figure 5.5, Table 5.2**).

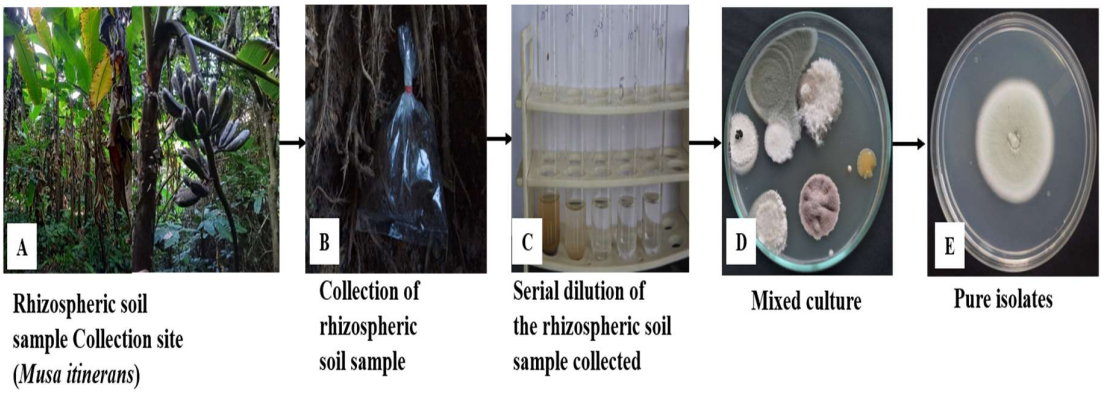

Figure 5.1: Isolation of fungal isolates from *Musa* rhizospheric soil sample using serial dilution and spread plate method. A. Rhizospheric soil sample collection site (*Musa itinerans*), B. Collection of rhizospheric soil in sterilized polythene bag, C. Serial dilution of the soil sample, D. Mixed culture plate obtained from the serial dilution technique and spread plate method and E. Pure fungal isolates cultured from the mixed culture plates.

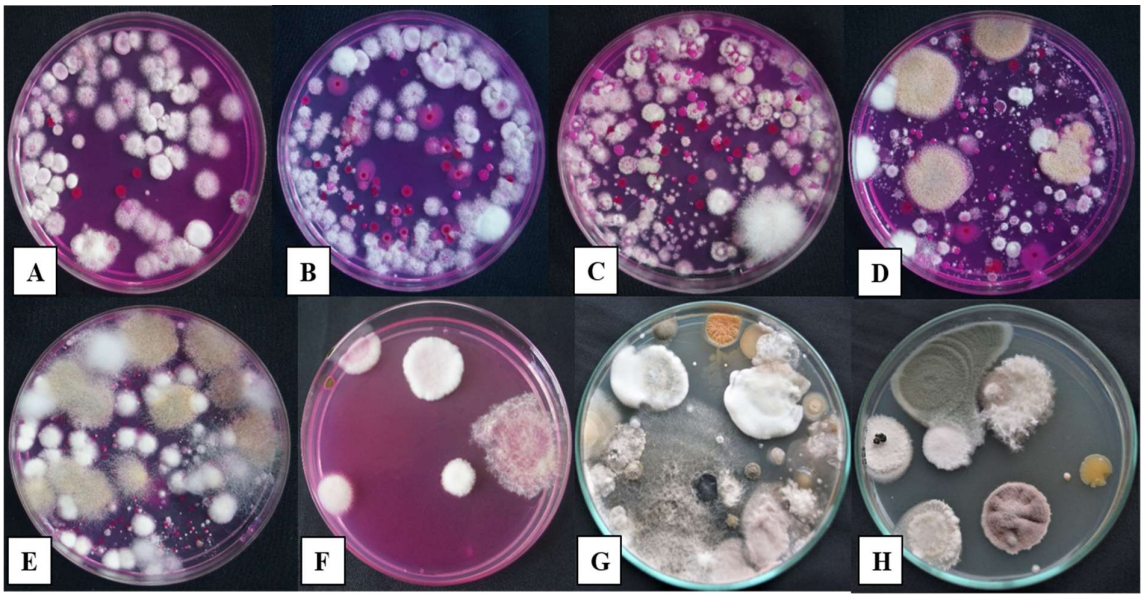

Figure 5.2: Mixed colony plates of fungal strains on Potato dextrose agar (PDA) and Rose Bengal Agar medium (RBA) cultured from rhizospheric soil sample collected from varied species of wild *Musa* (*M. itinerans, M. balbisiana, M. flaviflora,* and *M. velutina*) growing in different sites.

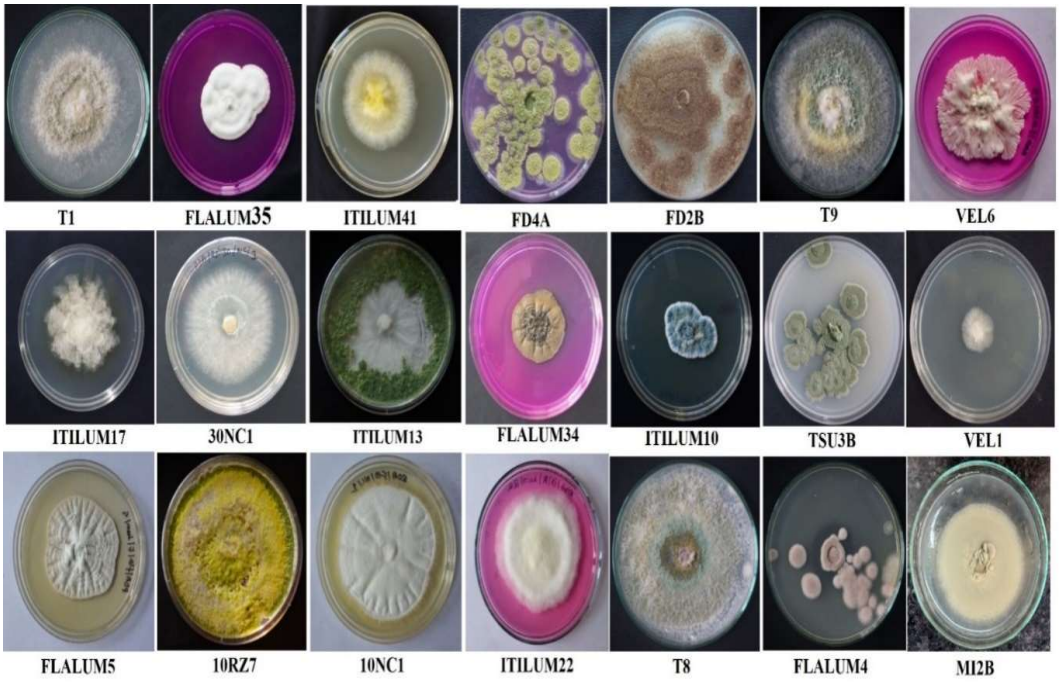

Figure 5.3A: Pure fungal strains isolated from *Musa* rhizospheric soil sample on PDA and RBA medium.

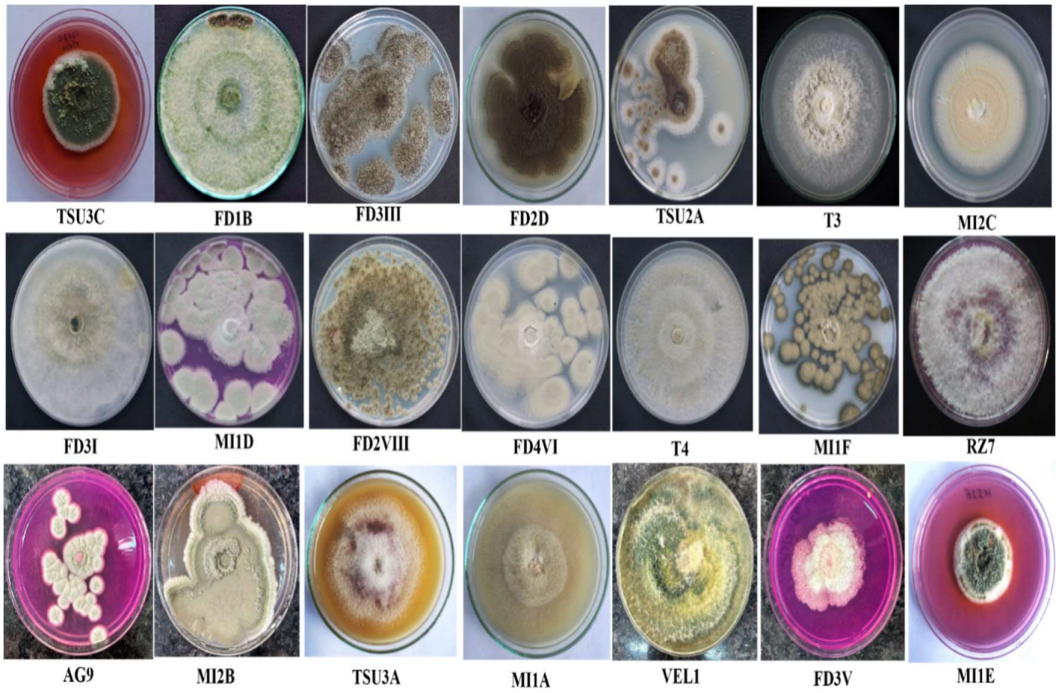

Figure 5.3B: Pure fungal strains isolated from *Musa* rhizospheric soil sample on PDA and RBA medium.

Table 5.1: Physicochemical analysis of the rhizospheric soil samples collected

District	Collection Site	Coordinates	pH ±SE*	Temp. (ºC)±SE*	Available Nitrogen (Kg/ha) ±SE*	Available Phosphorus (Kg/ha) ±SE*	Available Potassium (Kg/ha) ±SE*	Organic Carbon (%) ±SE*	Moisture Content (%)±SE*	Soil EC (dS/m) ±SE*
Wokha	**Site 1**	26.281483N, 94.374400E	5.70±0.03	27.98±1.34	170.03±1.23	3.45±1.23	76.03±1.11	0.24±0.09	39.02±1.09	0.087±0.01
	Site 2	26.287055N, 94.364751E	6.01±0.07	28.76±2.76	176.05±2.23	3.23±0.22	77.01±3.67	0.35±0.45	39.49±0.99	0.078±0.01
	Site 3	26.239112N, 94.315846E	5.55±0.02	30.58±1.78	169.03±1.9	3.36±0.94	69.01±2.89	0.32±0.22	40.01±1.04	0.076±0.02
	Site 4	26.215632N, 94.305030E	5.02±0.2	29.76±0.98	169.09±1.98	3.45±1.35	67.05±1.45	0.25±0.01	38.09±0.01	0.079±0.01
Mokokchung	**Site 1**	26.4828164N, 94.3913688E	4.50±0.09	27.65±1.45	198.67±1.89	9.67±2.00	490.12±4.76	0.65±0.01	35.78±1.34	0.032±0.01
	Site 2	26.4658641N, 94.3801225E	4.79±0.23	27.65±0.99	197.59±0.54	9.89±4.09	510.05±6.1	0.63±0.67	34.65±1.45	0.034±0.01
	Site 3	26.4861618N, 94.3541044E	4.99±0.12	28.98±2.52	212.78±0.98	10.99±1.34	523.02±3.78	0.63±0.22	40.45±2.87	0.032±0.02
	Site 4	26.43483N, 94.43353E	4.57±0.08	26.08±0.34	200.54±1.45	10.87±1.99	520.01±2.99	0.65±0.12	40.24±2.21	0.034±0.03
	Site 5	26.43383N, 94.43317E	4.89±0.34	26.18 ±0.98	200.64±1.76	10.97±0.67	527.05±3.54	0.67±0.23	40.27±1.1	0.035±0.02
	Site 6	26.48441N, 94.34359E	5.04±0.12	29.89±0.99	163.02±0.77	19.64±0.99	611.595±2.00	0.43±1.23	37.56± 0.99	0.033±0.01

	Site 7	26.4861618N, 94.3541044E	4.79±0.18	28.56±0.56	163.02±0.56	28.31±3.89	273.984±1.09	0.23±0.89	34.67±2.21	0.033±0.01
	Site 8	26.4658641N, 94.3801225E	4.38±0.42	28.78±1.56	163.02±1.89	3.31±3.33	78.01±0.99	0.23±0.99	33.78±2.34	0.025±0.02
	Site 9	26.48447N, 94.34347E	4.40±0.23	27.78±1.87	164.01±3.98	3.29±0.78	77.03±0.67	0.24±1.32	34.98±1.76	0.026±0.01
Zunheboto	**Site 1**	26.20469N, 94.48372E	4.78±0.09	27.19±1.9	212.78±3.56	36.56±0.88	657.02±3.88	0.67±1.56	40.23±3.89	0.087±0.02
	Site 2	26.20717 N, 94.48594 E	4.97±0.07	27.30±2.5	213.18±0.45	35.71±1.67	645.01±2.78	0.64±0.07	40.21±2.66	0.089±0.03
	Site 3	26.23487N, 94.28033E	4.94±0.45	26.20±3.54	413.82± 5.09	9.63±3.21	361.984±2.43	0.71±0.02	40.26±3.01	0.682±0.01

*±SE: Standard error from mean.

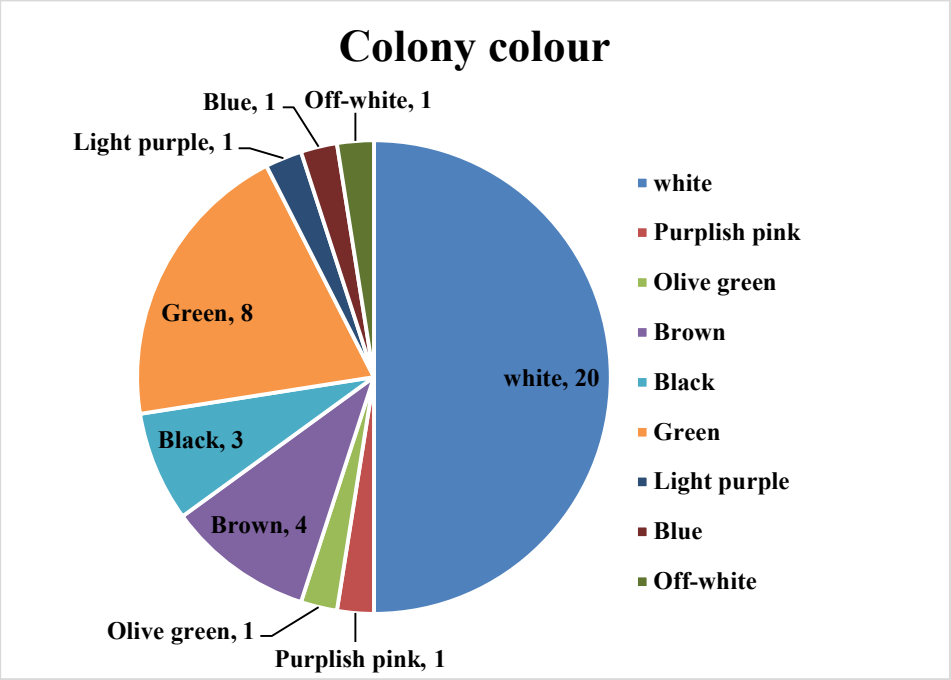

Figure 5.4: Pie chart depicting types of colony colour of the isolated fungal strains

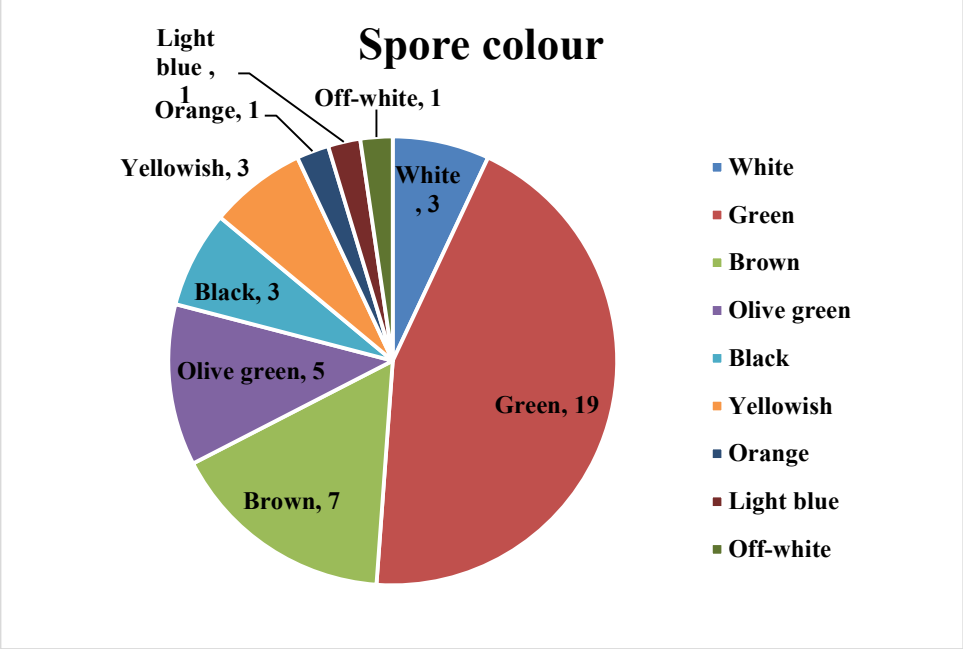

Figure 5.5: Pie chart depicting types of spore colour of the isolated fungal strains.

Twenty-eight isolates had round shape where (VEL6, T8, FD5XI, MI1D, AG3, MI1F, MI1A, TSU3B, FD2B, FD4D, FD4VI, FD5VII, TSU3A, MI2B, FLALUM4, FD3II, FD3III, VEL1, T1, T3, T4, NC3, MI3F, FD4XIII, RZ7, MI2C, FD2D and MI1B) and twelve were irregular in shape (AG9, FD1B, FD2X, TSU3C, TSU2A, FD3V, FD4A, AG1, AG8, AG2, AG5 and FD2A) and three had wavy shape (MI1E, AG6 and AG4) (**Figure 5.6, Table 5.2**).

Colony surface of 13 isolates (VEL6, MI1A, TSU3A, VEL1, T1, FD3V, T3, AG1, AG2, AG5, FD2A, FD2D and MI1B) among 43, were fluffy whereas powdery surface was found in case of twenty-five isolates (MI1E, MI2C, RZ7, MI3F, NC3, T4, AG8, FD4A, TSU2A, TSU3C, FD3III, FD3II, FLALUM4, FD2X, AG6, FD5VII, FD4VI, FD2B, TSU3B, MI1F, AG3, MI1D, FD5XI, AG9 and T8) andone isolate had rope like surface (AG4), and four had matte surface (FD2B, FD4D, MI2B and FD4XIII) (**Figure 5.7, Table 5.2**).

Cotton blue staining

With the help of cotton blue stainingmicroscopic study was done and isolates belonging to different genera was observed. Eleven isolates were found to belong to *Trichoderma* genera (T8, MI1D, AG6, FD4A, AG1, AG8, T4, NC3, AG2, AG5 and FD2D). While, three isolates belonged to *Aspergillus* genera (TSU3B, TSU3A and TSU3C), five belonged to *Penicillium* genera (FD4VI, FLALUM4, TSU2A, MI2C and MI1B), one belonged to *Fusarium* genera (VEL6), two belonged to *Mucor* genera (MI1A and MI2B), one *Gilmaniella* GENERA (FD2X), one *Acremonium* sp. (FD3II), one *Ustilago* sp. (FD3III), one *Clonostachysrosea* (T1 and FD3V), one *Chrysosporium* sp. (T3), one *Trichophyton* sp. (MI3F) and one *Talaromyces purpureogenus* (AG9) (**Figure 5.8A, 5.8B, Table 5.2**). Rest of the isolates were unidentified which includes isolates AG4, FD5XI, AG3, MI1F, FD2B, FD1B, FD4D, FD5VII, VEL1, FD2A, FD4XIII, RZ7 and MI1E.

Table 5.2: Morphological characteristics of the fungal isolates

Sl. No.	Fungal Isolates	Spore Colour	Colony Colour	Form	Surface	Identification with Cotton Blue Staining
1	VEL6	White	White	Round	Fluffy	*Fusarium sp.*
2	T8	Green	White	Round	Powdery	*Trichoderma* sp.
3	AG4	Brown	Purplish pink	Wavy	Rope like	Unidentified
4	AG9	Green	White	Irregular	powdery	*Talaromyces purpureogenus*
5	FD5XI	Olive green	Olive green	Round	Powdery	Unidentified
6	MI1D	brown	brown	Round	Powdery	*Trichoderma* sp.
7	AG3	Green	White	Round	Powdery	Unidentified
8	MI1F	Black	Black	Round	Powdery	Unidentified
9	MI1A	Green	White	Round	Fluffy	*Mucor fragilis*
10	TSU3B	Black	Black	Round	Powdery	*Aspergillus niger*
11	FD2B	Green	White	Round	Powdery	Unidentified
12	FD1B	Green	Green	Irregular	Matte	Unidentified
13	FD4D	Green	Green	Round	Matte	Unidentified
14	FD4VI	Green	White	Round	Powdery	*Penicillium* sp.
15	FD5VII	Olive Green	Green	Round	powdery	Unidentified
16	AG6	Olive Green	Green	Wavy	Powdery	*Trichoderma* sp.
17	TSU3A	Brown	White	Round	Fluffy	*Aspergillus* sp.
18	MI2B	Green	White	Round	Matte	*Mucor* sp.
19	FD2X	Green	White	Irregular	Powdery	*Gilmaniella subornata*
20	FLALUM 4	Olive green	Light purple	Round	Powdery	*Penicillium* sp.
21	FD3II	White	White	Round	Powdery	*Acremonium* sp.
22	FD3III	Green	Green	Round	powdery	*Ustilago*sp.
23	TSU3C	Brown	Brown	Irregular	Powdery	*Aspergillus* sp
24	TSU2A	Brown	Brown	Irregular	Powdery	*Penicillium*sp.
25	VEL1	Green	White	Round	Fluffy	Unidentified
26	T1	Yellowish	White	Round	Fluffy	*Clonostachys rosea*
27	FD3V	Green	White	Irregular	Fluffy	*Clonostachys rosea*
28	T3	Yellowish	White	Round	Fluffy	*Chrysosporium* sp.
29	FD4A	Orange	Green	Irregular	Powdery	*Trichoderma* sp.
30	AG1	Yellowish	Green	Irregular	Fluffy	*Trichoderma atro viride*
31	AG8	Green	green	Irregular	Powdery	*Trichoderma* sp.
32	T4	Green	White	Round	Powdery	*Trichoderma* sp.
33	NC3	Green	White	Round	Powdery	*Trichoderma* sp.
34	MI3F	Black	black	Round	Powdery	*Trichophyton* sp.
35	AG2	White	White	Irregular	Fluffy	*Trichoderma viride*
36	AG5	Green	White	Irregular	Fluffy	*Trichoderma* sp.
37	FD2A	Olive green	White	Irregular	Fluffy	Unidentified
38	FD4XIII	Light blue	Blue	Round	Matte	Unidentified
39	RZ7	Brown	White	Round	Powdery	Unidentified
40	MI2C	Green	White	Round	Powdery	*Penicillium*sp.
41	FD2D	Green	White	Round	Fluffy	*Trichoderma virens*
42	MI1E	Brown	Brown	Wavy	Powdery	Unidentified
43	MI1B	Off white	Off white	Round	Fluffy	*Penicillium* sp.

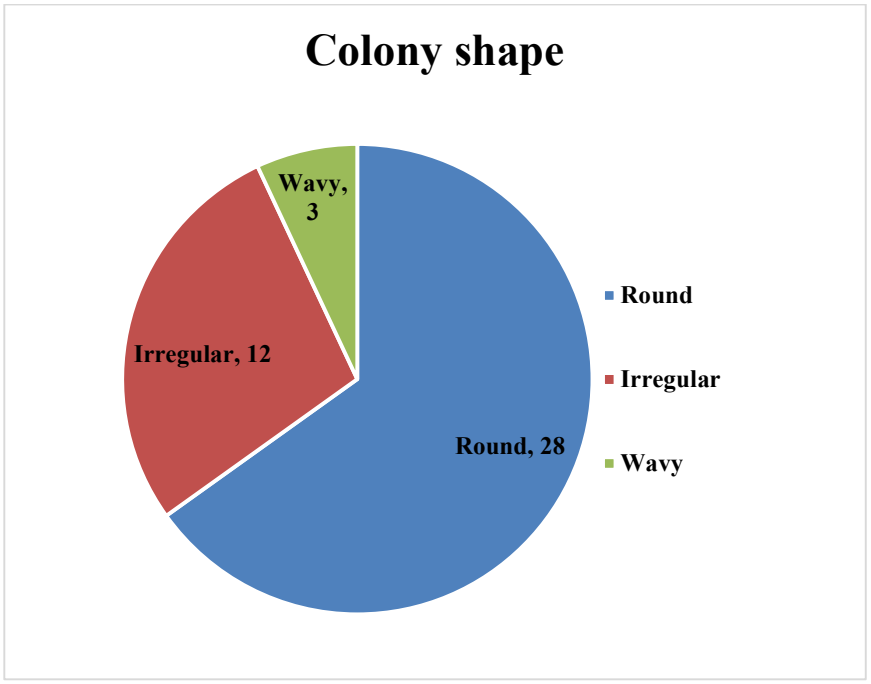

Figure 5.6: Pie chart depicting types of colony shape of the isolated fungal strains.

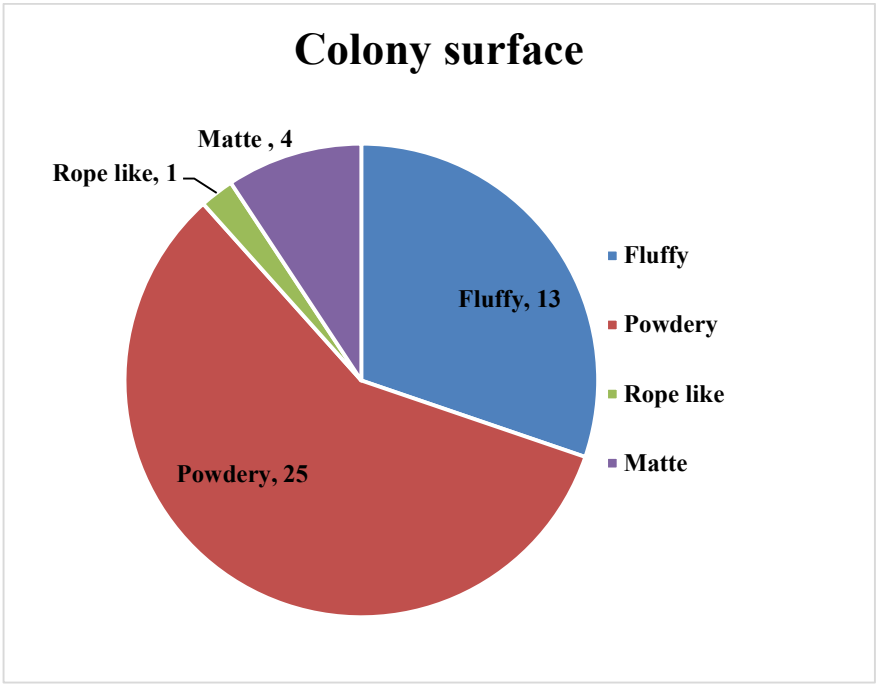

Figure 5.7: Pie chart depicting types of colony surface of the isolated fungal strains**.**

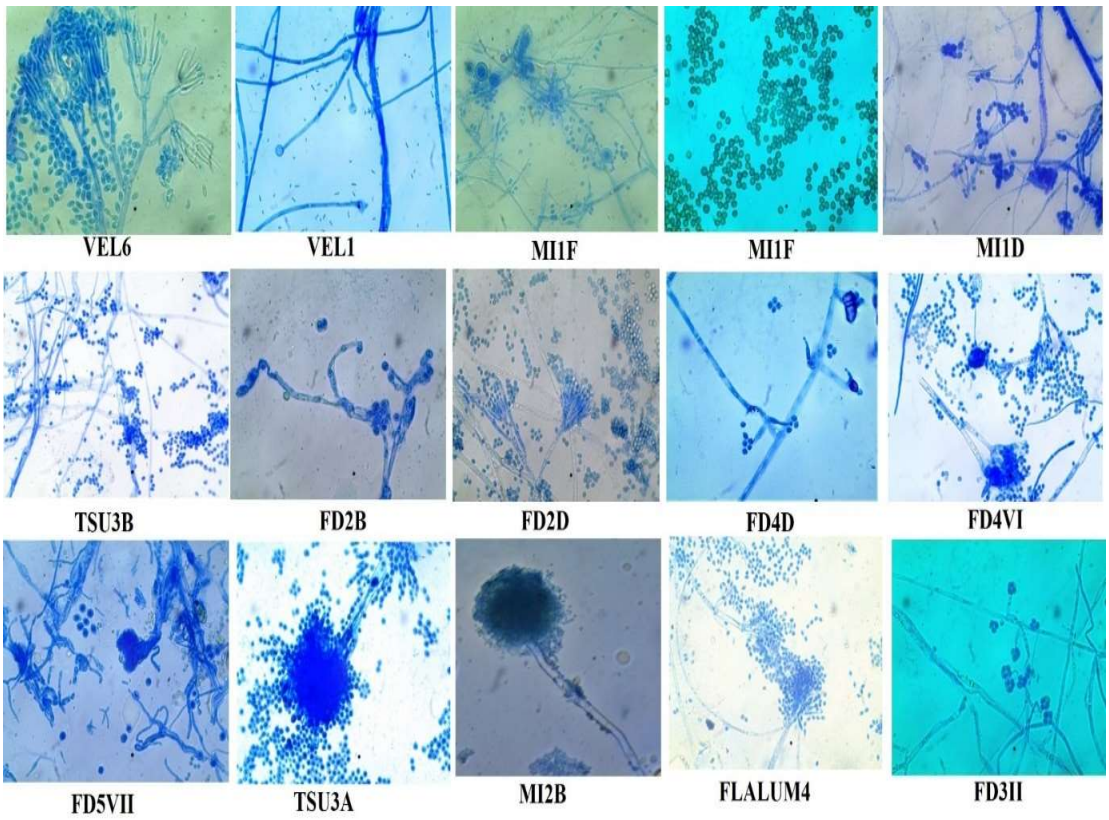

Figure 5.8A: Lactophenol cotton blue staining of the pure fungal isolates.

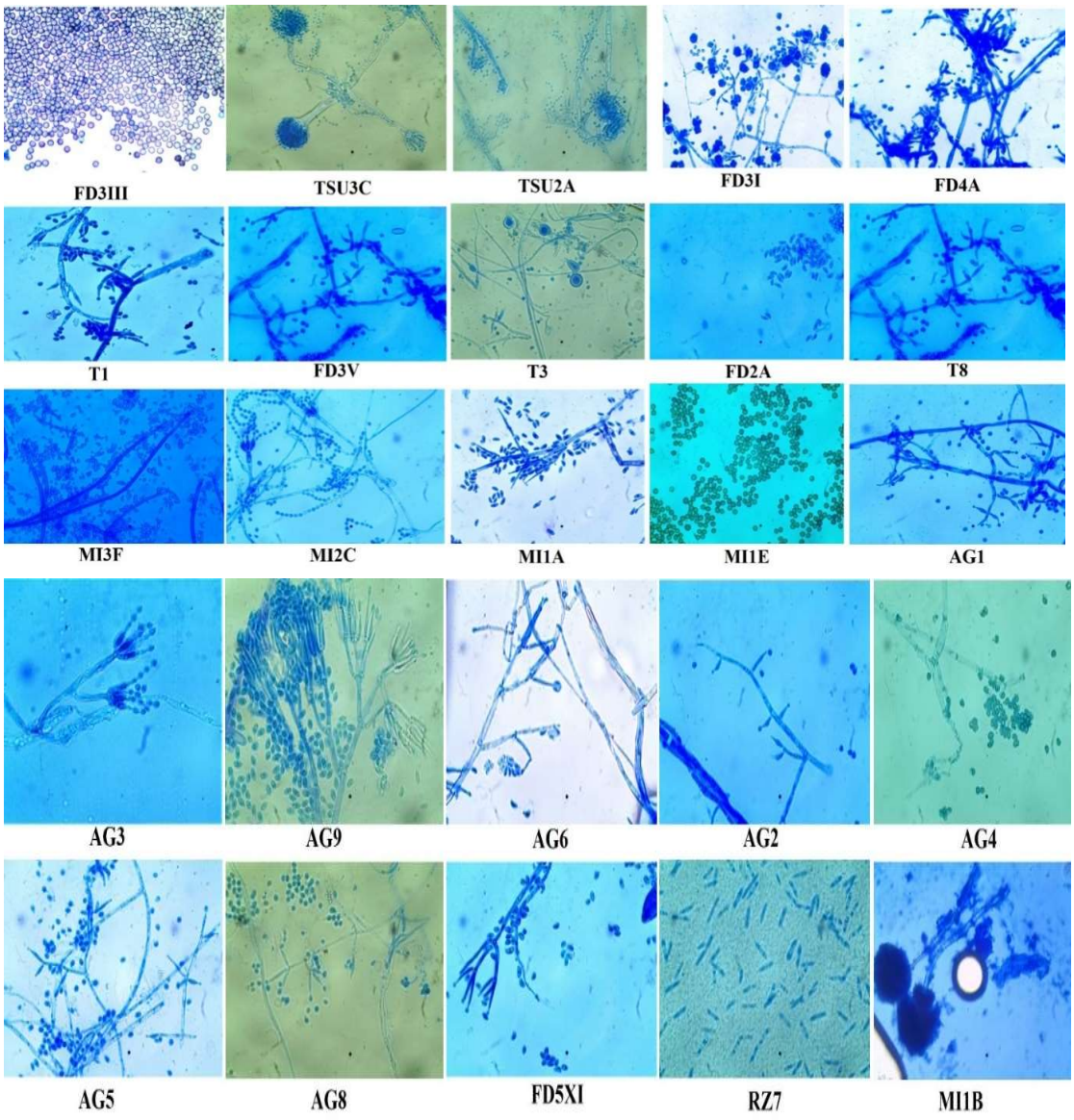

Figure 5.8B: Lactophenol cotton blue staining of the pure fungal isolates.

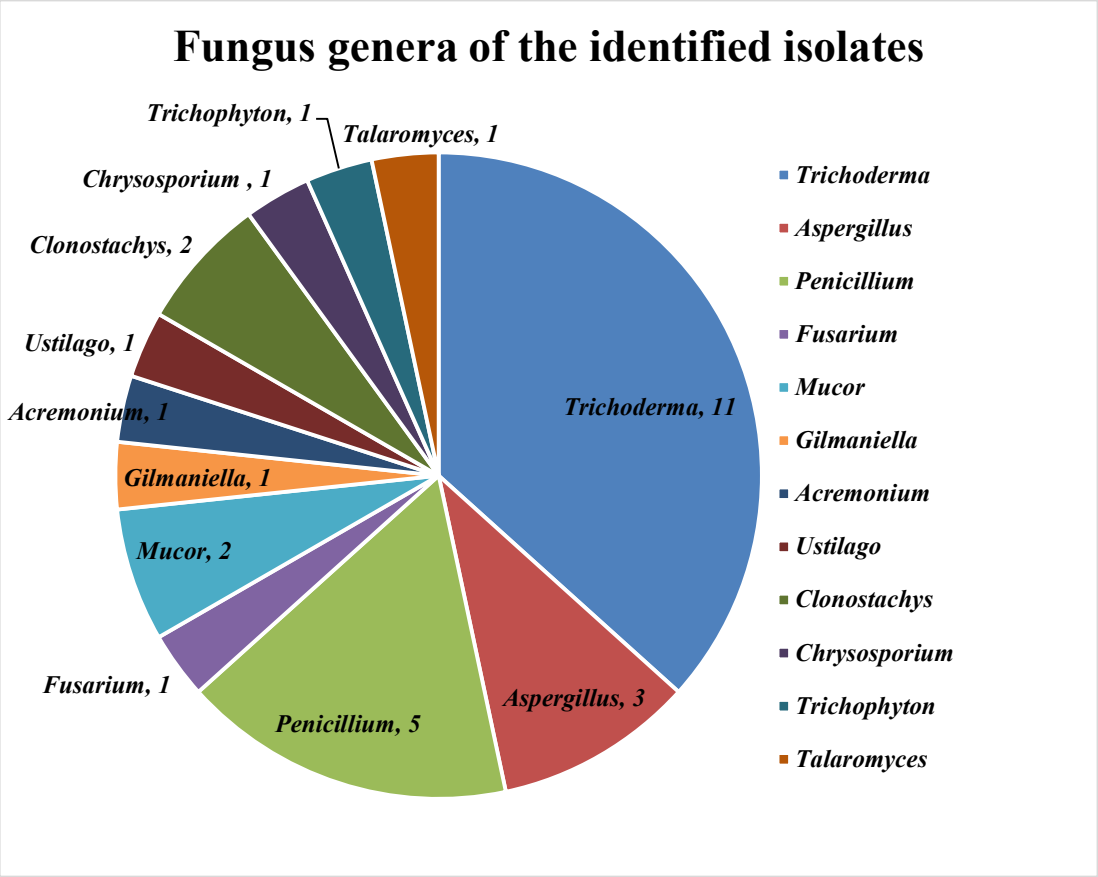

Figure 5.9: Pie chart depicting number of fungal isolates in the same genera isolated from *Musa* rhizospheric soil sample.

Discussion

The rhizospheric microbial community exhibits diverse members, including bacteria, fungi, nematodes, viruses, arthropods, oomycetes, protozoa, algae and archaea (Hossain et al., 2017). Root exudates play a crucial role in maintaining and supporting a highly specific diversity of microbes in the rhizosphere of a given particular plant species, suggesting a close evolutionary relationship. Additionally, root exudates act as signals initiating symbiosis with rhizobia and mycorrhizal fungi (Badri and Vivanco, 2009). Fungi are important components of soil microbes, often constituting a larger portion of the soil biomass than bacteria, depending on soil depth and nutrient conditions

(Mahadevamurthy et al., 2016). In the present study, a total of forty-three fungal isolates were cultured and purified using the Potato dextrose agar (PDA) and Rose Bengal agar (RBA) medium. Traditionally, fungal taxonomy was determined by phenotypic characteristics that could be observed. However, examination and interpretation of morphological characters may vary from person to person (Senanayake et al., 2020). For instances, it has been observed that some fungi such as *Candida albicans* alternate between several colony morphologies on their own at frequencies of 1.4×10^4 (Olsson, 2001). Therefore, in order to identify the fungal isolates and obtain additional confirmation, it is essential to sue molecular techniques and acquire additional confirmation. Because of the pigments they produce, different varieties of fungi produce different-looking colonies. Some colonies may be coloured, while others may be colourless. A wide array of pigments is produced by different fungi, which includes metabolites from several classes such as melanins, anthraquinones, naphthoquinone, azaphilones, carotenoids, oxopolyene, quinones and hydroxyanthraquinones (Kalra et al., 2020). It is now understood that a single fungal species can produce a mixture of different pigments, each with various biological properties, which is an important characteristic for their identification (Mukherjee et al., 2017). In the current study, the colony colours ofisolatesvaried: 20 isolates had white colonies, onehad a purplish pink colony, onewas olive green, fourwere brown, three hadblack colonies, 8 were green, 1 was light purple, 1 was blueand1 was off-white.

It has been reported that the choice of culture media plays a significant role fungal isolates in influence colony morphology and sporulation (Sharma and Pandey, 2010). In the present study, only two growth media were used and not significant differences in colony morphology were observed. Fungi reproduce by spores, produced either sexually or asexually, with most fungal spores adapted for airborne dispersal (Andersen et al.,

2009). In this study, 3 isolates had white spores, 19 had green spores, 7 had brown spores, 5 had olive green spores, 3 had black spores, 3 had yellowish spores, 3 had orange spores, 1 isolate with light blue spores and 1 isolate with off white.

The shapes of fungal colonies exhibit significant diversity, depending on substrate conditions and fungal species. Although the shapes and the surface textures of colonies provide valuable information for species identification or monitoring the state of growth, colony patterning is highly sensitive to the environmental factors (Mushimiyimana et al., 2016). In the present study, 28 isolates had round shapes, 12 were irregularly shaped, and 3 had wavy shapes. The Colony surface varied, 13 isolates, among 47were fluffy whereas powdery surface was found in case of 25 isolates, 1 had rope like surface and 4 had matte surfaces.

In addition to plant phylogeny and species identity, other plant traits, particularly root traits, are strong determinants of rhizosphere fungal community composition (Sweeney et al., 2020). In the current study, the structure of hyphae, spores, and conidiophores was examined using cotton blue staining, leading to the identification of 30 isolates out of 43 isolates. The most dominant genus was *Trichoderma,* with 8 isolates (T8, MI1D, AG6, FD4A, AG1, AG8, T4, NC3, AG2, AG5 and FD2D). This finding aligns with that of Kaushal et al. (2020), who reported *Trichoderma* as the most abundant fungal genus in rhizosphere, root and corm of *Musa* sp. Additionally, *Trichoderma* was reported to be dominant fungal group along with *Aureobasidium* and *Acaulospora* in the rhizosphere of wheat plant (Gqozo et al., 2020). The next most dominant genus was *Penicillium,* with five isolates (FD4VI, FLALUM4, TSU2A, MI2C, and MI1B). The genus *Penicillium* is among the most abundant and ubiquitous groups of soil fungi, phylogenetically represented by two subgenera and twenty-six sections under the family *Aspergillaceae* (Ashtekar et al., 2021). Followed by that was genus *Aspergillus*,

with three isolates (TSU3B, TSU3A and TSU3C). Among the rhizospheric fungi, *Penicillium* and *Aspergillus* spp. are the dominant P-solubilising filamentous fungi with other biotechnological applications such as biocontrol, biodegradation and phosphate mobilization (Elias et al., 2016). In soil suppressive to *Fusarium* wilt disease, *Penicillium, Aspergillus*, and *Trichoderma* were prevalent as beneficial fungi. These fungi can prevent pathogen invasion through enzymatic antifungal compounds, acting, indirectly acting as antifungal biological control agents (Jamil et al., 2023). Next, the genus *Mucor* was represented by two genera (MI1A and MI2B). The *Mucor* genus has shown very few isolates with plant growth promoting activities in many studies and is considered among the least effective PGPF (Hossain and Sultana, 2020; Debbarma et al., 2021). However, some studies have reported their plant growth promoting abilities, such as in potatoes (Utari, et al., 2018) and *Mucor moelleri* has been identified as a potential biocontrol agent against *Atheliarolfsii* and *Colletotrichum gloeosporiodes* (Nartey et al., 2021). Following this was the genus *Clonostachys,* with two isolates (T1 and FD3V). *Clonostachys* spp. is filamentous ascomycetes fungi with a multi-trophic lifestyle, commonly found as saprotrophs in various ecological niches, including soil and dead organic matter. They are known for their antagonistic activity against many plant parasitic nematodes (Piombo et al., 2023). The remaining isolates belonged to genera *Fusarium*, *Gilmaniella*, *Acremonium, Ustilago, Chrysosporium, Trichophyton* and *Talaromyces* genera with one isolateeach (VEL6, FD2X, FD3II, FD3III, T3, MI3F and AG9 respectively). The rest of the isolates were unidentified. However, morphology-based taxonomy sometimes fails to resolve species accurately due to overlapping characteristics, a high degree of phenotypic plasticity, cryptic species, and occurrence of different morphs for the same taxa. Cryptic species refer to the fungal species that have similar morphological characters, but are genetically different (Senanayake et al., 2020).

It is known that interactions between plants and soil fungi drive many essential ecosystem functions (Sweeney et al., 2020).

Conclusions

Based on the analysis of the fungal strains isolated form *Musa* rhizosphere, it is evident that the *Musa* rhizosphere hosts a diverse array of fungal communities, as shown by the varied colony morphologies and biochemical reactions observed. The isolates displayed a range of colony colours, including white, pink, green off-white, brown, black etc. produced by the pigments the fungi generate. Additionally, there was a significant variation in the shapes of the colonies and the colour of the fungal spores. Staining the hyphae and spores with lactophenol cotton blue provided further insights into the identification of the isolates. These characteristics are commonly used in traditional fungal identification techniques. Overall, these findings highlight the complexity and heterogeneity of the microbial community inhabiting the *Musa* rhizosphere, with important implications for nutrient cycling, plant-microbe interactions, and ecosystem functioning. The isolates from the rhizospheric region were identified as belonging to the genera *Trichoderma, Aspergillus, Mucor, Penicillium, Acremonium, Gilmaniella, Chrysosporium, Fusarium, Trichophyton, Clonostachys*, *Talaromyces* and *Ustilago*. However, abundance of plant growth promoting fungi varies significantly according to the host rhizosphere, and the dominating fungal genus is not necessarily the dominating PGPF in the rhizosphere population. Further research into the specific roles and interactions of these diverse fungal strains will contribute to a deeper understanding of rhizosphere ecology and agricultural sustainability.

Chapter – 6

Screening of Fungal Isolates for Plant Growth Promoting Traits

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Introduction

Unrestrained application of fertilizers, herbicides, and pesticides poses a serious risk to agricultural operations, leading to degraded soil and decreased crop yields (Banerjee and Dutta, 2019). Plant growth promoting fungi (PGPF) are a community of fungal species that live in the rhizosphere of plants and aid in the growth and development of those plants (Adedayo and Babalola, 2023). In comparison to using fertilizers and pesticides in the field, the use of plant growth-promoting fungus (PGPF) as biofertilizer is thought to be a more environmentally friendly approach. PGPFs have also demonstrated an effective function in sustainable agriculture (Hossain and sultana, 2020; Galeano et al., 2021). For example, after inoculating wheat and chickpea with *Aspergillus* sp. NPF7, the germination index and root and shoot length were greatly increased compared to the control due to the effects of gibberellic acid (GA), indole acetic acid (IAA), siderophore generating and phosphate (P) solubilising the fungus (Pandya et al.,

2018). In a different instance, it was discovered that inoculating tobacco plants with *Penicillium olsonii* improved their tolerance to saline stress and enhanced plant height, leaf area, dry weight and total chlorophyll content (Tarroum et al., 2022). The excretion of several extracellular compounds by soil fungus can affect the bioavailability and speciation of heavy metals in soil. These substances include enzymes and organic acids, such as fumaric acid, citric acid and gluconic acid (Manzoor et al., 2019). Numerous species of *Aspergillus*, *Penicillium* and *Trichoderma*, are some endophytes have already been used as PGPF (**Figure 6.1**).

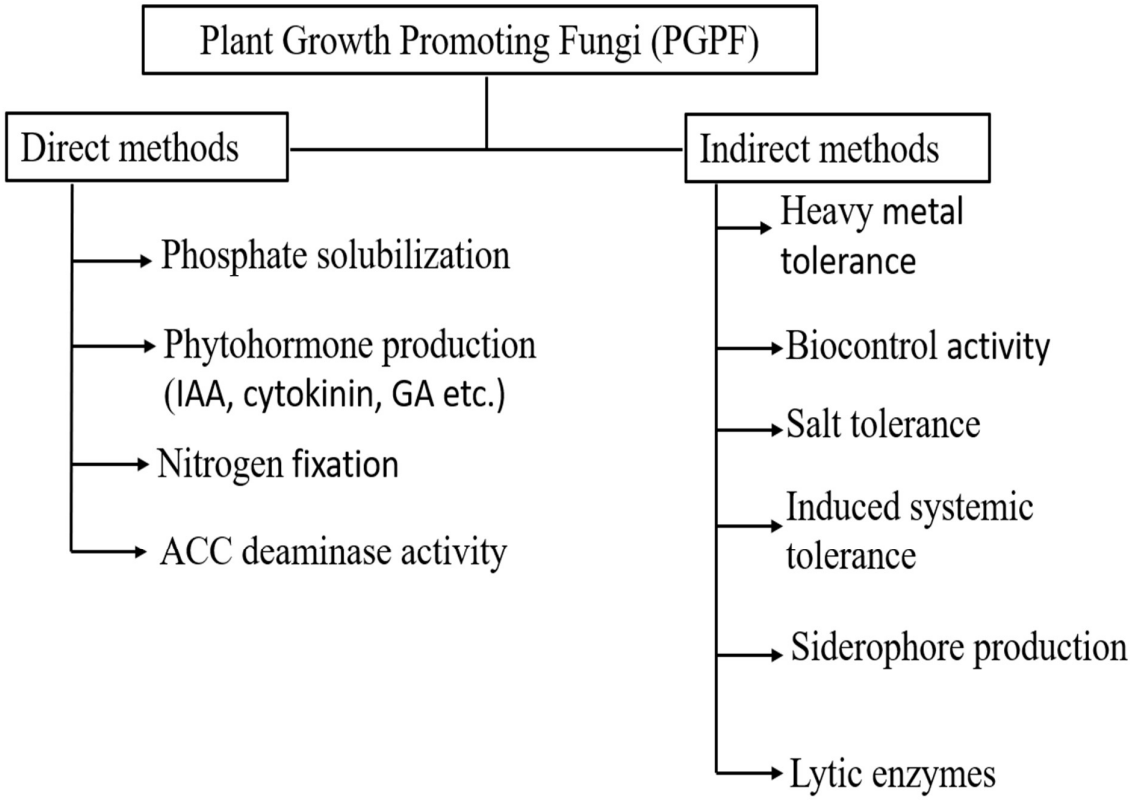

Figure 6.1: Plant growth promoting fungi (PGPF) enhanced plant's growth by various direct and indirect methods.

Plants collect phosphorus as orthophosphate. Because of the fixation process, which results in Fe-phosphate and Al-phosphate in acidic soil (Chang and Yang, 2009; Seshachala and Tallapragada, 2012; Sharma et al., 2013), the phosphate nutrient is typically low. The activities of different microorganisms that impact phosphorus mobilization in soil have a significant impact on the phosphorus cycle in nature. It is highly recommended that phosphate solubilizing microorganisms (PSM) be used to utilize native phosphate that is inaccessible, resulting in modifications to the soil that produce organic acid and chelating agents (Kumar et al., 2012a). Phosphate solubilizing microorganisms (PSM) are a broad category of soil fungus and bacteria that possess the ability to solubilize inorganic phosphate. The PSM is crucial for providing plants with extra phosphorus, which enables the long-term usage of phosphate fertilizer (Pradhan and Sukla, 2005). Phosphate-solubilizing fungi have been shown to enhance soil nutrient uptake, as demonstrated by the notable increase in phosphate and nitrogen uptake by haricot bean plants cultivated in soil infected with these fungi (Malviya et al., 2011). According to Tak et al. (2021), the use of PSF as phosphate fertilizer increased the need for agricultural production globally while lowering soil fertility, polluting water and accumulating toxic wastes.

Additionally, IAA, often referred to as exogenous IAA, can be produced by microorganisms, especially fungi that are taken from the rhizosphere. While plants can only generate a small quantity of endogenous IAA that is not used directly, exogenous IAA obtained via fungal isolation can be added to biological fertilizers to improve outcomes and offer maximum advantages (Gusmiaty et al., 2019). In wheat infected plants growing in heavy metal contaminated soil, *Penicillium ruqueforti*, an IAA generating fungus, produced significant resistance (Ikram et al., 2018). A few of the PGPF that produce IAA and promote plant growth are *Penicillium olsonii* (Tarroum et

al., 2022), *Acrophialophora jodhpurensis* (Daroodi et al., 2022), *Aspergillus* sp. and *Fusarium* sp. (Gusmiaty et al., 2019), *Talaromyces trachyspermus* (Chouhan et al., 2022), *Trichoderma viride* (Kumar et al., 2017) and *Bipolaris* sp. (Khan et al., 2022).

According to Crosa and Walsh (2002), siderophores are tiny peptidic molecule with side chains and functional groups that can serve as a high-affinity collection of ligands to coordinate ferric ions. The generation of siderophores in soil is a crucial factor in determining the capacity of various microorganisms to enhance plant development. According to Andrews et al. (2003), siderophores are produced in order to solubilize iron from theirsurroundings and form a complex ferric siderophore that can migrate by diffusion and return to the soil surface. Chlorosis, or yellowing of the leaves, is brought on by an iron shortage and costs money by destroying valuable income crops. A wide variety of interesting features can be seen in fungus siderophores. According to Renshaw et al. (2002), these siderophores have the ability to bind not just Fe (III) but also other metals such as Pb (II), Cr (III), Al (III) and actinide ions. This suggests that they may find application in the bioremediation of heavy metal contamination, pharmaceuticals, and industrial waste management.

According to Kisa et al. (2016), heavy metal toxicity is one of the main abiotic stresses endangering crop yield and sustainable agriculture. It also disrupts the native soil microbiota. Numerous fungi have been demonstrated to thrive in environments with elevated levels of hazardous metals (Deng and Cao, 2017). For example, when arbuscular mycorrhizal fungi were inoculated alongside *Calendula officinalis* plant, the uptake of heavy metals (Cd and Pb) was reduced and consequently, the beneficial secondary metabolites were boosted in contrast to non-mycorrhizal plants (Hristozkova et al., 2016). In a different instance, the fungus Pb3 (*Aspergillus terreus*), Cr8 (*Trichoderma*

longibrachiatum), isolate Ni27 (*A. niger*) and *T. viride* showed maximal uptake of 59.67, 16.25, 0.55 and 0.55 mg/g, respectively (Joshi et al., 2011). Higher resistance to Mo and Vanadium was demonstrated by *A. niger, A. foetidus* and *Penicillium simplicissimum* (Anahid et al., 2011). Hg biosorption capability was from 33.8 to 54.9 mg/g dry weight, with a removal capacity ranging from 47% to 97% in isolates of *Cladosporium* sp., *Didymella glomerata*, *F. oxysporum*, *Phoma costaricensis* and *Sarocladium kiliense* (Vãcar et al., 2021). According to Iram et al. (2021), *A. niger* isolates were found to be tolerant of Pb and Cr. Fungi that produce oxalate have the ability to precipitate metals as insoluble metal oxalates, which reduces metal bioavailability and enhances tolerance to harmful metals (Gadd et al., 2014).

In light of the aforementioned information, the following goals of the study were identified in this chapter: to screen the pure fungal isolates for features that promote plant growth and to identify specific PGPF strains with the following objectives:

Objectives

- Phosphate solubilisation test by the fungal isolatesfor qualitative and quantitative analysis.
- IAA production test by fungal isolates.
- Qualitative estimation for siderophore production.
- Screening for ammonia and amylase production by the fungal isolates.
- Molecular identification of the selected fungal isolates with plant growth promoting traits.

Materials and Methods

PGPF can promote plant development in two ways: directly and indirectly. Activities including phosphate solubilisation and indole 3-acetic acid (IAA) synthesis are

part of the direct approaches. Conversely, indirect techniques include the generation of siderophores, ammonia, and amylase, as well as the induction of systemic resistance and the tolerance of biotic and abiotic stressors such salinity and heavy metal tolerance.

Qualitative analysis for phosphate solubilization

Fungal isolates were grown on National Botanical Research Institute's phosphate (NBRIP) growth medium to solubilize phosphate supplemented in the media with media composition gL^{-1} ; 10g glucose, 5g $Ca_3(PO_4)_2$, 5g $MgCl_2$, $6H_2O$, 0.25g $MgSO_4$, $7H_2O$, 0.2g; KCl and0.1g $(NH_4)_2SO_4$ and 15g agar (You et al., 2020). Fungal isolates were incubated at 28±2ºC for 7 days after being inoculated on agar plates. Isolates with a clear halo zone around the colony were found to be phosphate solubilizing. The following formula was used to calculate the phosphate solubilizing index (PSI):

$$\text{Phosphate solubilizing index} = \frac{\text{Colony diameter + Halo zone}}{\text{Colony diameter}}$$

Quantitative assay of phosphate solubilization

Quantitative analysis for phosphate solubilization was done in liquid medium as described by Pande et al. (2017). Phosphate solubilization was estimated in 40 ml of NBRIP broth (Composition gL^{-1}; 10g glucose, 5g $Ca_3(PO_4)_2$, 5g $MgCl_2$, $6H_2O$, 0.25g $MgSO_4$, $7H_2O$, 0.2g KCl, 0.1g $(NH_4)_2SO_4$, pH 7.0) in 250 ml conical flask. The broth without culture inoculated has served as control. The test strains were grown overnight and incubated in NBRIP broth for 12 days at 28±2ºC.

After incubation, 1 ml of supernatant was taken out on 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th} and 12^{th} day. The supernatant was obtained by centrifugation at 10,000 rpm for 20 min and the 600µl of filtered supernatant was mixed with 1500µl of Barton's reagents and volume

was made up to 5ml with double distilled water (2.9 ml). After 10 min the intensity of yellow colour was read on spectrophotometer (Thermo Scientific Multiskan Go) at 430nm and the amount of P solubilized was extrapolated from standard curve. The experiments were conducted in triplicates and values were expressed as their mean.

Siderophore production by the fungal isolates

This assay was performed following the protocol of Hu and Xu (2011). CAS agar plates were prepared by mixing 100ml CAS reagent in 900ml sterilized NA agar medium. Fungal strains were spot inoculated on each plate. An un-inoculated plate was taken as control. After inoculation, plates were incubated at 28±2ºC for 7 days and observed for the formation of orange/pink halo zone around the fungal colonies. Formation of orange/pink halo zone around the fungal colony indicated positive result. The siderophore production index (SPI) was determined by subtracting the diameter of the colony from the total diameter (halo + colony)/colony dimeter

Qualitative assay of indole acetic acid (IAA) production

Ten ml of nutrient broth supplemented with tryptophan (0.1%, w/v) was inoculated with freshly grown fungal culture and incubated in a shaking incubator at 28±2ºC for 7 days to estimate IAA production. 10 ml nutrient broth with 0.1% tryptophan without inoculation was considered as control (Kumar et al., 2012a). After 7 days 1ml of the culture broth was taken and centrifuged for 10 min at 10,000 rpm. After that 1ml of the supernatant was taken and transferred to glass vial containing 2 ml of the Salkowski reagent. The mixture was then incubated for 25 min and the formation of pink colour solution was observed which indicated the production of IAA by the isolate.

Quantitative estimation of IAA production by the fungal isolates

Fungal isolates were kept for 12 days inside the shaker incubator at 28±2ºC. On 2nd, 4th, 6th, 8th, 10th and 12th day, 1 ml of the culture was taken and centrifuged at 10,000 rpm for 10 min. Supernatant was then transferred to a vial containing 2 ml of the Salkowski reagent to test tubes labelled with each strain (Kumar et al., 2012a). After 25 min of incubation, cultures showing pink colour formation were identified as positive for IAA production. 200µl of the mixture of reagent and bacterial culture supernatant was transferred to a 96 well microplate, and the O.D. was measured at 530nm. The absorbance at 530nm was plottedagainst the concentration for the standard curve, and the concentration (µg/ml) was obtained by plotting the absorbance of the fungal strains.

Ammonia production test

Purified fungal isolates were grown in 5ml nutrient broth and incubated in shaking incubator at 28±2º C for 48 h. Then 10ml of peptone water broth (Composition gL^{-1}; 20g peptone and 1g sodium chloride) was inoculated with the freshly grown isolates for 48hrs at room temperature with constant shaking (Sharma et al., 2021). After 48 h, Nessler's reagent was added to the culture broth to detect the ammonia production by the isolates. To prepare Nessler's reagent, 50 g of $HgCl_2$ and 35 g of potassium iodide was dissolved in 200 ml of distilled water in one conical flask. In another conical flask, 50g of sodium hydroxide was dissolved in 250 ml of distilled water. Both the solutions were mixed and made up to 500 ml. The solution was then allowed to stand for 10-15 min and the clear supernatant was then decanted and stored for analysis. One ml of the Nessler's reagent was added in the broth and incubated for 10 min. Positive ammonia production cases were considered where a faint yellow colour developed post Nessler's reagent addition and quantity of ammonia production is directly proportional with the depth of the yellow

colour. Isolates giving light yellow colour after adding the reagent was considered as isolates producing a small amount of ammonia whereas isolate producing dark yellow and orange colour indicated a medium and high amount of ammonia production, respectively.

Amylase activity

Starch-degrading activity of the cultures was estimated by following the method mentioned by (Imran et al., 2021). The hydrolysis of starch was conducted on a medium (Composition gL^{-1}; peptone, 5.0g; beef extract, 3 mg; starch (soluble), 2 g and agar20 g. Mycelium plug of Freshly grown (3 days old) culture were spot inoculated in the centre of the plate and incubated at 28° C for 7 days. After 7 days, fungal cultures were flooded with an iodine solution. The development of a pale-yellow zone around a colony in the blue medium indicated the starch-hydrolysing activityand isolates were considered positive for amylase production.

Molecular characterization of the PGPF strains

For molecular characterization of the fungal isolates genomic DNA was isolated following the cetyltrimethyl ammonium bromide (CTAB) method mentioned by Naziya et al. (2019). and was amplified using ITS-1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS-4 (5′- TCCTCCGCTTATTGATATGC-3′) with initial denaturation at 95°C (10 min), followed by denaturation for 35 cycles at 95° C (1 min), annealing at 55°C (1 min), primer extension at 72°C (2 min) and final extension at 72°C (8 min). The PCR-amplified products were electrophoretically detected on 1.5% (*w*/*v*) agarose gel containing $0.5\mu gmL^{-1}$ of EB (ethidium bromide) and subjected for sequencing. The sequence was deposited in GenBank, NCBI and accession number was acquired.

Statistical Analysis

The SPSS software was used for statistical analysis of the experimental data. All the reported results are the mean of the three replicates and deviations were calculated as the standard error of the mean (SEM).

Results

PGPF can support plant growth and development through a variety of direct and indirect means. While indirect ways include processes like siderophore generation, lytic enzymes, stress tolerance (e.g., heavy metal contamination), and salinity resistance, direct methods include their capacity to produce phytohormones like IAA, solubilize phosphate, fix nitrogen, etc. (**Figure 6.1**). The potential of the fungal strains to promote plant growth was assessed after they had been isolated and purified.

Phosphate solubilisation

Qualitative and quantitative estimation of the fungal isolates was done in NBRIP agar medium. On the agar medium, phosphate solubilisation was detected by formation of clear halo zone around the fungal colony indicating solubilisation of tricalcium phosphate. The highest PSI was exhibited by isolate AG5 (4.44±1.01), followed by FD4A (4.04±0.02), VEL6 (3.87±0.02), AG9 (3.78±0.02), T3 (3.67±1.07), TSU3B (3.33±1.92), FD3III (3.27±0.18), TSU3C (3.07±0.09), MI1B (3.03±0.06), TSU3A (3.02±1.22), FD4A (2.99±0.04), AG1 (2.99±0.14), FLALUM4 (2.98±0.17), FD5XI (2.93±0.02), FD4VI (2.66±0.04), AG2 (2.60±0.02), AG4 (2.48±0.02), RZ7 (2.43±0.04), T1 (2.19±0.01), MI1E (2.19±0.08), MI2C (2.15±0.02), T8 (2.05±0.01), FD4XIII (2.05±0.02), MI1D

(2.04±0.01), FD3V (2.03±0.06), FD2B (1.98±0.02), FD2A (1.89±0.01), FD2X (1.31±0.11), MI2B (1.23±1.09) and MI1A (1.02±0.02) (**Table 6.1 and Figure 6.2, 6.3**).

While, analysis of phosphate solubilization in liquid NBRIP medium, it was found that, different isolates started solubilization of tricalcium at different time interval (**Table 6.2**). Most of the isolates solubilized highest amount of tricalcium in the medium on the 4th day and highest solubilization was exhibited by the isolateT4 (146.43±25.59µg/ml), followed by VEL6 on 4thday (52.38±2.58µg/ml) and lowest was on the 2nd day (6.95±1.37µg/ml), followed by 9.95± 0.44µg/ml on 6thday, and 3.76±0.92µg/ml on the 8th day; while, no phosphate solubilization was detected on 10th and 12th day. For isolate VEL (36.72±3.15µg/ml), MI1D (49.48±1.47µg/ml), RZ7 (59.60±0.01µg/ml) and MI1A (50.68±3.33µg/ml) P solubilization was detected only on 4th day. For isolate MI1F, phosphate solubilization started from day 4 (9.40±1.19µg/ml) and highest being on 6th day (66.37±2.80µg/ml), followed by 11.57±0.72µg/ml, 9.49±1.54µg/ml and 0.76±2.54µg/ml on 8th, 10th and 12th day respectively. For isolate FD4XIII, highest solubilization was detected 58.01± 1.91µg/ml on the 4th day, while, on the 2nd day it was 18.38±1.96µg/ml and on 6th day 2.03±0.22µg/ml. For isolates TSU3B and FD2D, phosphate solubilization detected on 2ndday (4.78±0.65µg/ml and 4.80±4.78 µg/ml) and 4th day (60.07±1.55µg/ml and 3.80±0.89µg/ml) respectively. For FD2B isolate, solubilization started on 2nd day (12.16±1.22µg/ml), highest on 4th day (18.76±1.40µg/ml) followed by declining trend i.e., on 6th day (18.21±0.77µg/ml), 8th day (14.10±2.03µg/ml), 10th day (10.02±2.34µg/ml), and least on 12th day (3.59±1.02µg/ml). For isolate FD4D, least was on 12th day (8.46±1.27µg/ml) and highest solubilization was observed on the 6th day (23.92±1.46µg/ml), followed by 8th day (22.49±0.35µg/ml), 4th

day (18.20±2.19µg/ml), 10th (13.31±1.87µg/ml), 2nd (8.70±2.02µg/ml). For isolate FD4VI, solubilization stared from 4th day (24.21±1.82µg/ml), gradually decreased from 6th day (19.95±1.01µg/ml), 8th day (16.61±1.69µg/ml), 10th day (11.55±0.96µg/ml), till 12th day (3.62±2.01µg/ml). For isolate TSU3A, solubilization was only observed on 2nd (32.64±3.10 µg/ml), 4th (107.78±1.60µg/ml) and 6th day (6.13±1.69µg/ml).In case of isolate MI2B, highest solubilization was observed on the 4th day (75.74± 4.05µg/ml), followed by 6th day (2.86±0.09µg/ml), 8th day (2.84±1.13µg/ml) and 10th day (2.67±0.58µg/ml). For isolate FD2X, highest was observed on 4th day (33.69±0.52µg/ml), followed by 6th day (32.70±1.44µg/ml), 8th day (27.45±0.92µg/ml), 10th (22.99±1.27µg/ml) and 12th day (22.99±1.27µg/ml). For isolate FLALUM4, highest solubilization was observed on the 4th day (52.44±0.36µg/ml), followed by 6th day (3.33±1.55µg/ml), 2nd day (1.09±1.19µg/ml), 8th day (1.00±0.30µg/ml) and 10th day (0.14±0.44µg/ml). In case of isolate FD3III, highest solubilization was observed on the 4th day, followed by 2nd (4.60±0.35µg/ml) and 6th day (4.17±1.43µg/ml). For isolate TSU3C highest on the 4th day (67.28±1.20µg/ml) followed by 2nd day (2.25±1.83µg/ml). For TSU2A, highest was observed on the 4th day (50.21±5.43µg/ml), followed by 2nd day (8.26±0.99µg/ml), 6th day (6.17±0.75µg/ml), 8th day (5.56±1.87µg/ml), 10th day (3.36±1.18µg/ml) and 12th day (2.00±0.76µg/ml). For FD4A isolate, highest P solubilization was observed on 4th day (77.18±5.63µg/ml), followed by 2nd day (29.25±0.51µg/ml), 6th day (28.59± 1.88µg/ml), 8th day (24.08±0.74µg/ml), 10th day (22.54±1.64µg/ml) and 12th day (22.10±1.18µg/ml). For FD4A isolate, highest solubilization was observed on the 4th day (77.18±5.63µg/ml), followed by 2nd day (29.25±0.51µg/ml), 6th day (28.59±1.88µg/ml), 8th day (24.08±0.74µg/ml), 10th day (22.54±1.64µg/ml), and 12th day (22.10±1.18µg/ml). For isolate T1, highest

solubilization was observed on the 4th day (99.32±7.56µg/ml), followed by 6th day (13.57± 0.66µg/ml), 8th day (11.57± 0.72µg/ml), 10th day (9.45±1.86µg/ml) and 12th day (0.08±0.94µg/ml). For isolate FD3V, highest solubilization was observed on the 4th day (29.08±1.49µg/ml), followed by 6th day (26.77±1.89µg/ml), 8th day (26.36±1.16µg/ml), 2nd day (11.68±1.78µg/ml), 10th day (17.80±1.36µg/ml) and 12th day (2.38±2.60µg/ml). for isolate T3 and T8, highest solubilization were observed on the 4th day (99.32±7.56µg/ml and 126.53±8.42µg/ml), followed by 2nd day (18.14±2.08µg/ml and 19.83±2.32µg/ml), 6th day (11.11±1.10µg/ml and 7.08±3.17µg/ml), 8th day (7.68±0.39µg/ml and 6.29±2.76µg/ml), 10th day (5.36±0.98µg/ml and 1.00±0.34µg/ml), 12th day (5.09±0.05µg/ml and 0.56±1.09µg/ml) respectively. For isolate T4 and AG4, highest solubilization were observed on the 4th day (146.43± 25.59µg/ml and 10.86±0.93µg/ml), followed by 2nd day (13.08±0.38µg/ml and 6.55±0.53µg/ml) and 6th day (1.51±0.33µg/ml and 4.76±1.04µg/ml) respectively. For isolate FD2A, highest solubilization was observed on the 4th day (62.56±1.59µg/ml), followed by 6th day (27.79±0.87µg/ml), 8th day (20.57±1.32µg/ml), 10th day (19.97±0.23µg/ml), 2nd day (5.80±2.21µg/ml) and 12th day (3.04±2.49µg/ml). For isolate FD2VIII, highest solubilization was on the 4th day (39.62±0.46µg/ml), followed by 2nd day (21.69±0.78µg/ml). For isolate MI1E, highest solubilization was observed on the 4th day (24.38±0.94µg/ml), followed by 2nd day (21.48±1.25µg/ml), 6th day (14.08±0.82µg/ml), 8th day (11.16±0.72µg/ml) and 10th day (4.11±0.68µg/ml). For isolates AG1 and AG3, highest solubilization was observed on the 2nd day (4.23±2.97µg/ml and 29.34±0.22µg/ml respectively), followed by on the 4th day (2.57±0.61µg/ml and 11.13±0.53µg/ml respectively). AG9 isolate, had the highest solubilization was observed on the 4th day (52.53±1.46µg/ml), followed by 6th day (20.20±8.15µg/ml), 2nd day (4.72±1.55µg/ml),

8thday (3.40±1.03µg/ml), and 10th day (0.52±0.44µg/ml). For isolate AG6, highest solubilization was observed on the 8th day (18.13±2.75µg/ml), followed by 6th day (17.85±1.11µg/ml), 4th day (16.31±0.61µg/ml), 2nd day (14.20±2.50µg/ml), 10th day (11.83±0.80µg/ml), and 12th day (11.36±1.48µg/ml). For isolate AG2, highest solubilization was observed on the 4th day (13.78±1.14µg/ml), followed by 2nd day (11.36±1.11µg/ml), 6th day (10.28±0.45µg/ml), 8th day (7.31±1.46µg/ml) and 10th day (5.55±0.7µg/ml). For isolate AG5, highest solubilization was observed on the 6th day (31.13±0.61µg/ml) followed by 4th day (24.53±0.79µg/ml), 8th day (20.07±0.49µg/ml) and 10th day (18.69±1.00µg/ml). For isolate AG8 highest solubilization was observed on the 6th day (13.22±1.35µg/ml), followed by 8th day (9.38±2.18µg/ml), 4th day (7.24±0.86µg/ml), 2nd day (5.70±1.27µg/ml) and 10th day (3.05±0.77µg/ml). For isolate FD5XI, highest solubilization was observed on the 4th day (55.02± 0.81µg/ml), followed by 6th day (17.44± 1.70µg/ml), 8th day (17.43±0.64µg/ml), 10th day (14.70±1.36µg/ml) and 12th day (6.22±0.44µg/ml). For isolate FD1B, highest solubilization was observed on the 4th day (23.17±2.13µg/ml) followed by 2nd day (16.66±0.27µg/ml), 6th day (10.10±1.08µg/ml) and 8th day (6.03±2.37µg/ml).

Table 6.1: Qualitative analysis of plant growth promoting traits by the fungal isolates

Sl. No.	Fungal Isolates	Siderophore production index (SPI)±SE*	Phosphate Solubilizing Index (PSI) ±SE*	IAA Production	Ammonia Production	Amylase production
1	VEl6	-	3.87±0.02	-	++	-
2	VEL1	-	-	-	+++	-
3	MI1F	2.89±0.02	-	-	++	-
4	MI3F	-	-	-	++	-
5	MI1D	3.14±0.04	2.04±0.01	-	+++	+
6	FD4XIII	-	2.05±0.02	+	+	+

7	TSU3B	2.44±0.01	3.33±1.92	-	+	+
8	FD2B	-	1.98±0.02	-	+	+
9	FD2D	-	-	+	+++	+
10	FD4D	-	-	-	+	+
11	FD4VI	2.6±0.03	2.66± 0.04	+	+++	+
12	FD5VII	-	-	-	++	+
13	TSU3A	2.29±0.01	3.02±1.22	-	+	+
14	MI2B	3.19± 0.04	1.23±1.09	+	++	+
15	FD2X	2.1±0.03	1.31± 0.11	+	++	+
16	FLALUM4	2.09±0.02	2.98± 0.17	+	+	+
17	FD3II	-	-	+	+++	-
18	FD3III	2.4±0.07	3.27± 0.18	+	+++	-
19	TSU3C	2.33± 0.07	3.07± 0.09	-	++	-
20	TSU2A	1.90±1.07	2.99± 0.04	-	++	+
21	FD3I	1.89± 0.09	-	+	++	-
22	FD4A	2.62± 0.02	4.04± 0.02	+	+	-
23	T1	-	2.19± 0.01	+	+++	+
24	FD3V	-	2.03± 0.06	+	+++	-
25	T3	-	3.67± 1.07	-	+	-
26	FD2A	4.30±0.08	1.89± 0.01	-	++	+
27	T8	-	2.05± 0.01	+	++	+
28	MI3F	-	-	+	++	-
29	MI2C	3.2±0.06	2.15± 0.02	-	+++	-
30	MI1A	-	1.02± 0.02	-	+	+
31	MI1E	3.1±0.03	2.19± 0.08	+	++	+
32	AG1	3.5±0.12	2.99± 0.14	+	+	-
33	AG3	-	-	-	+	+
34	AG9	3.01±0.43	3.78±0.02	-	++	+
35	AG6	2.5±0.06	-	-	++	+
36	AG2	2.6±0.02	2.60±0.02	-	+	-
37	AG4	2.3±0.06	2.48± 0.02	-	++	-
38	AG5	3.01± 0.03	4.44± 1.01	-	++	-
39	AG8	2.7±0.09	-	-	+	-
40	FD5XI	-	2.93± 0.02	+	++	+
41	RZ7	1.90± 0.03	2.43±0.04	-	++	+
42	MI1B	-	3.03± 0.06	-	+	-
43	FD1B	-	-	-	++	+

Note: '+++': Indicates high ammonia production; '++': Indicates moderate ammonia production; '+': Indicates low ammonia production. [Isolates giving light yellow colour after adding the reagent was considered as isolates producing a small amount of ammonia ('+') whereas producing dark yellow indicated moderate amount of ammonia production ('++) and orange colour indicated a high amount of ammonia production ('+++')]. * ±SE: Standard error from mean.

Table 6.2: Phosphate solubilisation by the fungal isolates in PVK broth on 2nd, 4th, 6th, 8th, 10th and 12th day

Fungal Isolates	Concentration of PO_4^+(µg/ml)					
	2nd day	4th day	6th day	8th day	10th day	12th day
VEl6	6.95± 1.37	52.38± 2.58	9.95± 0.44	3.76±0.92	ND	ND
VEL1	ND	36.72± 3.15	ND	ND	ND	ND
MI1F	ND	9.40± 1.19	66.37± 2.80	11.57± 0.72	9.49±1.54	0.76± 2.54
MI1D	ND	49.48± 1.47	ND	ND	ND	ND
FD4XIII	18.38± 1.96	58.01± 1.91	2.03± 0.22	ND	ND	ND
TSU3B	4.78±0.65	60.07± 1.55	ND	ND	ND	ND
FD2B	12.16±1.22	18.76±1.40	18.21±0.77	14.10±2.03	10.02±2.34	3.59±1.02
FD2D	4.80±4.78	3.80±0.89	ND	ND	ND	ND
FD4D	8.70±2.02	18.20±2.19	23.92±1.46	22.49±0.35	13.31±1.87	8.46±1.27
FD4VI	ND	24.21±1.82	19.95±1.01	16.61±1.69	11.55±0.96	3.62±2.01
FD5VII	ND	ND	ND	ND	ND	ND
TSU3A	32.64± 3.10	107.78± 1.60	6.13± 1.69	ND	ND	ND
MI2B	ND	75.74± 4.05	2.86± 0.09	2.84± 1.13	2.67±0.58	ND
FD2X	18.15±1.00	33.69±0.52	32.70±1.44	27.45±0.92	22.99±1.27	16.02±2.33
FLALUM4	1.09± 1.19	52.44± 0.36	3.33± 1.55	1.00± 0.30	0.14±0.44	ND
FD3ii	ND	ND	ND	ND	ND	ND
FD3iii	4.60±0.35	14.84±1.37	4.17±1.43	ND	ND	ND
TSU3C	2.25± 1.83	67.28± 1.20	ND	ND	ND	ND
TSU2A	8.26± 0.99	50.21±5.43	6.17±0.75	5.56±1.87	3.36±1.18	2.00±0.76
TSU2C	ND	ND	ND	ND	ND	ND
T2	ND	ND	ND	ND	ND	ND
TSU2B	ND	ND	ND	ND	ND	ND
FD4A	29.25± 0.51	77.18±5.63	28.59± 1.88	24.08±0.74	22.54±1.64	22.10± 1.18
T1	ND	56.53± 6.47	13.57± 0.66	11.57± 0.72	9.45±1.86	0.08±0.94
FD3V	11.68±1.78	29.08±1.49	26.77±1.89	26.36±1.16	17.80±1.36	2.38±2.60
T3	18.14± 2.08	99.32± 7.56	11.11± 1.10	7.68± 0.39	5.36±0.98	5.09±0.05
T8	19.83± 2.32	126.53± 8.42	7.08±3.17	6.29± 2.76	1.00±0.34	0.56± 1.09
T4	13.08± 0.38	146.43±25.59	1.51±0.33	ND	ND	ND
NC3	ND	ND	ND	ND	ND	ND
MI3F	ND	ND	ND	ND	ND	ND
FD2A	5.80± 2.21	62.56± 1.59	27.79±0.87	20.57±1.32	19.97±0.23	3.04± 2.49
FD3C	ND	ND	ND	ND	ND	ND
MI2C	ND	ND	ND	ND	ND	ND
FD2VIII	21.69± 0.78	39.62± 0.46	ND	ND	ND	ND
MI1A	ND	50.68± 3.33	ND	ND	ND	ND
MI1E	21.48±1.25	24.38±0.94	14.08±0.82	11.16±0.72	4.11±0.68	ND
AG1	4.23±2.97	2.57±0.61	ND	ND	ND	ND

AG3	29.34± 0.22	11.13± 0.53	ND	ND	ND	ND
AG9	4.72±1.55	52.53±1.46	20.20± 8.15	3.40± 1.03	0.52±0.44	ND
AG6	14.20±2.50	16.31±0.61	17.85±1.11	18.13±2.75	11.83±0.80	11.36±1.48
AG2	11.36±1.11	13.78±1.14	10.28±0.45	7.31±1.46	5.55±0.7	ND
AG4	6.55±0.53	10.86±0.93	4.76±1.04	ND	ND	ND
AG5	ND	24.53±0.79	31.13±0.61	20.07±0.49	18.69±1.00	ND
AG8	5.70±1.27	7.24±0.86	13.22±1.35	9.38±2.18	3.05±0.77	ND
FD5XI	ND	55.02± 0.81	17.44± 1.70	17.43±0.64	14.70±1.36	6.22± 0.44
RZ7	ND	59.60± 0.01	ND	ND	ND	ND
MI1B	ND	ND	ND	ND	ND	ND
FD1B	16.66±0.27	23.17±2.13	10.10±1.08	6.03±2.37	ND	ND

* ±SE: Standard error from mean.

Siderophore production

Qualitative analysis of siderophore production by the isolates was done on CAS agar medium. On the CAS agar medium plates, 24 isolates out of 43 could produce orange/pink halo zone around the colony indicating siderophore production on the medium. Isolate FD2A showed the highest siderophore production index (SPI) with 4.30±0.08 value, followed by AG1 (3.5±0.12), MI2C (3.2±0.06), MI2B (3.19± 0.04), MI1D (3.14±0.04), MI1E (3.1±0.03), AG5 (3.01±0.03), AG9 (3.01±0.43), MI1F (2.89±0.02), AG8 (2.7±0.09), FD4A (2.62±0.02), AG2 (2.6±0.02), FD4VI (2.6±0.03), AG6 (2.5±0.06), TSU3B (2.44±0.01), FD3III (2.4±0.07), TSU3C (2.33±0.07), AG4 (2.3±0.06), TSU3A (2.29±0.01), FD2X (2.1±0.03), FLALUM4 (2.09±0.02), RZ7 (1.90± 0.03), TSU2A (1.90±1.07) and FD3I (1.89±0.09) (**Table 6.1 and Figure 6.4, 6.5**). The CAS reagent is what gives CAS agar media its blue colour. The blue colour of the HDTMA complexes is evident when they are coupled to ferric iron. But the colour changes from blue to orange when the iron in this dye complex is removed by a strong iron chelator, such as a siderophore (Louden et al., 2011).As a result, the orange/pink

halo zone in this study was created by the fungal isolates' siderophore, which effectively extracted the iron from the dye.

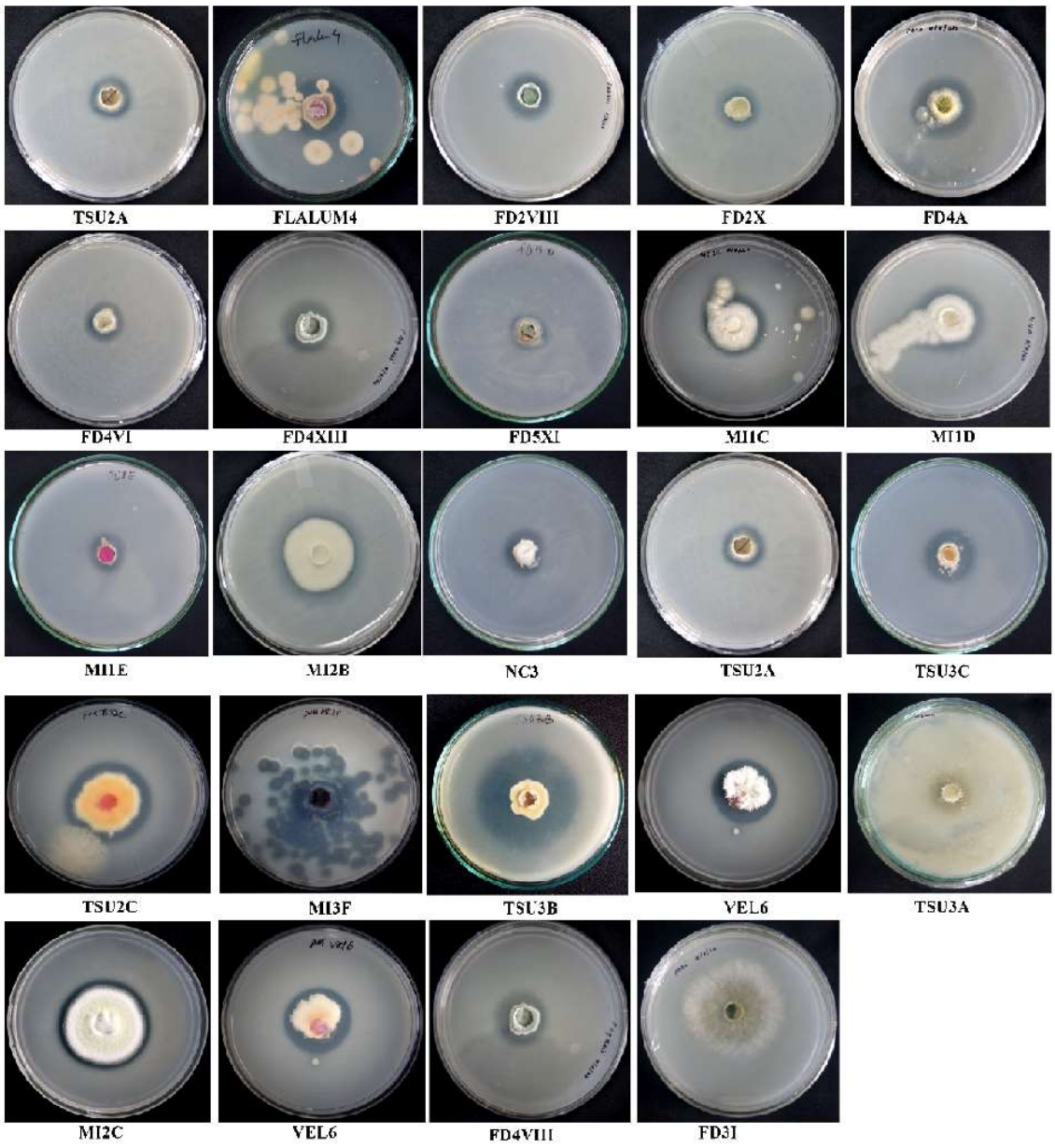

Figure 6.2: Phosphate solubilization test by the fungal isolates on NBRIP agar medium. Development of clear halo zone around the colony indicates the ability to solubilize phosphate by the isolates.

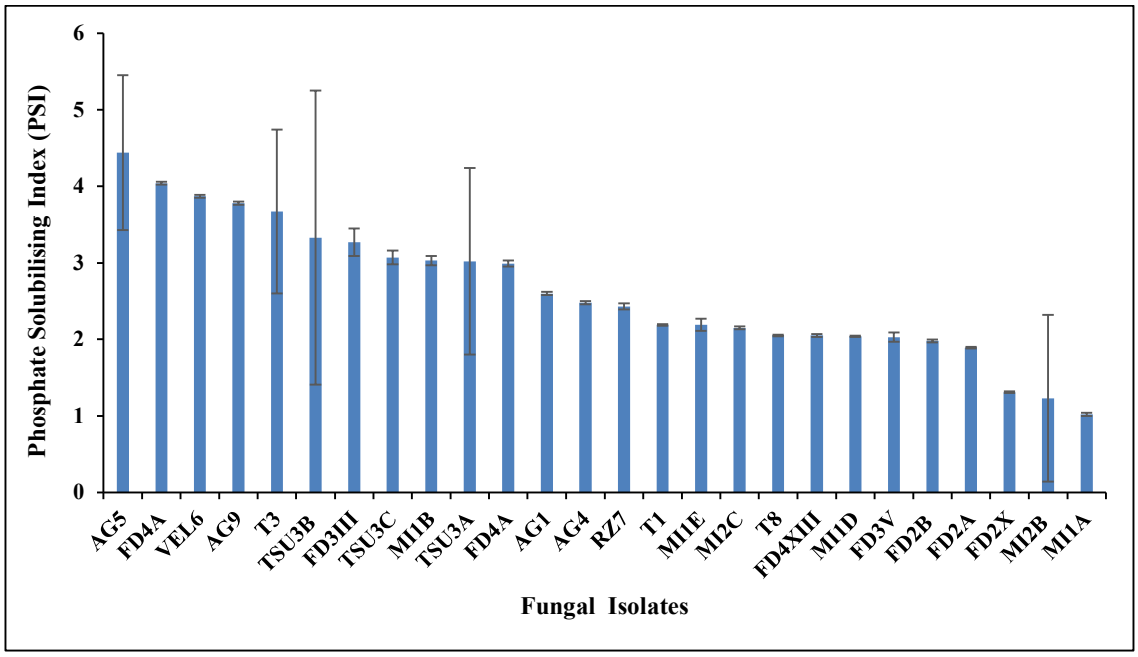

Figure 6.3: Phosphate solubilizing index (PSI) by the fungal isolates on NBRIP agar medium.

Figure 6.4: Siderophore production test by the fungal isolates indicated by development of orange/pink halo zone around the fungal colony.

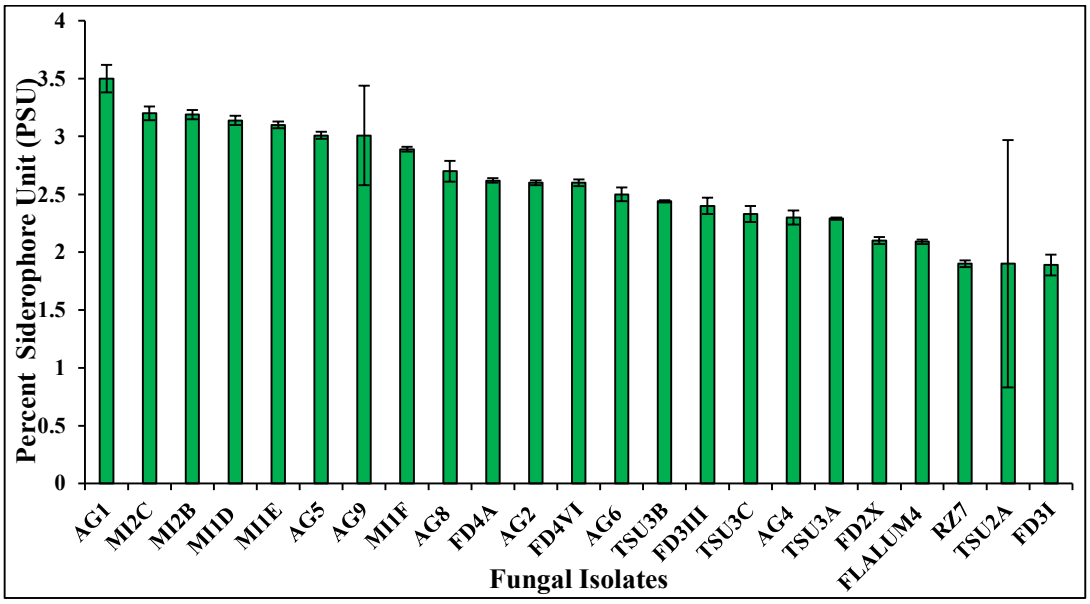

Figure 6.5: Percent siderophore unit by the fungal isolates.

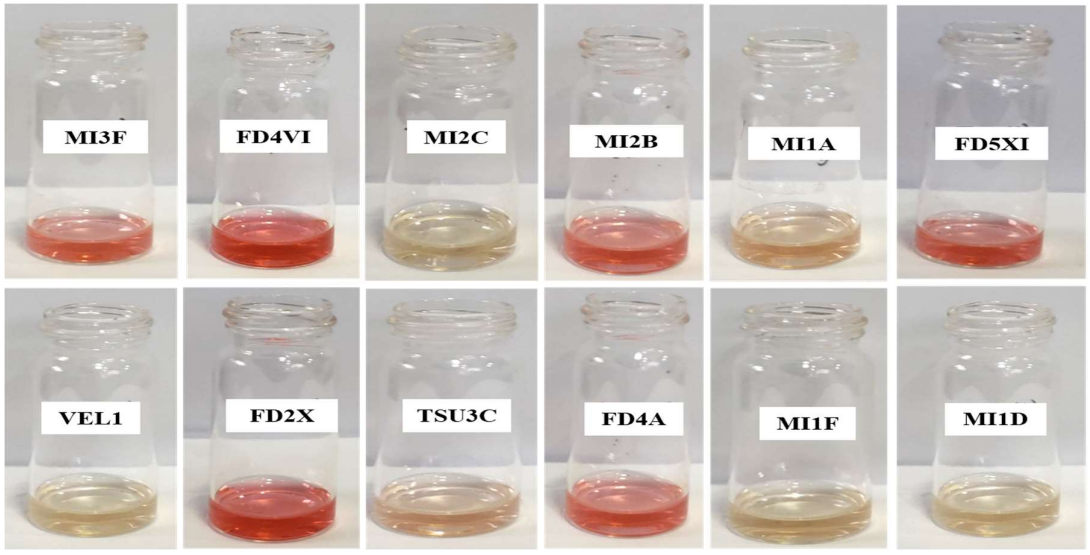

Figure 6.6: IAA production by the fungal isolates indicated by the development of pink colour after addition of Salkowski reagent. Colour change to dark pink, indication high IAA production while to faint pink, indicated moderate IAA production. No change in colour, indicating no IAA production.

Qualitative estimation of IAA production

All the 43 fungal isolates were screened qualitatively for IAA production. Of the 43 isolates, 17 isolates were tested positive for IAA production by producing pink colour after addition of the reagent to the culture supernatant (**Figure 6.6**). The positive isolates includeFD4XIII, FD2D, FD4VI, MI2B, FD2X, FLALUM4, FD3II, FD3III, FD3I, FD4A, T1, FD3V, T8, MI3F, MI1E, AG1 and FD5XI (**Table 6.1**).

Quantitative estimation of IAA production by the fungal isolates

Isolates which showed positive results for IAA production were further analyzed for quantitative analysis and IAA production was recorded from 2nd day till 12th day. Isolates were inoculated intonutrient broth supplemented with 0.1% L-tryptophan. Isolates started producing IAA at different time interval (**Table 6.3**). Most of the isolates had highest IAA production on the 8th day. Highest IAA production was shown by FD4XIII with 5.56±0.05µg/ml, followed by FD2D with 5.05±0.12µg/ml, on the 4th and 12th day respectively. For isolate FD4A, IAA production was detected from 6th day **(**1.34±0.13µg/ml), and on 8th day with highest production (1.67±0.09µg/ml) and till 10th day (0.89±0.05µg/ml). In case of isolates MI3F, FD3III, FD4VI and MI2B, IAA production was detected from 4th day **(**0.31±0.03µg/ml, 0.41±0.04µg/ml, 0.33±0.07µg/ml, and 0.24±0.05µg/ml respectively) and till 6th day with high IAA production on 6th day (3.24±0.05µg/ml, 4.23±0.05µg/ml, 3.34±0.08µg/ml and 0.99±0.04µg/ml respectively). Isolate FD5XI started producing IAA from 4th day (4.86±0.25µg/ml) and had the highest value calculated on that day. On the 6th day it was detected to be 3.89±0.14µg/ml and finally on the 8th day with 1.08±0.02µg/ml, further no IAA production was detected in the Media. For isolate FD4XIII, highest IAA was produced on the 4th day (5.56±0.05µg/ml), followed by the 2nd day (5.52±0.18µg/ml), 6th

day (4.99±0.12µg/ml), and on the 8th day (2.06±0.02 g/ml). In case of AG1, the highest IAA production was detected on the 10th day (1.09µg/ml), followed by 8th day (1.08±0.01 µg/ml) and on the 6th day (0.26±0.06 µg/ml). Isolates T8 and FLALUM4, had the highest IAA production on the 6th day (0.75±0.06 µg/ml and 0.44±0.02 µg/ml respectively) followed by on the 8th day (0.35 ±0.03 µg/ml and 0.07 ±0.02 µg/ml respectively). Isolate MI1E showed the highest IAA production on the 10th day (0.11±0.06 µg/ml), followed by the 8th day (0.04±0.07 µg/ml). Isolate FD3II showed highest IAA production on the 6th day (1.78 ±0.07 µg/ml), followed by 4th day (1.34 ±0.03 µg/ml) and 10th day (0.98 ±0.02 µg/ml). Isolate FD3V showed highest IAA production on the 8th day (3.24±0.05 µg/ml), followed by 6th day (0.31± 0.03 µg/ml) and 10th day (0.21±0.04 µg/ml). In case of isolate FD2X, highest IAA production was observed on the 8th day (0.09±0.05µg/ml), followed by 10th day (0.02±0.05µg/ml) and 12th day (0.01±0.02µg/ml). Isolate FD3I showed the highest IAA production on the 6th day (2.05 ±0.03µg/ml), followed by 8th day (1.03±0.02µg/ml), 10th day (0.08±0.01µg/ml) and 12th day (0.01±0.03µg/ml). In case of isolate FD2D, highest IAA production was detected on the 12th day (5.05±0.12µg/ml), followed by 8th day (2.43± 0.98µg/ml) and 6th day (1.45±0.12µg/ml). For isolates T1, highest IAA production was observed on the 8th day (1.23 ±0.04µg/ml), followed by 10th day (0.96±0.02µg/ml).

Ammonia production test

When screened for ammonia production by the fungal isolates, of the forty-three isolates, only eight isolates could produce large amount of ammonia indicated by the change of the Colour of the culture into brown colourand depicted by '+++'. The positive isolates were VEL1, MI1D, FD2D, FD4VI, FD3II, FD3III, FD3V and MI2C.While, moderate amount of the ammonia production was indicated by orange colour formation

and depicted with **'++'**.Nineteen isolates, which included VEl6, MI1F, MI3F, FD5VII, MI2B, FD2X, TSU3C, TSU2A, FD3I, FD2A, MI3F, MI1E, AG9, AG6, AG4, AG5, FD5XI, RZ7and FD1B produced moderate amount of ammonia in the media. Low amount of ammonia production was indicated by formation of yellow colourformation and depicted by giving **'+'**.Sixteen isolates were producing low amount of ammonia production which includes FD4XIII, TSU3B, FD2B, FD4D, TSU3A, FLALUM4, FD4A, T1, T3, MI1A, AG1, AG3, AG2, AG8 and MI1B (**Table 6.1 and Figure 6.7**).

Amylase production test

To check the amylase production by the fungal isolates qualitative analysis was done using iodine solution. From the amylase production test, it was found thatall the isolates were positive for α-amylase production. The isolates were able to hydrolyse starch in the medium which was indicated by the formation of halo zone around the fungal colony after adding iodine solution. The best performing isolates with halo zone forming in the media were T8, AG9, FD5XI, MI1D, AG3, MI1A, TSU3B, FD2B, FD1B, FD4D, FD4VI, FD5VII, AG6, TSU3A, MI2B, FD2X, FLALUM4, TSU2A,T1, FD2A, FD4XIII, RZ7, FD2D, MI1E) and other nineteen isolates were negative for the test which included MI1B, MI2C, AG5, AG2, NC3, T4, AG8, AG1, FD4A, T3, FD3V, VEL1, TSU3C, MI1F, AG4, VEL6) **(Table 6.1 andFigure 6.8.).**

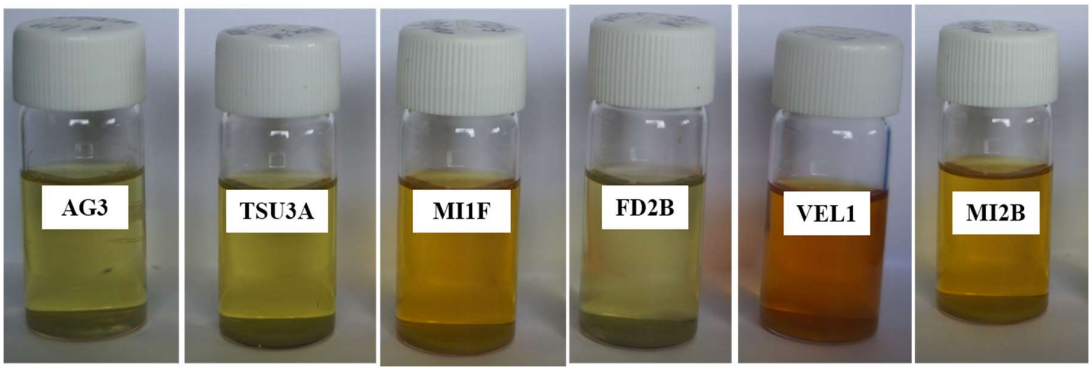

Figure 6.7: Ammonia production by the fungal isolates, where high amount of ammonia production was indicated by development of dark brown Colour, while moderate amount of ammonia production by development of orange Colour, and low amount of ammonia production was indicated by yellow Colour development of low amount of ammonia production**.**

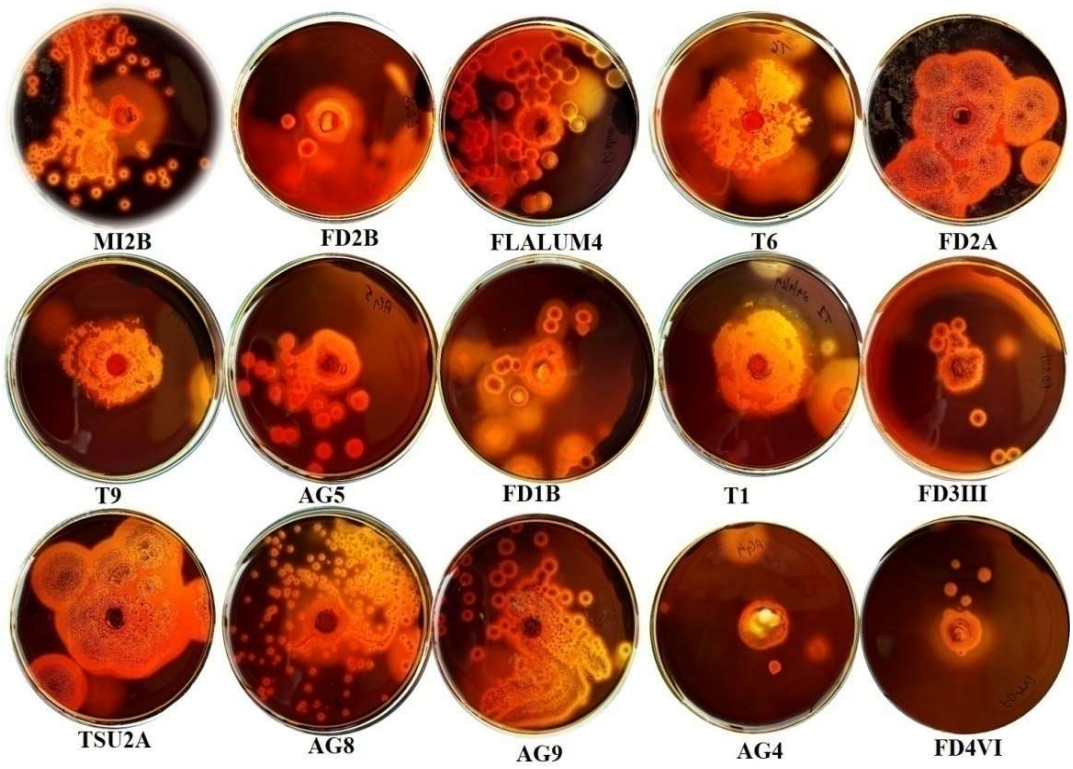

Figure 6.8: Amylase test by the fungal isolates. Development of clear halo zones around the fungal colonies on addition of iodine solution.

Table 6.3: IAA production by the fungal isolates on 2nd, 4th, 6th, 8th, 10thand 12th day.

Fungal Isolates	IAA Production (µg/ml)					
	2nd day	4th day	6th day	8th day	10th day	12th day
FD4A	ND	ND	1.34± 0.13	1.67±0.09	0.89±0.05	ND
MI3F	ND	ND	0.31± 0.03	3.24±0.05	ND	ND
FD5XI	ND	4.86± 0.25	3.89±0.14	1.08±0.02	ND	ND
FD4XIII	5.52± 0.18	5.56± 0.05	4.99±0.12	2.06±0.02	ND	ND
AG1	ND	ND	0.26±0.06	1.08±0.01	1.09 ±0.01	ND
T8	ND	ND	0.75±0.06	0.35 ±0.03	ND	ND
FD3III	ND	ND	0.41± 0.04	4.23±0.05	ND	ND
MI1E	ND	ND	ND	0.04±0.07	0.11±0.06	ND
FD4VI	ND	ND	0.33± 0.07	3.34±0.08	ND	ND
FD3II	ND	1.34 ± 0.03	1.78 ±0.07	0.98 ±0.02	ND	ND
FD3V	ND	ND	0.31± 0.03	3.24±0.05	0.21±0.04	ND
FD2X	ND	ND	ND	0.09±0.05	0.02±0.05	0.01±0.02
FD3I	ND	ND	2.05 ±0.03	1.03±0.02	0.08±0.01	0.01±0.03
FD2D	ND	ND	ND	1.45 ±0.12	2.43± 0.98	5.05±0.12
T1	ND	ND	ND	1.23 ±0.04	0.96 ±0.02	ND
MI2B	ND	ND	0.24 ±0.05	0.99 ±0.04	ND	ND
FLALUM4	ND	ND	0.44 ±0.02	0.07 ±0.02	ND	ND

* ±SE: Standard error from mean.

Molecular identification of the selected fungal isolates

Isolates were identified by performing a BLAST search analysis of the sequences of ITS (internal transcribed spacer) region. Fragments were amplified using Polymerase chain reaction (PCR) and generated by ITS1 and ITS4 primers. The amplified fragments were sequenced and blast with NCBI GenBank database. Nine isolates with plant growth promoting ability were selected for molecular identification and were identified as *Trichoderma atroviride* (AG1), *Trichoderma viride* (AG2), *Talaromyces purpureogenus* (AG9), *Clonostachys rosea* (T1), *Gilmaniella subornata* (FD2X), *Mucor fragilis* (MI1A), *Clonostachys rosea* (FD3V), *Aspergillus niger* (TSU3B) and *Trichoderma virens* (FD2D) with similarity 100%, 99.81%, 100%, 100%, 100%, 99.41%, 100%, 100% and 98.88% respectively (**Table 6.4 and Figure 6.9**).

Based on the sequences obtained a phylogenetic tree was constructed using MegaX software to show the evolutionary history between a set of species or taxa during a specific time and major transitions in the evolution.

Table 6.4: Molecular identification of the selected fungal isolates by 18S*rRNA* sequencing using ITS1 and ITS4 primers

Fungal Isolates	**Identification**	**GenBank Accession No.**	**Query Cover (%)**	**Percent Identity (%)**	**E-value**
AG1	*Trichoderma atroviride*	PP474513	100	100.00	0.0
AG2	*Trichoderma viride*	PP460514	89	99.81	0.0
AG9	*Talaromyces purpureogenus*	PP474455	100	100.00	0.0
T1	*Clonostachys rosea*	PP474559	98	100.00	0.0
FD2X	*Gilmaniella subornata*	PP474570	100	100.00	0.0
MI1A	*Mucor fragilis*	PP474579	100	99.41	0.0
FD3V	*Clonostachys rosea*	PP474658	100	100.00	0.0
TSU3B	*Aspergillus niger*	PP474432	97	100.00	0.0
FD2D	*Trichoderma virens*	PP460553	95	98.88	0.0

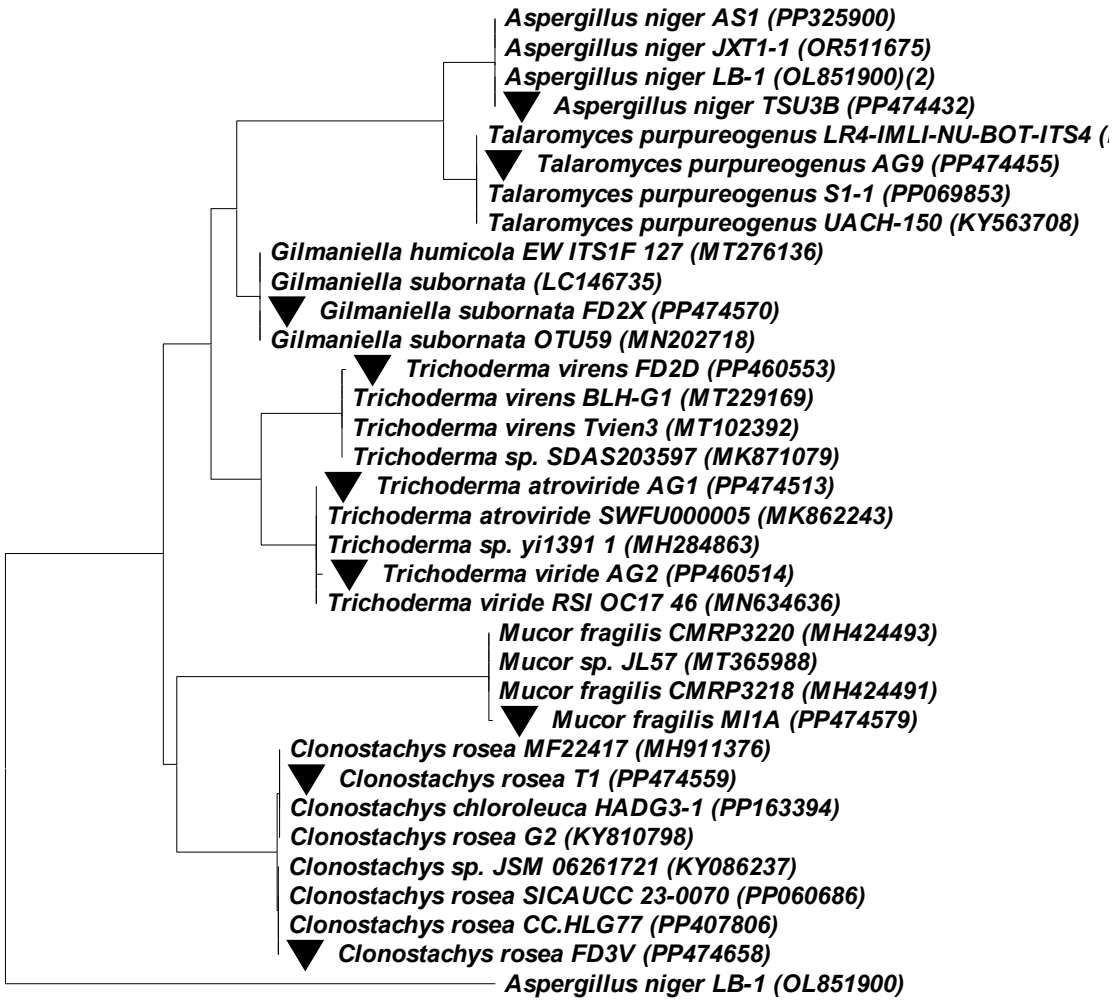

Figure 6.9: The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model [1]. The tree with the highest log likelihood (-3589.21) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura 3 parameter model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 34 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 638 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [2].

Discussion

Chemical fertilizers are costly and raise the cost of production, but they also present health risks and have an adverse effect on the microbial population in the soil by deteriorating the physical structure of the soil and causing a shortage of oxygen in the plant root zone (Mahadevamurthy et al., 2016). Recent years have seen the patenting and registration of a number of mycofungicides for the management of plant diseases, as well as the registration of fungal biofertilizers for use in crop production (Kaewchai et al., 2009). The hardest thing to do is rehabilitate the cultivable lands because most of them have already been contaminated by artificial fertilizers and pesticides due to the rapid population growth (Tatung and Deb, 2021).

According to reports, phosphate solubilizing fungi not only supply phosphorus but also significantly increase the production of nitrogen and compounds that stimulate plant growth in the rhizosphere (Kucey et al., 1998). Additionally, it was discovered that co-inoculation of Penicillin and *Aspergillus* species lengthens shoots. Similar results were also reported (Mittal et al., 2008). The increase in shoot and root length contributes to increased solubilization of phosphorous generated by the PSF's secretion of enzymes and organic acid that are used by cell proliferation division and enlargement. In their most recent research, Hao et al. (2021) also found that, in bleach plum environments under salt stress, arbuscular mycorrhizal fungi and phosphate solubilizing fungi increase nutrient absorption, improve gasses exchange, and improve the chl fluorescence parameter. PSF *Penicillium oxalicum* strain ZP6 efficiently bioremediated Cd pollution in phosphate mining marsh, according to research by Zheng et al. (2022). According to a recent study, co-inoculation of *P. oxalicum* and tricalcium phosphate efficiently increases the concentration of urea and accessible phosphate in red soil that has been intentionally contaminated with lead. By increasing soil AP concentration, it also reduces Pb

bioavailability, bio-accessibility, leaching ability, and mobility (Hao et al., 2022). Phosphate solubilisation is noticeably higher in Pikovskaya's medium containing tricalcium phosphate (TCP) than in media containing rock phosphate, according to Elias et al. (2016).

In the current investigation, 43 fugal isolates in total were extracted from the mixed culture and subsequently screened for the generation of siderophores on CAS agar medium, phosphates on NBRIP media, IAA on Salkowski reagent, ammonia and amylase production.

According to Walpola and Yon (2013), phosphate is one of the most important micronutrients for plant cell development, root formation, flowering, fruiting, and seed production, as well as accumulation and energy release. Plant growth disruption, particularly in fine roots, is caused by phosphorus deprivation. Phosphate must first be transformed into a simpler form before plants can utilize it, as they can only absorb it in soluble form (Elfiati et al., 2021). One of the essential macronutrients for plant growth and development is phosphate, and phosphate-solubilizing fungi (PSF) in the soil can increase plant bioavailability of this nutrient (Kumar et al., 2020). A large amount of P is applied as fertilizer worldwide improving soil fertility, which enters into immovable pools due to precipitation response with extremely reactive Al and Fe in acidic soil and Ca in calcareous or normal soil (Chittora et al., 2020). Reportedly, a wide range of soil fungi are reported to solubilize insoluble phosphorous such as *A. niger* and *Penicillium* sp., which are the most common fungi capable of phosphate solubilization (Mahadevamurthy et al., 2016). Hence these phosphate solubilizing fungi can be used to make the phosphate available the plants and help in increased crop production. Fungi have been reported to have greater ability to solubilize insoluble phosphate than bacteria.

In the present study, when screened for phosphate solubilization, thirty isolates were able to produce clear halo zone around the fungal colony in the NBRIP agar media. However, when tested in the liquid NBRIP media thirty-eight isolates showed phosphate solubilizing ability. similarly, in many other reports, PSM have shown to solubilize phosphate more efficiently in liquid media than in solid media suggesting testing on liquid media more reliable (Nautiyal., 1999). Different isolates start solubilizing phosphate in the liquid media in different time interval. However, most of the isolates produced the highest on the 4^{th} day number being thirty isolates; four isolates produced highest solubilization on the 6^{th} day and three isolates produced highest on the 2^{nd} day. Several phosphate solubilizing fungi isolates belonging to genera *Aspergillus sp., Acremonium roseolum, Cladosporium* sp*., Fusarium* sp*., Penicillium* sp. and *Trichoderma* sp*., Talaromyces* sp. were identified to produce clear halo zone around their colonies (Arias et al., 2023). Phosphate solubilizing fungi *Aspergillus* sp. also produces important growth regulator such as indole acetic acid, which are widely used in fermentation process including producing organic acid and also in producing enzymes (Reddy et al., 2014). Das et al. (2013), while studying the mangrove isolate *A. niger* MPF-8 has concluded that *A. niger* have the ability to convert insoluble tri calcium phosphate to soluble form which was greatly enhanced by incorporation of glucose and ammonium sulphate in the medium.

In addition to the synthesis of IAA by the plant, microorganisms can also influence the auxin level of plants through de novo biosynthesis of IAA. In order for microbes to produce IAA, they must either reside within a host plant or take up the IAA through the roots (Jahn et al., 2021). Seventeen isolates, including FD4XIII, FD2D, FD4VI, MI2B, FD2X, FLALUM4, FD3II, FD3III, FD3I, FD4A, T1, FD3V, T8, MI3F, MI1E, AG1 and FD5XI, were shown to be capable of producing IAA when screened for

IAA production. However, a variety of environmental elements, such as temperature and pH level, can affect the production of IAA (Fu et al., 2015). IAA facilitates the fungi's root colonization of the plant. When *Aspergillus awamori* was inoculated onto maize, the plant growth was promoted. IAA was found to be important in colonizing the root, as seen by lower root colonization when IAA production was suppressed, and increased root colonization when IAA was applied (Mehmood et al., 2019).

Microorganisms have developed extremely specialized pathways that use siderophores, which are low molecular weight iron chelators, to meet their nutritional needs for iron. According to Andrews et al. (2003), these siderophores are produced in order to solubilize iron from their surroundings and create a complex ferric siderophore that can migrate by diffusion and return to the soil surface. In the present study, twenty-four isolates were capable of producing the siderophores which was indicated by an orange/pink halo zone around the fungal colony, as shown in **Table 6.1**. According to reports, fungi that produce siderophores can boost plant development when the amount of iron in the plant is limited. Iron-sensitive Fur proteins, the global regulators GacS and GacA, the sigma factors RpoS, PvdS and FpvI, quorum-sensing auto inducers such N-acyl homoserine lactone, and site-specific recombinases are generally responsible for the strict regulation of siderophore production (Saraf et al., 2014).

Ammonia production is another important feature of fungi helps in the plant growth by making nitrogen available to the plant (Tatung and Deb, 2023). Additionally, ammonia can supply plants with a sufficient of ammonia required for root and shoot elongations and consequently promote plant growth (Khalil et al., 2021). Herein, all isolated fungal strains isolated from *Musa* rhizosphere had the ability to produce ammonia with a varying degree after adding Nessler’s reagent to broth media as indicated in the **Table 6.1**. While testing, large amount of ammonia production was indicated by

the change of the Colour of the culture into brown Colour which includes VEL1, MI1D, FD2D, FD4VI, FD3II, FD3III, FD3Vand MI2C. Other 19 isolates produced moderate amount of the ammonia production which included (VEl6, MI1F, MI3F, FD5VII, MI2B, FD2X, TSU3C, TSU2A, FD3I, FD2A, MI3F, MI1E, AG9, AG6, AG4, AG5, FD5XI, RZ7 and FD1B). Rest sixteen isolates showed low amount of ammonia production (FD4XIII, TSU3B, FD2B, FD4D, TSU3A, FLALUM4, FD4A, T1, T3, MI1A, AG1, AG3, AG2, AG8 and MI1B).

Amylases are among the most important hydrolytic enzymes, used extensively in various industries, from food to pharmaceuticals (Ahmed et al., 2020). These enzymes degrade starch by specifically cleave the α-glycosidic linkage in starch (Saleem and Ebrahim, 2014). Most amylases are produced by soil fungi such as *Aspergillus, Penicillum* and *Rhizopus* (Sunitha et al., 2012). Amylase-producing fungi solubilize and utilize starch, co-mineralize nitrogenous compounds to enhance their availability to plants in the rhizosphere (Imran et al., 2021). In the resent study, twenty-four isolates fungal isolates tested positive for amylase production, using starch plate method. This was indicated by a clear zone of starch hydrolysis in the Petri dishes after iodine treatment. The positive isolates included T8, AG9, FD5XI, MI1D, AG3, MI1A, TSU3B, FD2B, FD1B, FD4D, FD4VI, FD5VII, AG6, TSU3A, MI2B, FD2X, FLALUM4, TSU2A, T1, FD2A, FD4XIII, RZ7, FD2D, MI1E. Nineteen isolates were negative for the test, including MI1B, MI2C, AG5, AG2, NC3, T4, AG8, AG1, FD4A, T3, FD3V, VEL1, TSU3C, MI1F, AG4 and VEL6. This indicates higher fungal community with starch hydrolyzing ability in the *Musa* rhizosphere. In the study by Saleem and Ebrahim (2014), *A. niger* and *R. stolonifer* were the most active in producing amylase from the seeds of five different beans. Similar findings were reported by Galeano et al. (2021), with *A.*

niger being the best isolates producing amylase and exhibiting other plants growth promoting traits, enhancing growth of common bean.

Among the molecularly identified isolate's genera the highest number of isolates was represented by *Trichoderma* sp. with the total number of three isolates including *T. atroviride* (AG1), *T. viride* (AG2) and *T. virens* (FD2D). *Trichoderma* species are free-living fungus that helps plants by promoting their growth and ability to withstand illness in both the rhizosphere and the aerial portions of the plant (Guo et al., 2020). Because of their well-known biological control mechanism and reputation for boosting crop development and productivity, *Trichoderma* spp. have found widespread use in agricultural applications (Zin and Badaluddin, 2020). During interactions with other organisms, they establish communication via various molecules, including effectors proteins. One such effector was discovered to enhance *Trichoderma'*s mycoparasitic ability or modify plant physiology to colonize plant roots (Guzmán-Guzmán et al., 2024). *T. virens* (FD2D) produced a considerable amount of ammonia and demonstrated a positive outcome for IAA generation in the current investigation. On the other hand, *T. viride* (AG2) and *T. atroviride* (AG1) both had the ability to solubilize phosphate, create IAA, siderophores, and minimal amounts of ammonia. According to a Bedine et al., (2022), study, when compared to untreated common beans, *Trichoderma* sp. dramatically increases phosphate uptake, photosynthetic pigment, and overall protein content.

Many reports have revealed that *Trichoderma* sp. played a significant role in high stress environmental conditions and provided host species with abiotic stress (tolerance). *Trichoderma citronoviridae* demonstrated the greatest ability to absorb Cu in a study by Liaquat et al. (2020b). However, *Trichoderma reesei* was able to withstand elevated Pb levels. With a total of two isolates, both of which were *Clonostachys rosea*, *Clonostachys* sp. was the second-highest isolate genera found in the present study. The results of this

investigation indicated that *C. rosea* produces a good amount of ammonia, phosphate solubilization and IAA.

Other genera of isolates that were found were *Gilmaniella* sp., *Aspergillus* sp., *Mucor* sp. and *Talaromyces* sp. *Gilmaniella subornata* (FD2X), *Aspergillus niger* (TSU3B) and *Mucor fragilis* (MI1A) each had one isolate. When examined, *Talaromyces purpureogenus* (AG9) produced a little amount of ammonia and siderophore and shown the ability to solubilize phosphate. Similar findings were discovered by Sun et al. (2023), who reported that *T. purpureogenus* significantly increased the dry weight of the shoots (37.93%), roots (31.25%) and plant height (13.03%) for low-P sensitive inbred line when co-cultivated on different genotypes of maize seedlings, but not for the low-P tolerance inbred line. Furthermore, for both inbred lines, it also markedly raised the total P-concentration in the roots (3.10%~9.77%) and shoots (22.4%~32.9%).

In the present investigation, it was discovered that *A. niger* (TSU3B) could solubilize phosphate, produce siderophores, and produce little ammonia. One of the most widely employed PGPFs in sustainable agriculture methods is *A. niger*, which is also utilized in the food industry to produce a variety of enzymes and metabolites, including citric acid (Galeano et al., 2021). A study by Klaic et al. (2021) found that using *A. niger* as a biofertilizer increased the production and nutrient uptake in *Lolium multiflorum* Lam. According to a different study on chickpeas, *A. niger* significantly improved plant growth by demonstrating strong inhibitory effect against *Fusarium oxysporum* f. sp. Ciceris through the production of organic acids (Nayak and Vibha, 2017).

G. subornata (FD2X) in the present study was found positive for all the screening (IAA production, siderophore production, phosphate solubilization and moderate amount of ammonia production). *G. subornata* has shown promising activities in plant growth promotion (Wang et al., 2012; Yuan et al., 2016). In a report by Jamil et al. (2023), it was

found that healthy rhizospheric soil of *Musa* had high population of fungal community of *Penicillium* sp. As compared to soil infected with *Fusarium* wilt which might indicate the antagonistic activity of the *Penicillium* and maintain a healthy plant.

In the present study, *M. fragilis* (MI1A), could only solubilize phosphate and produce low amount of ammonia production. Currently, there are relatively few studies on *M. fragilis*on plant growth and promotion. However, some studies have reported its significant involvement in changes in the primary and secondary metabolites (Xu et al., 2021). Additionally, it also produces two key pharmaceutical agents; podophyllotoxin and kaempferol which may help protect the overexploitation of endangered plant *Podophyllum* sp. which is highly endangered for its production of these compounds (Huang et al., 2014). In another study, *Mucor* sp. enhanced the plant height, tuber weight and root fresh weight of Potato plants infected with juveniles of *Meloidogyne* spp. (Utari et al., 2018).

The present finding established thatrhizospheric soil sample collected from wild *Musa* sp. of Nagaland support various phosphate solubilizing, IAA producing, siderophore producing and ammonia producing fungi. These properties can enhance plant growth and development.

Conclusions

Plant growth promoting fungi is a great alternative to chemical fertilizers to allow the plants to take nutrients available in the soil which usually are present in the soil as insoluble form. The PGPF are also environmentally friendly, less expensive and more convenient as compared to chemical fertilizer.In this study, we have isolated few fungal isolates including *Trichoderma atroviride* (AG1), *Trichoderma viride* (AG2), *Talaromyces purpureogenus* (AG9), *Clonostachys rosea* (T1), *Gilmaniella subornata* (FD2X), *Mucor fragilis* (MI1A), *Clonostachys rosea* (FD3V), *Aspergillus niger* (TSU3B)

and *Trichoderma virens* (FD2D) from wild *Musa* species growing in the jungle of Nagaland. These isolates showed the potential to act as biofertilizer for sustainable agriculture system which included production of IAA, siderophore, amylase, ammonia, and phosphate solubilization. However, to properly measure the effect of the isolated fungi inoculation in the plants is required before introducing into the fields.

Chapter – 7

Effect of Selected PGPF Strains on Plant Growth Promotion

Introduction

Environmental disruptions and public health concerns have been linked to rapid urbanization, dwindling agricultural areas, significant climate change and extensive use of agrochemicals in agricultural practices (Kumar et al., 2022). One important tool for reducing this problem is biofertilizer (Tatung and Deb, 2021). Microbial strains function as biofertilizers by using a variety of mechanisms, including nitrogen fixation, potassium and phosphorus solubilisation, excretion of phytohormones, production of substances that suppress phytopathogens, protection of plants from abiotic and biotic stresses and detoxification of subsurface pollutants, to improve nutrient uptake, improve soil fertility, and increase crop yields (Mącik et al., 2020). Rhizospheric fungus are a notable group of microbiota colonizing the rhizosphere, however their exploration is not as extensive as that of rhizospheric bacteria (Pattnaik and Busi, 2019). Plant growth promoting fungi (PGPF) are non-pathogenic soil-dwelling fungi, and studies have shown that plants benefit greatly from their connections with multipurpose PGPF (Pandya and Saraf, 2010;

Hossain et al., 2017). The growth of plant can be promoted by direct mechanisms like fixation of atmospheric nitrogen (Mohamed et al., 2022), solubilisation of minerals such as phosphate (Bashan et al., 2013), stress tolerance (Iram et al., 2012; Manzoor et al., 2019), inducing systemic resistance (Hossain et al., 2017), and production of plant growth regulators like auxins (Mehmood et al., 2019), gibberellins (Salazar-Cerezo et al., 2018), cytokinins (Anand et al., 2022) and ethylene (Chagué, 2010).

According to Fu et al. (2015), IAA is the most prevalent in plants and controls several facets of plant growth and development. Fungal isolates that produce IAA have also shown to be quite effective at assisting plants in surviving in soil contaminated with heavy metals (Ikram et al., 2018). Certain fungal isolates have the capacity to create ammonia, which can further stimulate plant development (Murali et al., 2012). Fungiplay a crucial part in ammonification, the process by which nitrogen elements are further cycled, as evidenced by their ability to produce ammonia (Imran et al., 2021).

It is known that fungi are better at solubilising phosphate than bacteria are (Nahas, 1996). Murali et al. (2012) found that applying conidial suspension and culture filtrate of phosphate-solubilising fungus *Penicillium* sp. to crops significantly improved the plants' vigour and seed germination of pearl millet. According to Yadav et al. (2011a), *Aspergillus niger* is a fungal species that possesses a high solubilisation of phosphate, making it a promising agent for biofertilizer and biocontrol. In a recent study, the inoculation of *Penicillium oxalium* with phosphate-solubilising fungi in rock phosphate supplemented alkaline soil increased the phosphate level and wheat and maize production. According to Singh et al. (2011), PSF *P. oxalicium* also improves the phosphate concentration of the soil. According to a recent study, using *Aspergillus niger* as a biofertilizer increases plant production and phosphorus uptake when several PSF inoculants were employed (Wang et al., 2015).

Numerous microorganisms produce siderophores, which are categorized into four primary classes (catecholates, hydroxamates, carboxylates, and mixed type) based to their ligand types, functional groups, and structural characteristics (Arora and Verma, 2017). The two main siderophore kinds that are produced by fungi are hydroxamate and carboxylate and these have mostly been researched in *Aspergillus species*. As an example, *A. fumigatus* and *A. nidulans* synthesize over fifty-five different kinds of siderophores (Pecoraro et al., 2021). According to Huschka et al. (1985), data on absorption kinetics imply that specific recognition of the different siderophores is necessary for siderophore transport into fungal hyphae.

Aspergillus spp., *Penicillium* spp., and their combination were found to significantly boost plant development in a recent Mung bean study when compared to the uninoculated control group (Vibha et al., 2014). *Aspergillus fumigatiaffinis*, *Chaetosphaeronema achilleae*, *Alternaria botrytis* and *Botryotrichum atrogriseum* were infected on wheat in a different study, and the results showed improved growth and development compared to the control group (Mohamed et al., 2022). *Aspergillus awamori* also considerably inhibited the growth of *V. dahliae* and *P. drechsleri*. Moreover, *Paraconiothyrium flavescens* dramatically boosted the root and shoot growth of wheat seeds, demonstrating the biocontrol agent's efficacy (Rezvani., 2020). With a maximum improvement and protection against *Colletotrichum capsici* of 78.75%, *Talaromyces* sp. NBP-61 demonstrated a considerable improvement in seed and plant growth metrics (Naziya et al., 2019).

With the aforementioned information in mind, the following objectives were set forth in this chapter for study the effects of some of the selected PGPF isolates on the promotion of two different test plants.

Materials and Methods

Effect of selected PGPF isolates on growth of different crops plants

The PGPF isolates with various growth promoting traits were selected and inoculated on different plants including *P. vulgaris* L. and *B. vulgaris* L. to see their effect on the plant growth parameters (shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight).

Selection of fungal strains for inoculation

Fungal isolates exhibiting plant growth promoting abilities were selected for plant inoculation onto *B. vulgaris* L. and *P. vulgaris* L. The isolates selected were *Trichoderma atroviride* (AG1), *Trichoderma viride* (AG2), *Talaromyces purpureogenus* (AG9), *Clonostachys rosea* (T1), *Gilmaniella subornata* (FD2X), *Mucor fragilis* (MI1A), *Clonostachys rosea* (FD3V), *Aspergillus niger* (TSU3B) and *Trichoderma virens* (FD2D). All the isolates were inoculated onto the plants singly and effect of its inoculation on the above-mentioned plants were analyzed after 30 days of plantation**.**

Seed sterilization and inoculation with fungal suspension

Seeds were surface-sterilized with 70% ethanol for 1 min followed by 2 % sodium hypochlorite for 2 min, and washed thoroughly 7 times with sterilized deionized water. From the last washings, 100µL of the aliquot was checked for the presence of microbial contamination and there was no microbial growth, indicating the complete surface sterilization of the seeds. Sterilized seeds were then immersed in eachfungal suspension for 3½ h in shaking conditions at room temperature. After seeds inoculation with PGPF strains, seeds were then sown in pots containing mixture of soil and sand in 1:1 ratio. Plantlets in each pot were later trimmed to 3 plantlets.

Inoculums preparation and pot experiments

Fungal inoculantswere prepared by inoculating mycelial plugs of selected fungal

strains into 50 ml of Sabouraud dextrose broth media, followed by a 3-day incubation in a shaking incubator at 28±2ºC. Subsequently, each fungal isolate was introduced into individual pots containing 3 plantlets each of *B. vulgaris* L. and *P. vulgaris* L. The pots were regularly watered with autoclaved tap water. A control treatment without fungal inoculation received only autoclaved tap water. Selected isolates were evaluated for their impact on the growth and development of the two crop plants. After one month, plants were harvested, and various growth parameters such as shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight were measured.

Inoculation of PGPF strains as individual inoculums on *Beta vulgaris* L.

Eight PGPF strains were selected and inoculated on to *B. vulgaris* L**.** singly. The isolates selected for this experiment were *Clonostachys rosea* (FD3V)*, Trichoderma atroviride* (AG1)*, Trichoderma viride* (AG2)*, Talaromyces purpureogenus* (AG9)*, Aspergillus niger* (TSU3B)*, Gilmaniella subornata* (FD2X)*, Clonostachys rosea* (T1)*, Trichoderma virens* (FD2D). The effects of the investigated rhizofungal isolates on plants growth of model plant *B. vulgaris* L. in pot experiment were recorded**.** For each treatment, 30 seeds were sown in each potand later trimmed to three plantlets each. Experiments were repeated thrice. Seedlings were harvested after 30 days of sowing and morphological characteristics were determined. For the purpose, vegetative characteristics including shoot length, root length, root and shoot fresh biomass, and dry biomass were measured after uprooting all the plantlets. Under the control condition, these metrics were compared with PGPF treated and untreated plantlets. Booster shots were administered two times a week and plantlets were watered regularly with autoclaved sterilized water.

Inoculation of PGPF strains as individual inoculum on *Phaseolus vulgaris* L.

Seven PGPF strains were selected and inoculated on to plant singly. The isolates selected were *Trichoderma atroviride* (AG1), *Trichoderma viride* (AG2), *Clonostachys rosea* (T1), *Aspergillus niger* (TSU3B), *Mucor fragilis* (MI1A), *Trichoderma virens* (FD2D), *Gilmaniella subornata* (FD2X). The effects of the investigated rhizobacterial isolates on plants growth of model plant *P. vulgaris* L. in pot experiment. For each treatment, 30 seeds were sown in each pot and experiments were repeated thrice and later trimmed to three plantlets each. Once the seedlings had been kept for thirty days, their morphological features were identified and recorded. After removing all of the plantlets, measurements were taken of the vegetative properties, such as shoot length, root length, fresh biomass from the roots and shoots, and dry biomass. These measurements were compared with plantlets that were treated with PGPF and those that were not under the control condition. Plantlets received two weekly booster doses and regular irrigations with autoclaved, sterilized tap water.

Statistical Analysis

The SPSS software was used for statistical analysis of the experimental data. All the reported results are the mean of the three replicates and deviations were calculated as the standard error of the mean (SEM). For assessing the importance treatment effect was done following one-way ANOVA and Least Significance Test (LSD) at the 0.05 level of confidence was used to compare means in cases where the F values were significant. When the p value was ≤ 0.05, differences were deemed significant.

Results

The PGPF strains used in this investigation were extracted from the rhizosphere of *Musa* plants that were growing in the jungle of Nagaland. After PGPF species were screened for growth-promoting characteristics, several species were found. Following the

molecular characterization of the chosen fungal isolates, two experiments were set up and two agricultural crop plants were inoculated.

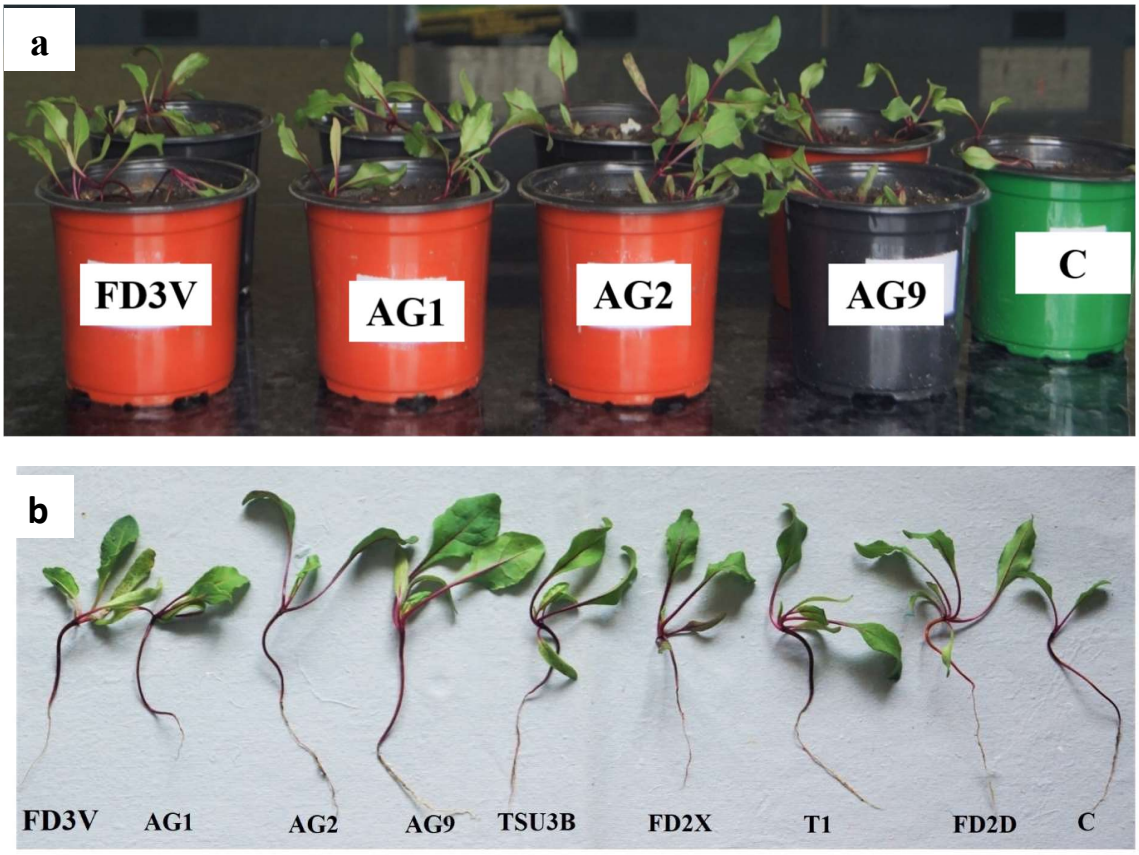

Figure 7.1: a. Pot inoculation of *Beta vulgaris* L. with PGPF strains. **b.** Effects of PGPF inoculation on beet root (*Beta vulgaris* L.) growth parameters such as root lengths, shoot length, shoot fresh, and shoot dry weight over controlled treatment. The fungal isolates (*Clonostachys rosea* (FD3V), *Trichoderma atroviride* (AG1), *Trichoderma viride* (AG2), *Talaromyces purpureogenus* (AG9), *Aspergillus niger* (TSU3B), *Gilmaniella subornata* (FD2X), *Clonostachys rosea* (T1), *Trichoderma virens* (FD2D), Control (C). showed promote and increase in (a) (uninoculated pot).

The following PGPF strains were used in the first pot trial: FD2X (*Gilmaniella subornata*), T1 (*Clonostachys rosea*), AG9 (*Talaromyces purpureogenus*), AG1 (*Trichoderma atroviride*), AG2 (*Trichoderma viride*), TSU3B (*Aspergillus niger*), FD3V (*Clonostachys rosea*), and FD2D (*Trichoderma virens*) (**Figure 7.1**). After 30 days of

plantation, platelets were uprooted, and plant growth parameters were recorded. Highest root length was observed in the treatment with AG9 (*Talaromyces purpureogenus*) with 7.70 cm root length, followed by T1(6.67cm), FD2D (6.50cm), AG1 (6.20cm), AG2 (5.83cm), TSU3B (5.33cm), FD3V (4.37cm) and Control (4.33cm). The lowest root length was observed in treatment with FD2X. However, significant increase in root length was observed only by AG9, T1 and FD2D. In case of shoot length, highest increase was observed in AG9 (13.27cm), followed by FD3V (12.03cm), FD2D (11.87cm), AG1 (11.63cm), AG2 (11.50cm), T1 (10.87cm), TSU3B (10.73cm) and Control (9.03cm). The lowest shoot length was observed by FD2X (7.40cm). Only FD3V, AG9, AG1, AG2 and FD2D showed significant enhancement in shoot length ($p \leq 0.05$). In regards to shoot fresh weight, the highest value was observed by AG9 (0.87g), followed by T1 (0.77g), FD3V (0.64g), FD2D (0.56g), TSU3B (0.55g), AG2 (0.50g), AG1 (0.47g), FD2X (0.31g) and CONTROL (0.14g). All the treatments showed significant increase in shoot fresh weight. Finally shoot dry weight was highest in treatment with T1 (0.48g), followed by AG9 (0.33g), TSU3B (0.31g), AG2 (0.26g), AG1 (0.24g), FD2D (0.24g), FD2X (0.14g), FD3V (0.08 g) and CONTROL (0.02g). Significant increase in shoot dry weight was observed in the treatment AG9, TSU3B, and T1. In case of root fresh weight AG9 showed the highest root length (0.25±0.01g), followed T1 (0.23±0.01g), FD2D (0.18±0.01g), AG1 (0.18±0.02g), TSU3B (0.16±0.01g), AG2 (0.13±0.01g), FD3V (0.15±0.01g), FD2X (0.08±0.01g) and control (0.08±0.01g). All the isolates showed significant growth in the root fresh weight over control treatment ($p \leq 0.05$). Whereas, in case of root dry weight highest value was observed by T1 (0.09±0.02g), followed by AG9 (0.08±0.01 g), FD2D (0.05±0.01 g), AG1 (0.05±0.01 g), FD3V (0.03±0.01 g), AG2 (0.03±0.01 g), TSU3B (0.03±0.01 g), FD2X (0.02±0.01g) and control (0.02±0.01g).

However, T1 and AG9 have shown significant growth over control ($p \leq 0.05$) (**Figure 7.2. and Table 7.1**).

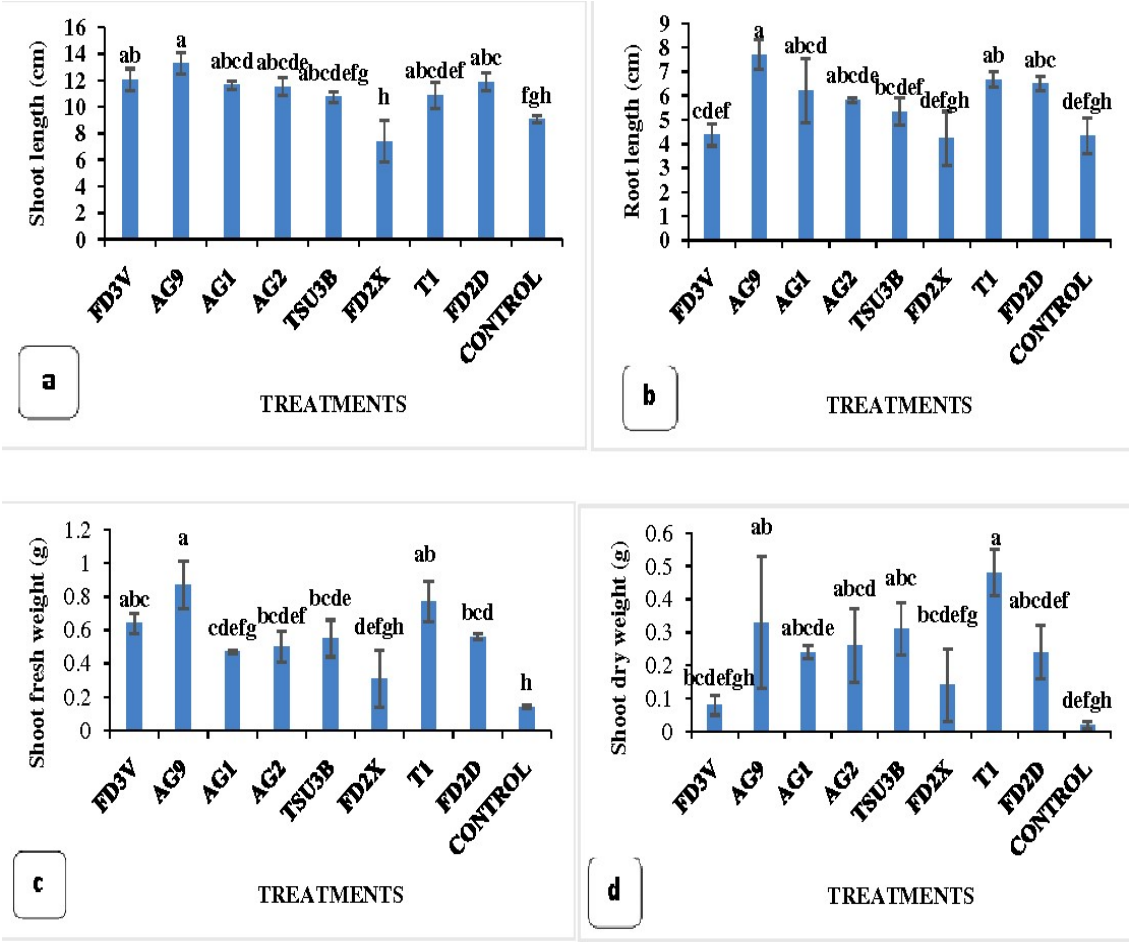

Figure 7.2: Effects of different PGPF isolates [(*Trichoderma atroviride,* FD3V), (*Talaromyces purpureogenus,* AG9), (*Trichoderma atroviride,* AG1), (*Trichoderma viride,* AG2), (*Aspergillus niger,* TSU3B), (*Gilmaniella subornata,* FD2X), (*Clonostachys rosea,* T1), and (*Trichoderma virens,* FD2D)] on the growth promotion of *Beta vulgaris* L.(**a**. Shoot length, **b.** Root length, **c.** Shoot fresh weight and **d.** Shoot dry weight).

Table 7.1: Effect of PGPM inoculation on growth parameters of *Beta vulgaris* L.

Test Plants	PGPF Isolates	Root Length (cm) *±SE	Shoot Length (cm) *±SE	Shoot Fresh Weight(g) *±SE	Shoot Dry Weight (g) *±SE	Root fresh weight (g) *±SE	Root dry weight (g) *±SE
***Beta vulgaris* L.**	AG9	7.70± 0.62^{a}	13.27± 0.77^{a}	0.87± 0.14^{a}	0.33±0.20^{ab}	0.25±0.01^{a}	0.08±0.01^{ab}
	FD3V	4.37± 0.45^{cdef}	12.03± 0.84^{ab}	0.64± 0.06^{abc}	0.08±0.03^{bcdefgh}	0.15±0.01^{efg}	0.03±0.01^{cde}
	AG1	6.20± 1.33^{abcd}	11.63± 0.32^{abcd}	0.47± 0.01^{cdefg}	0.24±0.02^{abcde}	0.18±0.02^{cd}	0.05±0.01^{bcd}
	AG2	5.83± 0.09^{abcde}	11.50± 0.66^{abcde}	0.50± 0.09^{bcdef}	0.26±0.11^{abcd}	0.13±0.01^{ef}	0.03±0.01^{cdef}
	TSU3B	5.33± 0.57^{bcdef}	10.73± 0.43^{abcdefg}	0.55± 0.11^{bcde}	0.31±0.08^{abc}	0.16±0.01^{cde}	0.03±0.01^{cdefg}
	FD2X	4.23± 1.12^{defgh}	7.40± 1.56^{h}	0.31± 0.17^{defgh}	0.14±0.11^{bcdefg}	0.08±0.01^{h}	0.02±0.01^{cdefgh}
	T1	6.67± 0.33^{ab}	10.87± 0.99^{abcdef}	0.77± 0.12^{ab}	0.48±0.07^{a}	0.23±0.01^{ab}	0.09±0.02^{a}
	FD2D	6.50± 0.29^{abc}	11.87± 0.69^{abc}	0.56± 0.02^{bcd}	0.24±0.08^{abcdef}	0.18±0.01^{c}	0.05±0.01^{abc}
	CONTROL	4.33± 0.73^{defgh}	9.03± 0.26^{fgh}	0.14± 0.01^{h}	0.02±0.01^{defgh}	0.08±0.01^{i}	0.02±0.01^{cdefgh}
***Phaseolus vulgaris* L.**	FD2X	8.03±1.41^{abcd}	38.77±1.01^{a}	1.69±0.19^{a}	0.87±0.18^{a}	0.10±0.02^{abcd}	0.03±0.01^{abcd}
	FD2D	7.33±0.60^{abcedfg}	33.10±0.95^{abcd}	1.20±0.24^{abcdef}	0.54±0.17^{abcdef}	0.10±0.02^{ab}	0.03±0.01^{abc}
	MI1A	7.90±1.02^{abcdef}	31.17±3.77^{abcde}	1.28±0.21^{abcde}	0.58±0.08^{abcd}	0.11±0.03^{a}	0.04±0.02^{ab}
	TSU3B	7.97±0.50^{abcde}	31.10±3.15^{abcdef}	1.57±0.27^{ab}	0.82±0.22^{ab}	0.10±0.01^{abc}	0.04±0.01^{a}
	T1	7.40±0.64^{abcdefg}	27.50±2.46^{cdefg}	0.95±0.14^{bcedfg}	0.37±0.12^{bcdefg}	0.05±0.01^{efg}	0.01±0.02^{abcdefg}
	AG2	9.33±0.42^{a}	36.57±3.78^{ab}	1.57±0.39^{abc}	0.65±0.20^{abc}	0.08±0.01^{abcde}	0.02±0.02^{abcde}
	AG1	8.67±0.60^{abc}	35.13±3.83^{abc}	1.47±0.17^{abcd}	0.56±0.18^{abcde}	0.08±0.01^{abcdef}	0.02±0.01^{abcdef}
	CONTROL	9.10±1.10^{ab}	27.23±3.12^{cdefg}	0.76±0.04^{efg}	0.39±0.07^{abcdefg}	0.06±0.01^{efg}	0.02±0.01^{abcdefg}

**±SE: Standard error from mean; Means followed by the same letter in the coloumare not significantly different at $p \leq 0.05$.

Figure 7.3: a. Pot inoculation of *Phaseolus vulgaris* L. with PGPF strains. **b**. Effects of PGPF inoculation on *Phaseolus vulgaris* L. The PGPF isolates *Trichoderma atroviride* (AG1), *Trichoderma viride* (AG2), *Clonostachys rosea* (T1), *Aspergillus niger* (TSU3B), *Mucor fragilis* (MI1A), *Trichoderma virens* (FD2D), *Gilmaniella subornata* (FD2X) and Control (C) exhibited promotion in root lengths, shoot length.

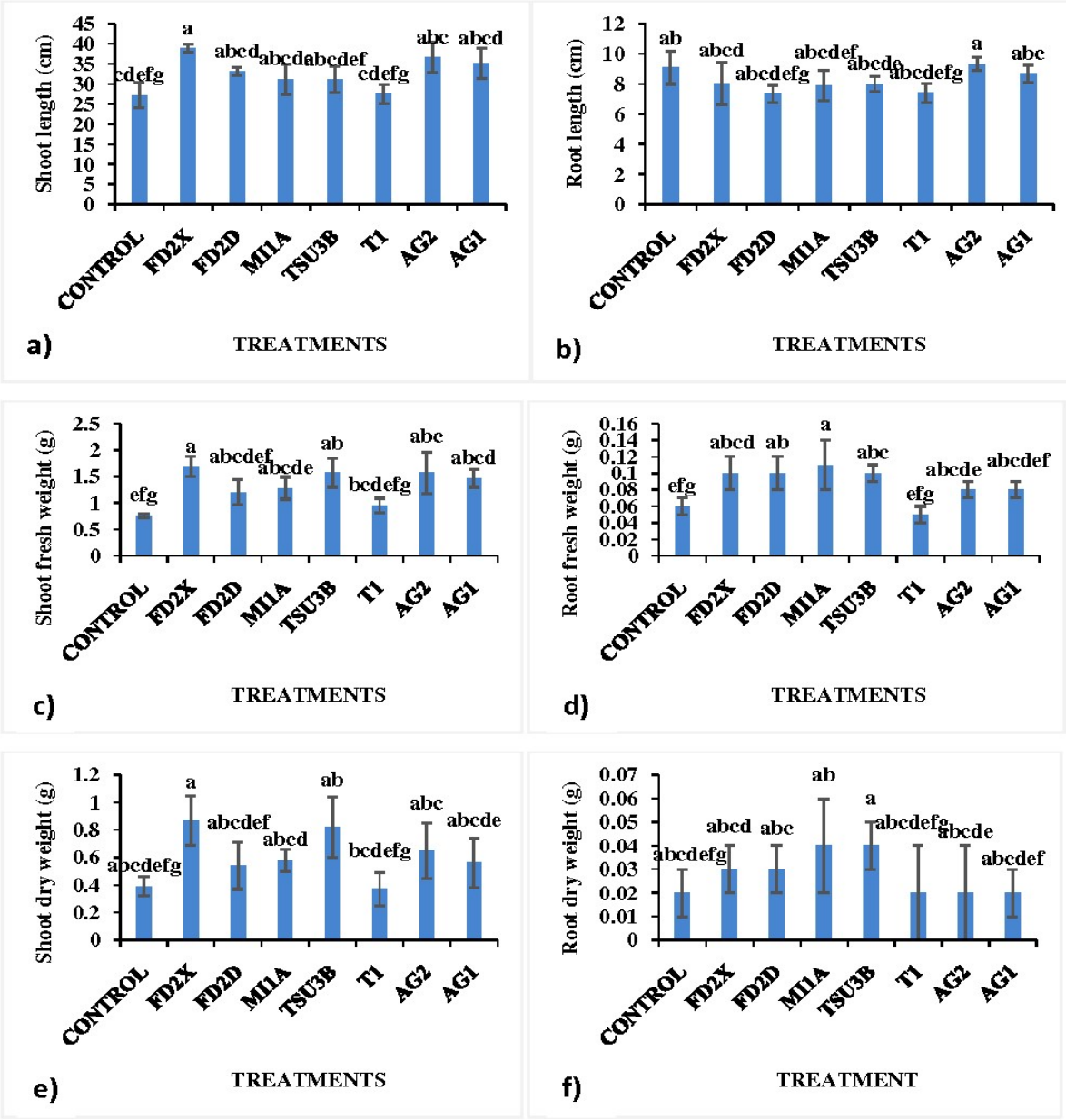

Figure 7.4: Effects of different PGPF isolates on growth parameters such asshoot length (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e) and root dry weight (f) of *Phaseolus vulgaris* L. PGPF isolates inoculated were FD2X (*Gilmaniella subornata*), FD2D (*Trichoderma virens*), MI1A (*Mucor fragilis*), TSU3B (*Aspergillus niger*), T1 (*Clonostachys rosea*), AG2 (*Trichoderma viride*), AG1 (*Trichoderma atroviride*) and C (Control treatment).

In the second pot experiment, *P. vulgaris* L was used as the host plant, and the PGPF strains that were chosen were AG2 (*Trichoderma viride*), TSU3B (*Aspergillus niger*), MI1A (*Mucor fragilis*), FD2X (*Gilmaniella subornata*), and AG1 (*Trichoderma atroviride*) (**Figure 7.3).** Following 30 days of planting, plantlets were pulled up, and growth parameters including shoot length, root length, fresh weight at the shoot and root, shot dry weight, and root dry weight were measured.In this pot trial highest root length was observed in the treatment AG2 (9.33cm), followed by CONTROL (9.10cm), AG1 (8.67cm), FD2X (8.03cm), TSU3B (7.97cm), MI1A (7.90cm), T1 (7.40cm) and FD2D (7.33cm). However, no significant difference was observed in root length ($p \geq 0.05$). In case of shoot length, highest increase was observed in treatment FD2X (38.77cm), followed by AG2 (36.57cm), AG1 (35.13cm), FD2D (33.10cm), MI1A (31.17cm), TSU3B (31.10cm) and T1 (27.50cm), over control (C) (27.23cm). Significant increase in shoot length was observed in FD2X and AG2 ($p \leq 0.05$).In case of shoot fresh weight, highest increase was observed in the treatment FD2X (1.69g), followed by TSU3B and AG2 (1.57g), AG1 (1.47g), MI1A (1.28g), FD2D (1.28g), T1 (0.95g) and Control (0.76g) and significant increase in shoot fresh weight was only observed in treatments FD2X, TSU3B, AG1 and AG2 ($p \leq 0.05$). In case of root fresh weight highest increase was observed in treatment MI1A (0.11g), followed by FD2X, FD2D, and TSU3B (0.10g), AG1 and AG2 (0.08g), CONTROL (0.06g) and T1 (0.05g). In case of shoot dry weight, highest increase was observed in FD2X (0.87g), followed by TSU3B (0.82g), AG2 (0.65g), MI1A (0.58g), AG1 (0.56g), FD2D (0.54g), CONTROL (0.39g) and T1 (0.37g). In case of root dry weight highest value was observed in treatment MI1A and TSU3B (0.04g), followed by FD2X and FD2D (0.03g), CONTROL, AG2 and AG1 (0.02g),

and T1 (0.01g). However no significant difference was observed in case of shoot and root dry weight ($p \leq 0.05$) (**Figure 7.4 and Table 7.2**).

Discussion

In order to increase productivity, conventional farming methods frequently rely largely on chemical fertilizers, which pose serious risks to agro-ecosystems by contaminating food chains, degrading soil quality, and contaminating water supplies (Kumar et al., 2022). The potential of PGPF as bioinoculants to promote plant growth and development has been shown in numerous studies and *Rhizopus, Fusarium, Aspergillus, Penicillium, Gliocladium*, and *Trichoderma* are a few PGPF that are frequently investigated (Larekeng et al., 2019).

In the present study, *B. vulgaris* L. and *P. vulgaris* L. were inoculated with selected PGPF, resulting in enhanced plant growth parameters. Especially, *B. vulgaris* treated with isolate AG9 (*Talaromyces purpureogenus*) exhibited significant improvements in root length, shoot length, shoot fresh weight and root fresh weight ($p \leq 0.05$).Similar findings were discovered by Zhao et al. (2021), where *T.purpureogenus* was found to be able to colonize in the soil of the rhizosphere and the tissue of cucumbers, thereby improving the growth parameters of the seedlings and producing siderophores and phosphates. Another study discovered that using *T. purpureogenus* might increase bitter gourd growth while reducing *Fusarium* wilt development. The control efficacy was as high as 63.7%. (susceptible bitter gourd '9208′) and 48.7% (resistant bitter gourd '09-3-55') (Tian et al., 2022).

According to a study by Kaur and Saxena (2023), *Talaromyces purpureogenus* is a fungus that can withstand drought stress. When inoculated onto *Triticum aestivum* L., it significantly increased a number of physio-biochemical growth parameters under both normal and drought-stressed conditions. The results of this investigation also showed that

isolate T1 (*Clonostachys rosea*) significantly increased the length of the shoots, roots, fresh weight, and dry weight of the *B. vulgaris.* This significant fungal isolate demonstrates potent biological control over a variety of fungal plant pathogens, nematodes, and insects. It also demonstrates strong biological control over the biodegradation of plastic waste, the biotransformation of bioactive compounds, the use of fermentation as a bioenergy source, and the biodegradation of plastic waste (Sun et al., 2020, Jensen et al., 2021). Pepper, tomato, and eggplant treated with *C. rosea* improved plant growth and seed germination (Türkölmez et al., 2023). However, in case of *P. vulgaris,* even though there is an increase in the growth parameters, these were not statistically significant by treatment T1 (*Clonostachys rosea*).

In *B. vulgaris*, isolate AG1 (*Trichoderma atroviride*) considerably increased the shoot length and shoot fresh weight; in *P. vulgaris*, however, these same parameters were significantly increased. According to Sui et al. (2022), the application of *Trichoderma atroviride* and a chemical fungicide containing 6% tebuconazole on wheat treated with *Fusarium pseudograminearum* and *Rhizoctonia cerealis* resulted in a reduction of *Fusarium pseudograminearum*. However, wheat treated with *T. atroviride* exhibited higher yield and fewer instances of white heads compared to the chemical treatment. This suggests that *T. atroviride* has the potential to replace chemical fungicides for controlling a broader spectrum of soil-borne diseases in wheat and improving wheat yield. The bio-stimulatory activity of *T. atroviride* LZ42 formed in talc was seen in another investigation, where tomato seedlings exhibited higher aerial and root dry weights in greenhouse trials following treatment. With an 82.69% control efficiency which is comparable to the carbendazim treatment - *T. atroviride* LZ42 successfully reduced Fusarium wilt disease in tomato seedlings (Rao et al., 2022). *T.*

atroviride and *T. virens* were applied alone or in combination with PGPR strains (*P. koreensis*, and *B. subtilis*) to observe its effect on growth and wilt disease severity caused by *Verticillium dahliae* and plant defence-related enzymes (peroxidase, polyphenol oxidase, phenylalanine ammonium lyase, and β-1,3 glucanase) of eggplant. The most successful applications were the isolates of *T. atroviride* and *T. virens* in combination with bacteria (Bilginturan and Karaca, 2021). In the present study, while *P. vulgaris* showed a significant increase in shoot length, shoot fresh weight, and root fresh weight, FD2X (*Gilmaniella subornata*) inoculation on *B. vulgaris* resulted in an increase in shoot fresh weight and dry weight that was not statistically significant. According to Yuan et al. (2016), the genus *Gilmaniella* is present in both the rhizospheric and endophytic regions of plants. There aren't many publications on plant growth-promoting actions in this genus, although there are a few species that have been researched in this area.

In one study, for example, Wang et al. (2012), found that when *Gilmaniella* sp. was inoculated onto *Atractylodes lancea*, the activities of phenylalanine ammonia lyase and polyphenol oxidase grew gradually and peaked in the latter stages, while the activity of peroxidase peaked in the first few days. Furthermore, chitinase and β-1,3-glucanase activities were much higher than control plants' (induced by fungal elicitor) activities. *A. lancea*, when inoculated with *Gilmaniella* sp., increases plant development and sesquiterpenoid biosynthesis of *A. lancea*, according to a different study conducted by Yuan et al. (2016). Before being infected with *F. oxysporum*, *A. lancea* plantlets were inoculated with *Gilmaniella* sp. AL12 (AL12), which inhibited the narcotization of root tissues and the plant growth retardation that are often linked to *Fusarium* root rot (Ren et al., 2016). When it came to *B. vulgaris* L., FD2D (*Trichoderma virens*) significantly increased the growth of shoot

length, root length and shoot fresh weight, while *P. vulgaris* L. exhibited a substantial rise in root fresh weight. Beneficial fungus *T. virens* is well-known for its herbicidal, growth-promoting and biocontrol properties (Bansal et al., 2023). The growth of pathogenic *Fusarium proliferatum* was prevented by the fermentation extract of *Trichoderma virens*, whereas the growth of *Arabidopsis thaliana* lateral roots and the number of hairy roots were both increased (Wang et al., 2022a). Auxin-related behaviours, such as enhanced biomass production and promoted lateral root development, were seen in wild-type *Arabidopsis* seedlings infected with either *T. virens* or *T. atroviride* (Contreras-Cornejo et al., 2009). Only the shoot fresh weight and shoot dry weight in *B. vulgaris*, as well as the shoot fresh weight and root fresh weight in *P. vulgris*, were substantially increased by TSU3B (*A. niger*). One of the most effective fungus bioinoculants that aids in plant growth and promotion is *A. niger*. As per Rani and Jain, (2017), *A. niger* that was isolated from the rhizosphere of the medicinal plant *Azadirachta indica*, also known as neem, had the highest level of antibacterial activity against the test bacterium *E. coli*. Galeano et al. (2021), found that the inoculation of common bean plants with *A. niger* resulted in increased height and fresh and dry mass of the aerial part and root when compared to the non-inoculated treatment. When *A. niger* and *Aspergillus caespitosus* were combined to inoculate *Trigonella foenum-graecum* L, the result was more desirable physiological properties, including higher levels of protein, carbohydrate, total phenolic content, and antioxidant activity, as compared to treatment with individual PGPFs or distilled water. As shown by HPTLC, even the extract from fenugreek plants treated with the consortium had the highest diosgenin content ($342.374 \pm 0.67 \mu g ml^{-1}$) (Thakor et al., 2023). In a substrate without phosphorus limitation, *A. niger* can stimulate the growth of coffee (*Coffea arabica*) seedlings. After 30 weeks, the seedlings that were injected

with a granular formulation of *A. niger* showed notable increases in height (5.6%), stem diameter (6.1%), number of leaves (8.5%), root dry mass (13%) and volume (15%) (Araújo et al., 2020).

In the present study, AG2 (*T. viride*) markedly increased the fresh weight and shoot length of *B. vulgaris* L. and *P. vulgaris* L. The most commonly employed species is *T. viride*, which has been used to combat 87 different crops, 70 soil-borne diseases, and 18 foliar infections, respectively (Sharma et al., 2014). It is well-known for its antagonistic properties against a variety of plant pathogenic fungi (Awad et al., 2018). For instance, potato plants that were bio-primed with *Trichoderma viride* and challenged with *Alternaria solani* had a considerable improvement in a number of growth metrics. This improvement was attributed to a modulation of the activities of antioxidant enzymes, which in turn improved redox homeostasis (Kumar et al., 2022). *T. viride* colonized in the leaves of wild-type *Arabidopsis* and enhanced biomass production, elevated nutrient uptake, and accelerated leaf and seedling development, according to a study by Guo et al. (2020). Only the fresh weight of the roots was significantly increased in the current investigation by MI1A (*M. fragilis*). Xu et al. (2021), report that *M. fragilis* promotes the accumulation of salvianolic acid B, rosmarinic acid, stearic acid and oleic acid in *S. miltiorrhiza* hairy roots, thereby acting as an effective endophytic fungal elicitor with excellent application prospects for medicinal plant cultivation. Nevertheless, there aren't many published studies on this fungus.

Findings of the pot experiment of the present study revealed that several isolates *viz., T. atroviride*, *T. viride*, *T. purpureogenus*, *C. rosea*, *G. subornata*, *A. niger*, *T. virens* and *M. fragilis* have multiple growth-promoting properties that positively impact the growth and development of *B. vulgaris* and *P. vulgaris*. These reports highlight the potential of the

chosen strains as bio-inoculants with greater potency and field trials on other crop plants, particularly *G. subornata* (FD2X) and *T. purpureogenus* (AG9), on which the best results have been obtained among the tested isolates but on which not much research has been done regarding their capacity to promote plant growth. On all criteria, MI1A (*M. fragilis*) exhibited the least amount of increase. These outcomes are explained by the PGP characteristics that they have.

Conclusions

Based on the current investigation, it can be said that certain PGPF isolates have the potential to be used as PGPF bioinoculants, including AG1 (*Trichoderma atroviride*), AG2 (*Trichoderma viride*), AG9 (*Talaromyces purpureogenus*), T1 (*Clonostachys rosea*), FD2X (*Gilmaniella subornata*), MI1A (*Mucor fragilis*), FD3V (*Clonostachys rosea*), TSU3B (*Aspergillus niger*) and FD2D (*Trichoderma virens*). When it comes to *Beta vulgaris* AG9 has demonstrated the greatest growth, whereas FD2X has demonstrated a substantial improvement in shoot length, fresh weight, and dry weight in *P. vulgaris.* While, some isolates produced significantly higher growth characteristics than others and produced superior outcomes overall, certain isolates' increases in growth parameters were not statistically significant. When inoculated onto *B. vulgaris* L. and *P. vulgaris* L., their various growth-promoting traits of the selected isolates had a positive impact on plant growth and production. These PGPM strains exhibit higher efficacy, as demonstrated by both plants' better growth under controlled conditions. To sum up, these isolates show potential as inoculants to promote plant growth. Nevertheless, more research is required to comprehend the physio-chemical characteristics of the soil and the interactions between plants and mushrooms.

Chapter – 8

Summary and Conclusions

Agriculture is one of the human activities that contribute most to the increasing amount of chemical pollutant via excessive use of synthetic chemical fertilizers and pesticides which cause further environmental damage with potential risks to human health. So, in order to prevent further damage to the environment, sustainable agriculture should be practice with the help of soil microbes such as Plant growth promoting microorganisms. The use of PGPM as biofertilizers is the most eco-friendly way for agricultural practice in the long run. However, for better efficiency, a great deal of understanding and further research is necessary. To develop good bio-inoculants many of PGPM isolates must be cultured and tested for the possession of growth- promoting traits. According to Singh et al. (2015), the coating of PGPR strains positively influenced on wheat germinations. The *Azotobacter* PGPM improved wheat seed germination up to 100% in less time compared to control, *Azotobacter* was proved to be more effective for disease suppression and wheat root rot than *Azospirillum.* Once the bacterial/fungal isolates pass the test for being PGPM,

molecular and biochemical characterization are required to be done to identify the isolate. An emerging field to improve and explore the PGPR strain is genetic engineering which enables to over-express the traits so that strains with required characters are obtained (Kundan et al., 2015).

Hence in the present study an attempt was made to isolate PGPM strains from different species of Wild Musa (*M. balbisiana, M. itinerans, M. velutina and M. flaviflora*) growing in the jungle of Nagaland. Nagaland which is located in the northeast region of India has a diverse range of wild *Musa* species which grows robustly in the jungle form dense *Musa* grooves. One significant attribute to these healthy growths can be a result of Plant-microbe interaction in the root system. The present study was aimed at isolating plant growth promoting microorganism from *Musa* rhizospheric soil samples, studying and quantifying their PGP traits such as IAA production, phosphate solubilization, siderophore production, ammonia production, amylase production, heavy metal and salinity tolerance, characterizing them on morphological and biochemical basis, identify them using molecular tools and evaluate their efficacy in promoting plant growth under pot conditions. From this perspective rhizospheric soil samples were collected from different districts (Wokha, Zunheboto, and Mokokchung) of Nagaland.

Numerous growing media have already been created to detect the PGPM; in the current investigation, phosphate solubilization was accomplished using NBRIP (You et al., 2020; Pande et al., 2017) and siderophore synthesis test was accomplished using CAS medium (Srimathi and Suji, 2018; Hu and Xu, 2011; Payne, 1993). Salkowski reagent was utilized for the test of indole-3-acetic acid synthesis (Kumar et al., 2012a). The amylase test was conducted in accordance with Imran et al. (2021). Test for tolerance to salinity

conducted in accordance with Sharma et al. (2021). The heavy metal tolerance test by Yadav et al. (2022) and the ammonia production test as stated by Sharma et al. (2021) were conducted. Following PGPM ability screening, molecular identification of the isolates was carried out (Thanh and Diep, 2014; Naziya et al., 2019).

Present study showed that not all bacterial isolates turned out positive for growth promoting traits. From the qualitative estimation of bacterial traits, majority of the bacterial isolates turned out positive for phosphate solubilization and siderophore production compared to IAA production test, probably indicating that these two traits might have played a dominant role in promoting the growth of the species used in this study wild *Musa* species. So, it may be suggested that the PGPM isolates can be induced in the species of cultivar *Musa* with an aim to increase the plant growth and yield. From this study, it is clear that Rhizospheric microbes can provide a rich source of phosphate solubilizing, siderophore producing, ammonia producing, amylase producing and IAA producing bacterial and fungal isolates. Other traits such as heavy metal stress and salinity stress tolerant isolates can also be used to be used for plants growing in stressed environment.

A total of 136 bacteria and 43 fungi were isolated using serial dilution technique on nutrient agar medium for bacteria and PDA and RBA agar media for fungal isolates. Later after purifying both the bacterial and fungal isolates were screened for plant growth promoting traits which included IAA production, siderophore production, phosphate solubilization, ammonia production, amylase production, heavy metal and salinity stress tolerance. Isolates from rhizospheric soil showed multiple plant growth promoting (PGP)traits. Some isolates all showed all PGP traits like phosphate solubilization,

siderophore, IAA, amylase or ammonia production. Some showing only a single trait while others had two or three traits.

In soil, the effectiveness of applied phosphorus (P) fertilizers is typically limited to around 30% due to its fixation in soils. In acidic soils, P is fixed in the form of iron/aluminium phosphate, on the other hand in neutral to alkaline soils. This fixation process hinders the availability of P for plants, leading to reduced efficiency of P fertilizers (Sharma et al., 2013). In the present study, in case of PGPR isolates, 94 isolates were able to display the capability to solubilise phosphates when cultured onNational Botanical Research Institute's phosphate medium (NBRIP). While, among the PGPF isolates, 30 isolates were able to produce halo zone on NBRIP agar medium indicating phosphate solubilizing ability. However more isolates could solubilize phosphate in liquid NBRIP media. In liquid medium phosphate solubilizing capacity varied amongst all selected isolates at different incubation period. Maximum phosphate solubilization for PGPF isolates was observed by isolates T4, T8, TSU3A and T3(146.43, 126.53, 107.78 and 99.32µg/ml) all isolates on 4^{th} day. Whereas, in case of PGPR isolates, fifteen highest phosphate solubilizer were 10RZ9 (360.1±45µg/ml), followed by 30EZ3 (356.66±0.89µg/ml), 30RZ10 (342.86±1.34µg/ml), 30E24 (329.07±1.33µg/ml), 30E6 (298.03±4.32µg/ml), TSU2(1) (291.15±0.68µg/ml), 30E22 (291.14±0.67µg/ml), 30E5 (287.68±3.11µg/ml), 30E23 (273.9±4.21µg/ml), 10E9 (273.9±1.67µg/ml), TSU3(7) (263.56±1.77µg/ml), 30E18 (263.55±1.78µg/ml), RZ15 (260.1±4.09µg/ml), 10RZ16 (246.31±7.09µg/ml) and 30E1 (242.86±1.45µg/ml). All these isolates were also able to reduce the pH of medium that increase solubilization of tricalcium phosphate.

Auxin is a plant hormone that exerts significant influence over various processes related to plant tissue formation, including growth, cell division, cell differentiation, and protein synthesis. It is produced as secondary metabolites. PGPF isolates were qualitatively analysed for IAA production and out of 43 isolates 17 were positive for IAA production. Seventeen positive isolates were further analysed quantitatively. Highest IAA production was observed by FD4XIII (5.56±0.05), followed by FD2D (5.05±0.12), FD5XI (4.86±0.25), FD3III (4.23±0.05), FD4VI (3.34±0.08), MI3F (3.24±0.05), FD3V (3.24±0.05), FD3I (2.05 ±0.03), FD3II (1.78 ±0.07), FD4A (1.67±0.09), T1 (1.23 ±0.04), MI2B (0.99±0.04), T8 (0.75±0.06), FLALUM4 (0.44±0.02), MI1E (0.11±0.06) and FD2X (0.09±0.05) on 4^{th} day, 12^{th} day, 8^{th} day, 8^{th} day, 8^{th} day, on 8^{th} day, on 6^{th} day, 6^{th} day, 8^{th} day, 10^{th} day, 8^{th} day, 6^{th} day, 6^{th} day, 10^{th} day and 8^{th} day respectively. Out of 136 PGPR isolates 77 isolates were able to produce IAA while integrating Salkowski reagent. Out the positive isolates 58 isolates were selected for quantitative estimation ranging from 0.10-149µg/ml. Seven bacterial isolates (TSU12, TSU11, NC7, TSU7, TSU3(10), LUMBB8 and LBS23) produced the highest IAA under in vitro conditions (149.86µg/ml, 126.35µg/ml, 22.38µg/ml, 10.09µg/ml, 8.80µg/ml, 4.65µg/ml and 4.29µg/ml) on 6^{th} day, 4^{th} day, 12^{th} day, 6^{th} day, 4^{th} day, 8^{th} day and 10^{th} day respectively. It was noted that as compared to phosphate solubilising trait very few isolates were shown to have IAA producing ability.

Siderophore production is another important attribute of PGPM. Rhizospheric microbes synthesize and release siderophore that binds Fe^{3+} and chelate Fe3+ and makes it less available for other species in the microbial community of the rhizosphere (Dutta et al.,2015). In soil, siderophore production activity plays a central role in determining the ability of different microorganisms to improve plant development by enhancing the iron

uptake by plants. Additionally, it helps in biocontrol activity against pathogen. The active transport system through the membrane begins with the recognition of the ferric- siderophore by specific membrane receptors of Gram-negative and Gram-negative bacteria (Boukhalfaand Crumbliss, 2002).In the present study, 24 PGPF isolates were positive for siderophore production when screened on CAS agar media and fifteen isolates,FD2A (4.30±0.08), AG1 (3.5±0.12), MI2C (3.2±0.06), MI2B (3.19±0.04), MI1D (3.14±0.04), MI1E (3.1±0.03), AG5 (3.01± 0.03), AG9 (3.01±0.43), MI1F (2.89±0.02), AG8 (2.7±0.09), FD4A (2.62± 0.02), AG2 (2.6±0.02), FD4VI (2.6±0.03), AG6 (2.5±0.06), TSU3B (2.44±0.01), were found most prominent for siderophore production with highest siderophore production index. In case of PGPR strains 90 strains were shown positive for siderophore production when tested on CAS agar plates. Furthermore, selected isolates were screened for quantitative analysis and EZ30 (197.138), 10EZ28 (110.350), 30EZ18 (48.995), EZ24 (46.132), 10RZ3 (45.327), 10RZ15 (40.594), 10EZ18 (40.392), 10EZ3 (40.392), 10EZ9 (39.618) and 20EZ13 (39.170)were the ten isolates with highest percentage siderophore unit.

Isolates were also tested for ammonia production and varying degree of production was observed among the isolates with TSU8, LUMB2, 5NC8, 5NC6, LUMB9, LUMITI6, LBS36, LBS16, LBS44A, 25EZ22, EZ27, 5R9, RZ27, RZ7, 5E9, 5R12, 5R15, 5R3, TSU4, TSU6, TSU3(4), TSU3(2), TSU3(6), TSU3(7), TUL12, NC11, 5NC6, 5NC13, VEL1, MI1D, FD2D, FD4VI, FD3II, FD3III, FD3V and MI2C displaying the highest ammonia production (+++) indicated by the brownish colour formation when Nessler's reagent was added to the culture broth.

Abiotic and biotic stress, in context of plants is an unfavourable condition that results in the reduction of their growth and development. Stress is classified into two type's abiotic

stress and biotic stress. Abiotic stress includes salinity, drought, and unfavourable temperatures. Excessive uptake of essential or non-essential heavy metals from soil by plants can cause adverse effects. Many researches showed that the change of soil microbial community structure was driven by multiple factors—the comprehensive effect of soil physical-chemical properties and toxic contaminants such as heavy metals (Beattie et al., 2018; Luo et al., 2019). Bacterial isolates were tested for Cd^{2+}($CdCl_2.H_2O$), Cu^{2+}($CuSO_4$), Ni^{2+}($NISO_4.H_2O$), As^{3+}($NaAsO_2$), Sb^{3+}(K_2 $(SbO)_2C_8H_4O_{40}$, $3H_2O$), Zn^{2+}($ZnSO_4$, $7H_2O$) and Cr^{2+}(K_2CrO_4) tolerance using the minimum inhibitory concentration (MIC). Isolate TSU7 and TSU11 had the highest tolerance for Cu (770µg/ml), for Cr it was LUMITI2, LUMBB4 and LUMBB7 (10010 µg/ml), for Zn highest tolerance was shown by LUMBB2, LUMBB9 and LUMBB7 (10010µg/ml), for Cd highest tolerance was shown by TSU7 (570µg/ml). For Ni highest tolerance was shown by LUMITI3 and LUMBB2 (560µg/ml). Whereas, for An, highest tolerance was shown by LUMB10 (270 µg/ml) and for arsenic highest was shown by LBS23, LBS4 and TSU3 (850µg/ml).The acquired results of study significantly complement the physiological characteristics of the representatives of the PGPM isolates, which can be practically applied in biotechnological processes, especially in the development of new prospective biodegradation strains of microorganisms applicable in the processes of decontamination of industrial localities contaminated by toxic organic substances. The bacterial isolates were screened for salinity resistant test with highest (14%), salinity tolerance displayed by TUL11, TUL6 and RZ27 followed by isolates TUL12, TSU4, RZ20 and EZ11 which showed tolerance up to 12%.

PGPM isolates were selected for molecular identification using 16srRNA sequencing and 18Sdna sequencing technique for PGPR and PGPF strains respectively. The BLAST

analysis of gene sequences of these isolates showed similarity with *Bacillus cereus* (LUMBB7), *Serratia marcescens* (TSU1), *Pseudomonas teessidea* (TSU2(4)), *Bacillus cereus* (TSU3), *Bacillus safensis* (TSU4), *Bacillus pumilus* (TSU8), *Pseudomonas putida* (M5), *Agrobacterium larrymoorei* (M7), *Bacillus safensis* (M1), *Burkholderia cepacian* (M6), *Kosakonia arachidis* (M14), *Cupriavidus necator* (M5), *Pseudomonas putida* (M8), *Pseudomonas orientalis* (M9), *Pseudomonas monteilii* (M11), *Pseudomonas gessardii* (LUMITI4), *Pseudomonas gessardii* (LBS23), *Bacillus* sp.(LUMITI1), *Chryseobacterium cucumeris* (LUMBB2), *Cedecea neteri* (NC7), *Bacillus subtilis* (LUMITI6), *Proteus terrae* (TSU6), *Proteus terrae* (TSU2(1)), *Lelliottia amnigena* (TSU12), *Providencia rettgeri* [TSU3(7)], *Pseudomonas koreensis* (TSU7), *Klebsiella variicola* (TSU11), *Burkholderia cepacia* (TUL6), *Bacillus cereus* (LBS4), *Proteus terrae* (TSU1(9)), *Bacillus safensis* (TUL12), *Burkholderia cepacia* (TUL11), *Alcaligenes faecalis* (LBS36), *Pseudomonas fluorescens* (LBS12A), *Pseudomonas* sp. (LBS16), *Trichoderma atroviride* (AG1), *Trichoderma viride* (AG2), *Talaromyces purpureogenus* (AG9), *Clonostachys rosea* (T1), *Gilmaniella subornata* (FD2X), *Mucor fragilis* (MI1A), *Clonostachys rosea* (FD3V), *Aspergillus niger* (TSU3B) and *Trichoderma virens* (FD2D). Gene sequences of these isolates were deposited in GenBank and Accession numbers have been obtained.

In today's world, huge amount of synthetic fertilizers has been used in crop field; they reduce the risk of pathogens, nutrient deficiencies increasing the crop yield. But on the other hand, these synthetic fertilizers cause lots of hazard on environment, also developing pest resistance (Aktar et al., 2009). In that scenario, microorganisms that help in growth promotion are very good alternative of these hazardous chemical fertilizers. The efficacies of

the selected PGPM isolates were tested on various crop plants such as *B. juncea*, *H. annuus*, *P. vulgaris*, *B. vulgaris* and *C. arietinum* either in singly or in combination.

For the first pot experiment on *C. arietinum* L. with PGPR strains singly, *K. arachidis* was shown to have the highest shoot length, shoot fresh weight, root length, and root dry weight; whereas, root fresh was highest in *P. putida* treatment. Second set of experiment with PGPR strains in *H. annuus* L. and *B. juncea* combination of PGPR strains *B. cereus* (TSU3), *B. safensis* (TSU4), *B. pumilus* (TSU8), *K.variicola* (TSU11), *L.amnigena* (TSU12), *P.koreensis* (TSU7), *S. marcescens* (TSU1)) have also shown improvement in plant growth with PGPM inoculation along with tolerance to heavy metal stress. Among four treatments, 'C' or control treatment (no heavy metal and PGPR in the potting mix), P (PGPR + no heavy metal in the potting mix), H (no PGPR + heavy metal in the potting mix (150mg/ml of Cd/Cu)) and P+H (PGPR + heavy metal in the potting mix (150mg/ml of Cd/Cu)). In both the plants species, shoot and root length, root fresh weight, and root dry weight was highest in P treatment (only PGPR consortia). In *Brassicajuncea* shoot fresh and dry weight was highest in P+H treatment ($p \leq 0.05$). Whereas in *H. annuus,* shoot fresh weight, dry weight was highest in P treatment. In the third experiment, *Brassica juncea* was inoculated by consortium of LUMITI6 (*B. subtilis*), LUMBB2 (*C. cucumeris*), LUMBB7 (*B. cereus*). It was found that significant increase only in shoot length and shoot fresh weight. In the 4th experiment selected PGPR strains, upon inoculation into common bean plants (*P. vulgaris*), demonstrated effectiveness in enhancing plant growth. Amongst the 5 treatments evaluated, the treatment with *P. orientalis* exhibited the most significant growth improvements in terms of shoot and root length, as well as shoots and root fresh weight, whereas, RZ23 enhanced the shoot fresh weight. This was followed by treatments with *B.*

cepacian and *P. taiwanensis,* all of which outperformed the control treatment though it was not statistically significant. PGPF strains were inoculated singly onto *B. vulgaris* L. and *P. vulgaris* L. *B. vulgaris* L. treated with isolate AG9 (*T. purpureogenus*) showed significant improvements in root length, shoot length, shoot fresh weight and root fresh weight. Isolate T1 (*C. rosea*), also exhibited significant increase in shoot length, root length, shoot fresh weight and shoot dry weight in *B. vulgaris* L. AG1 (*T. atroviride*), in *B. vulgaris* L. significantly enhanced the shoot length and shoot fresh weight, whereas in case of *Phaseolus vulgaris* significantly enhanced the shoot length and shoot fresh weight. Root fresh weight in *B. vulgaris* L. was highest by AG9 and all the isolates showed significant growth in the root fresh weight over control treatment. In case of root dry weight highest value was observed by T1. Only T1 and AG9 have shown significant growth over control FD2X (*G.subornata*) when inoculated on *B. vulgaris* L. have shown increase in shoot fresh weight and dry weight, however it was not statistically significant, while in *P. vulgaris* L. significant increase was observed in terms of shoot length, shoot fresh weight and root fresh weight. FD2D (*T. virens*), showed significant increase in the growth of shoot length, root length, shoot fresh weight in case of *B. vulgaris* L., whereas, in case of *P. vulgaris* L. significant increase in root fresh weight. TSU3B (*A. niger*)*,* enhanced only the shoot fresh weight and shoot dry weight in *B. vulgaris* significantly, and in *P vulgrais* L. shoot fresh weight and root fresh weight. Isolates AG2 (*T. viride*), significantly enhanced shoot length, and shoot fresh weight in both *B. vulgaris* L. and *P. vulgaris* L. Isolates MI1A (*M. fragilis*), in the present study, significantly enhanced only the root fresh weight. These reports indicate the potential of the selected strains as bio-inoculants with more pot and field trials on other crops plants especially *T.purpureogenus* (AG9) and FD2X (*G.subornata*) on which not many works have

been done regarding its plant growth promoting ability but have shown the best results amongst the tested isolates.However, there are still many limitations that have to be consider while attempting to formulate bioinoculants from PGPM which are as follows.

Conclusions

As PGPM through several studies have been proven to be helpful in establishing the natural condition of the soil by not only reducing the use of the number of chemical pesticides and fertilizer but also by taking up the harmful compounds deposited in the soil. However, there are some points to keep in mind, for instance, the results it provides in-vitro and in-vivo might be quite different sometimes as PGPM inoculation is to a great extent influenced by plant's characters and by the chemical, physical and biological properties of the soil. With enough proof in the literature by many researchers that PGPM's can be one of the best alternatives of chemical fertilizer for the sustainable and eco-friendly agricultural systems, there is a lot of scope for a microbiologist to develop bio-fertilizers, biopesticides, bio-remediators by using consortia of these microbes. However, its implementation can only be successful on a large scale if the farmers are being given proper knowledge about its use as they are the primary workers directly involved with agriculture. One of the big challenges in the way is to develop a cost-effective method for the preparation of PGPM bio-inoculants so that it can be made available to people at a very low price compare to synthetic fertilizers. Another challenge is to create awareness about the long-term harmful effects of chemicals they use both on our health and soil.Considering the whole study, it can be concluded that, in our study, PGPR strains *P. putida* (EZ11), *K. arachidis* (EZ27), *P. monteilii* (EZ30), *S. marcescens* (TSU1), *B. cereus* (TSU3), *K. variicola* (TSU11), *L. amnigena* (TSU12), *P.*

koreeensis (TSU7), *B. pumilus* (TSU8), and *B. safensis* (TSU4), *B. subtilis* (LUMITT6), *C.cucumeris* (LUMBB2), *B. cereus* (LUMBB7) and *P. gessardii* (LUMITI4), *C. neteri* (NC7), *P. orientalis* (RZ3), *A. larrymoorei* (RZ23), *B. cepacian* (RZ27), *P. taiwanensis* (RZ5), and PGPF strains *T. atroviride* (AG1), *T. viride* (AG2), *T. purpureogenus* (AG9), *C. rosea* (T1), *G. subornata* (FD2X), *M. fragilis* (MI1A), *C rosea* (FD3V), *A. niger* (TSU3B) and *T. virens* (FD2D), were very potent PGPM strains as they had many plant growth promoting abilities when tested such as IAA production, siderophore production, phosphate solubilization, amylase production, ammonia production, salinity and heavy metal tolerant. These isolates could promote the growth and productivity of experimented crops plants which included *B. juncea*, *H. annuus*, *P. vulgaris*, *C. arietinum* and *B. vulgaris*. These strains proved their growth promoting capacity when applied singly or in consortium. So, our idea of developing a consortium has given us a fruitful result and that can raise a single step ahead towards sustainable agriculture. Many isolates were shown to be highly tolerant to heavy metal and salinity stress proving its potential for helping plants to growth in such contaminated areas. Therefore, these isolates could potentially be developed as inoculants to mitigate metal toxicity in plants grown in metal polluted soil with a better understanding of the soil physio-chemical properties and plant-bacteria interaction. Also, expanding the knowledge about the microbial genetics taking part in resistance of heavy metal is required to be studied to develop bacterial candidates for multifunctional PGPR - based formulations. In addition, stress tolerance was more prevalent in bacteria growing in the rhizosphere of *Musa* plant growing closest to the river. By understanding the mechanisms involved in mobilization and transfer of heavy metals, future strategies can be developed and use in the bioremediation process. Although the isolates were naturally isolated from the rhizosphere of

wild *Musa* plants, it must be cautiously considered since some isolates has been reported as a human pathogen. Further investigations are needed.

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ANNEXURE -I

Primers Used in the Study

Gene	Primer	Forward	Annealing (ºC)	Primer	Reverse	Annealing (ºC)
16S rRNA	18F	5’AGAGTTTGATCCTCAG3’	54.3	1492R	5'GGTTACCTTGTTACGACTT3'	52.5
18S rRNA	ITS-1	5′-TCCGTAGGTGAACCTGCGG-3′	55.0	ITS-4	5′- TCCTCCGCTTATTGATATGC-3′	55.0

ANNEXURE -II

List of Publications

1. Tatung, M., and Deb, C.R. (2021). Plant growth promotion by Rhizobacteria: a potential tool for sustainable agriculture. In: Deb, C.R. and Paul, A. (Eds.), Bioresources and Sustainable Livelihood of Rural India. Mittal publications, India, Pp. 29- 49.

2. Tatung, M., and Deb, C.R. (2023). Isolation, characterization, and investigation on potential multi-trait Plant growth promoting rhizobacteria from wild banana (*Musa itinerans*) rhizospheric soil. *Journal of Pure and Applied Microbiology*, *17*(3), 1578-1590. https://doi.org/10.22207/JPAM.17.3.19

3. Deb, C.R., and Tatung, M. (2024). Siderophore producing bacteria as biocontrol agent against phytopathogens for a better environment: A review. *South African Journal of Botany*, *165*, 153-162. https://doi.org/10.1016/j.sajb.2023.12.031

4. Tatung, M., and Deb, C.R. (2024). Screening and characterization of heavy metal tolerant rhizobacteria from wild Musa rhizosphere from coal mining area of Changki, Nagaland, India and assessment of their growth promoting potential under Cd/Cu contaminated conditions. *South African Journal of Botany*, *165*, 217-227. https://doi.org/10.1016/j.sajb.2023.12.039

5. Tatung, M., and Deb, C.R. (2024). Bacterial siderophores as potential biocontrol agent against phytopathogens. In: Deb, C.R., Talijungla and Puro, N. (Eds.) Bioresources: Conservation and Sustainability. Mittal Publications, India, Pp. 423-436.

ANNEXURE -III

Patents published

1. Tatung, M. and Deb, C.R. A Novel Bacterial Composition for Plant Growth. Indian Patent (Appl. No. 202431028204 dated 05.04.2024).
2. Tatung, M. and Deb, C.R. A Novel Bacterial Consortium for Plant Growth in Heavy Metals and Salinity. Indian Patent (Appl. No. 202431029655 dated 12.-4.2024).
3. Tatung, M. and Deb, C.R. A novel bacterial consortium for plant growth. Republic of South Africa. (Appl. No. 2024/03428).
4. Tatung, M. and Deb, C.R. A novel bacterial consortium for plant growth in heavy metals and salinity. Republic of South Africa. (Appl. No. 2024/03427).

ANNEXURE -IV

List of Seminars, Webinars, Workshops Attended and Paper Presented

1. Work shop on 'Gene Cloning, Protein Biochemistry, Structure Biology and Bioinformatics'. Tata Memorial Centre, Advanced Centre for Treatment, Research and Education in Cancer, Kharghar, Navi Mumbai-410210.
2. National webinar on 'World Environment Day' on June 5th, 2021 organised by Department of Botany, Rajiv Gandhi university, Arunachal Pradesh, India.
3. National webinar on 'Black fungus diseases (Mucormycesis)' on 14th June, 2021 organised by Department of Botany, Rajiv Gandhi University, Arunachal Pradesh, India.
4. Virtual event, on 15th July, 2021, iThenticate - Publish with Confidence in partnership with Turnitin South Asia. Noida, India
5. Webinar on 'Technologies for processing safe foods, entrepreneurial prospects and ministry support: sufficing the demands of food industry'. Organised by Department of Food Technology, Rajiv Gandhi University, Rono hills, Doimukh 791112, Arunachal Pradesh, India in collaboration with National Institute of Food Technology Entrepreneurship and Management (NIFTEM), Sonepat 131028, Haryana, India on 19th July, 2021.
6. 10th Annual Meeting of Asian Council of Science Editors Hosted on 18th August 2024 at Khalidia Palace Hotel, Deira Dubai, UAE.
7. Mum Tatung. 2022. Isolation of Plant Growth Promoting Rhizobacteria from *Musa* Rhizospheric Soil Collected from Changki Coal Mining Area, Nagaland, India. International Conference 'Bioresources & Bioeconomy' (ICBB-2022), Organized by: Department of Botany, Nagaland University, Lumami-798627, Nagaland, India.
8. Mum Tatung. 2024. Assessment of heavy metal tolerant Plant Growth Promoting Rhizobacteria from the *Musa* rhizosphere and effects of its inoculation in the growth of *Brassica juncea.* National Seminar on Current Understandings of Plant Health

May 12, 2024, organised by Department of Botany, School of Life Sciences, Manipur University Canchipur, Imphal – 795003, Manipur held on May 12, 2024.

9. Mum Tatung. 2024. Enhnaced Growth of *Phaseolus vulgaris* by inoculation with Phosphate Solubilizing Plant Growth Promoting Rhizobacteria from *Musa* Rhizosphere. 5th Asian Conference of Science, Technology & Medicine hosted from 17-18 August 2024, Khalidia Palce Hotel, Deira Dubai, UAE.

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