ISOLATION AND MOLECULAR CHARACTERIZATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) FROM DIFFERENT REGIONS OF MAHARASHTRA.

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Biotechnology

By

Gavande Sharda Shivaji

Registration Number: 42100288

Supervised By

Dr. Shashikant Sharma (26755)

School of Bio Engineering and Bioscience

Lovely Professional University

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

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DECLARATION

I, hereby declared that the presented work in the thesis entitled "**Isolation and molecular characterization of plant growth promoting rhizobacteria (PGPR) from different regions of Maharashtra.**" in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision of **Dr. Shashikant Sharma,** working as Assistant Professor, in the **School of Bio Engineering and Bioscience** of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

(Signature of Scholar)

Name of the scholar: Gavande Sharda Shivaji

Registration No.: 42100288

Department/school: Biotechnology, School of Bio Engineering and Bioscience

Lovely Professional University,

Punjab, India

CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled "**Isolation and molecular characterization of plant growth promoting rhizobacteria (PGPR) from different regions of Maharashtra**" submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the Biotechnology, is a research work carried out by **Gavande Sharda Shivaji**, 42100288, is bonafide record of her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

(Signature of Supervisor)

Name of supervisor: Dr. Shashikant Sharma

Designation: Assistant Professor

Department/school: School of Bio Engineering and Bioscience

University: Lovely Professional University

ABSTRACT

Since ancient times, microbes have been utilized to promote human health; this practice is still carried out today, albeit with more advanced techniques. Groups of free-living bacteria known as PGPR (Plant Growth-Promoting Rhizobacteria) have a number of beneficial characteristics that make them ideal to be utilized as biofertilizer agents.

The current investigation described the rhizobacteria linked to several locations of Maharashtra's cereal, pulse, along with vegetable crop plants, for example, maize (*Zea mays*), soybean (*Glycine max*), chilli (*Capsicum annuum*) & mung bean (*Vigna radiata*).

The study was aimed at isolating and characterizing Plant Growth Promoting Rhizobacteria from soil samples. From this perspective, the rhizobacteria linked to the vegetable, pulse, along with cereal crops from several Maharashtra regions were isolated & characterized. Total of 12 Rhizospheric soil samples was collected from economically important crops (Maize, Soybean, Chilli & mung bean) of different regions of Maharashtra Marathwada, Western Maharashtra, Vidarbha and Konkan). Using the serial dilution approach on nutrient agar media, 1174 bacterial colonies had been isolated and evaluated for characteristics that promote plant development. For various PGP traits overall 36 isolates had beenobserved positive for example potassium, zinc, phosphate solubilization, production of siderophore and Amylase.

The largest phosphate solubilization zone as well as phosphate solubilization index (PSI) among the 36 bacterial isolates that were assessed for phosphate solubilization were displayed by CA4a in the current investigation (25mm & 13.50mm, correspondingly). Highest (12) solubilized potassium concentration of supplemented insoluble potassium has been demonstrated by GM4b with high value of KSI on 7th DAI. Amongst all selected isolates, maximum zone of clearance is 51mm & Zinc Solubilizing Index (11.20) was displayed by CA2c. CA1b (4.62) and GM2c (4.87) isolates were found most efficient siderophore producers, GM2c demonstrate maximum halo zone diameter that is 31mm. Out of every isolate that had been examined for Amylase Production, CA2c showed highest zone diameter (12 mm).

Total of 12 isolates had been finally chosen on the basis of morphological as well as some biochemical tests and was further screened for Indole-3-acetic acid (IAA) production, Catalase activity, Urease and Oxidase test. Four bacterial isolates (GM2, GM3, ZM3 and ZM4)

produced more than 15 µg/mL IAA under *in vitro* conditions. Catalase activity was observed to be positive in all isolates (Hydrogen Peroxide Test) except CA2 isolates. Isolates GM2, CA2 and CA3 were found positive for Urease test. Among all the bacterial isolates, GM1, CA2 and CA3 were found positive for Oxidase test.

These twelve isolates were chosen for the molecular characterization utilizing 16S rRNA gene sequencing approach. Their 16S rRNA gene sequences exhibited similarities to certain previously identified bacteria on the basis of BLAST analysis. Phylogenetic tree was constructed of four isolates using MEGA version 11 software. *PGPR isolates were showing maximum similarity with- Pseudomonas* species (*Pseudomonas guariconensis, Pseudomonas plecoglossicida, Pseudomonas mosselii* and *Pseudomonas aeruginosa*), *Aeromonas*sp (*Aeromonas caviae- 2, Aeromonas hydrophila*), *Acinetobacter*sp, *Delftia tsuruhatensis* and *Stenotrophomonas maltophilia.*

In a pot and field experiment, six PGPR isolates were chosen to test their effectiveness in plant growth promoting activity in maize, soybean, and chilli plants. Application of the PGPR isolates to chilli, soybean, maize and mung bean enhances growth of the plant, biochemical as well as yield parameters compared to seeds that were untreated.

Once field tests on various economically valuable crops under agroclimatic conditions in the Maharashtra region are completed, these chosen PGPR isolates may be utilized in biofertilizer formulations. Research and implementation of PGPR strategies can serve as a model for knowledge sharing, enabling farmers to adopt sustainable practices & enhance whole agricultural efficiency.

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This thesis represents the culmination of years of hard work, dedication, and learning, and it would not have been possible without the support, guidance, and encouragement of many individuals. I would like to take this opportunity to express my heartfelt gratitude to all those who contributed to the success of this project.

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Sharda Shivaji Gavande

26/10/2025.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	: Percentage
µL	: Microlitre
°C	: degree centigrade
ACC	: I -aminocyclopropane-1 -carboxylate
ANOVA	: Analysis of Variance
BLAST	: Basic local alignment search tool
CAS	: Chrome Azurol S
CFU	: Colony forming unit
CMC	: Carboxy-methyl cellulose
cm	: Centimeter
CTAB	: Cetyl trimethylammonium bromide
DAS	: Days after sowing
DAI	: Days after inoculation
DNA	: Deoxyribonucleic acid
DNS	: 3,5- dinitrosalicyclic acid
DW	: Dry weight
HCN	: Hydrogen cyanide
EDTA	: Ethylene diamine tetra acetic acid
FW	: Fresh weight
HCN	: Hydrogen cyanide
lAA	: Indole acetic acid
M	: Molar
mg	: Milligram

min : Minutes

ml : Millilitre

mm : Millimetre

NA : Nutrient agar

nm : Nanometer (Wavelength)

OD : Optical Density

P : Phosphorus

PCR : Polemerase chain reaction

PGPR : Plant growth promoting rhizobacteria

PGP : Plant growth promoting

pH : Negative log of hydrogen ions

RCBD : Randomized Complete Block Design

ppm : Parts per million

rpm : Rotations per minute

TAE : Tris- acetate EDTA

TCP : Tricalcium Phosphate

TE : Tris EDTA

µg : Microgram

µM : Micromolar

LIST OF APPENDICES

Chapter 1

Introduction

The world faces challenges that include overcrowding, industrialization, urbanization, food scarcity, along with drought, indicating substantial obstacles for the agriculture sector, which is essential for assuring food accessibility. The rapidly growing population, along with the reduction of agricultural area because of urbanization and industrialization, has posed an enormous threat to food safety. The yield and productivity of agricultural crops must rise in tandem with the production of agricultural food to fulfil the growing demand for sustainable agriculture (Basu *et al.,* 2021). Consequently, our sole remaining alternative is to enhance agriculture yield by utilizing the existing land and water resources.Climate change poses several environmental difficulties to plants that impede their growth & productivity (Gao *et al.,* 2007).

The rhizosphere is the small area of soil that surrounds plant roots and influences bacterial activity, proliferation, and interactions. It is characterized by rhizobacteria that promote plant growth, an overly complex root structure, and interactions among microflora & fauna. Because rhizospheric bacteria devour different substances generated through root cells as nutrients, rhizospheric soil has a considerably greater number of these microorganisms than subsoil. Plant growth promoting rhizobacteria, or PGPRs, are free-living soil microbes that thrive in the rhizosphere and rapidly colonize plant root systems (Kloepper and Scroth 1978).

PGPR stimulates plant development through production of phytohormones (Sivasakthi *et al.,* 2014). Auxin, gibberellins, & cytokinins are examples of phytohormones produced by some rhizobacteria (Rupaedah*et al.,* 2014). For cell division, growth, along with root initiation, IAA (indole acetic acid) is essential. (Salisbury 1994). The production of siderophores and chemicals that cause antibiosis by rhizobacteria has been the focus of various investigations aimed at understanding more about PGPR (Maksimov *et al.,* 2011).

The climate in Vidharbha and Marathwada regions is hot & dry, contrasting with the mild and humid climates of Konkan and Western Maharashtra. This climatic diversity significantly influences the agricultural landscape, leading to distinct crop systems. Marathwada predominantly cultivates cotton, bajra, and jowar, whereas Konkan and western Maharashtra specialize in rice, sugarcane, and coconut cultivation. The Konkan region experiences a tropical

monsoon climate due to its coastal location. It has heavy rainfall during the monsoon season, which supports lush vegetation and agriculture. The microbial flora in Konkan's soil is diverse and includes various beneficial microorganisms. The Western Ghats and Western Coastal regions are considered to be the most important parts of Maharashtra State. Western Maharashtra has a hot and semi-arid climate. Major agricultural development indices indicate Western Maharashtra to be ahead of other regions. Understanding these regional differences underscores the significance of adapting agricultural practices to local conditions.

There are frequent droughts in Marathwada. Unpredictable rainfall and elevated temperatures brought on by climate change have only made this agonizing scenario worse. But now, things are shifting. There is currently a potential method for enhancing plant growth and drought tolerance. Because osmo-tolerant PGPR help organisms utilize water more efficiently, they can be employed as inoculants to alleviate water stress. Controlling the physiological response to water deprivation, preserving plant life, and boosting the resilience and growth of agricultural products are the key functions of PGPR. The PGPR promotes drought tolerance by enhancing the shape and architecture of the root system. Farmers are shifting their attention from cultivating cash crops, which require a lot of inputs, to crops like maize, soybeans, and chillies, which offer greater profits to farmers. These crops are cultivated with both irrigation & rainfall.

Recent investigations have unequivocally demonstrated the beneficial effects of PGPR on the increasing production of several agricultural crops, that include cereals, in a variety of settings with varying ecological conditions. This has raised awareness of the beneficial rhizobacteria linked to cereals (Ozturk *et al.,* 2003; Marques *et al.,* 2010; Mehnaz *et al.,* 2010; Zhang *et al.,* 2012). An awareness of the collective bacterial population, their characterisation, and identification are required to comprehend the distribution and variety of native microorganisms across the rhizosphere of certain agricultural plants (Chahboune, 2011). In view of the growing awareness of agricultural practices that rely on chemical fertilizers, it is imperative to investigate region-specific strains of bacteria that could be employed as a growth-promoting or -enhancing inoculum to achieve targeted crop production (Deepa *et al.,* 2010).

Some PGPR have the capacity to create various bioactive chemicals, that include fungicidal as well as antibiotic compounds, along with their ability to stimulate growth of plants (Dey *et al*., 2004; Lucy *et al.,* 2004).

Ideal PGPR strain should have certain remarkable characteristics as discussed below:

- It should be eco-friendly and highly competent with rhizosphere.
- It must be capable of encouraging growth and development of plants.
- It must be in harmony with other rhizospheric bacteria in the soil.
- After inoculation, it must be capable of colonizing the plant roots in significant numbers.
- It must have wide spectrum of action.
- It must be able to tolerate physicochemical factors for example heat, oxidants, radiations, & desiccation.
- It must have aggressive functions over the existing rhizobacterial strains.
- It must be used as biofertilizers (Basu *et al*., 2021).

The microbes evolve faster than other organisms and also exhibit more complex correlations between strains. Therefore proper scientific identification of microbes considering stable characters is needed. Recently 16S rRNA technique has a widely used as reliable technique for the study of the taxonomy and phylogeny of soil microbial diversity (Srinivasan *et al*., 2001). The metagenomic technique for sequencing the conserved 16S rRNA region is effective and accurate. It also indicates reliable identification of bacterial groups in conjecture with conventional phenotype and biochemical assay procedures (Boivin-Jahns *et al*., 1995). 16S rRNA technique includes calculating the relevance of identical DNA in the whole genome by categorizing similarities between different patterns of restriction, specifically the ribotyping and study of related homologous gene sequences. Ribotyping and resemblance to DNA-DNA are the high-quality procedures to categorize various bacterial strains.

Moreover, the approach for identifying any rhizobacterial isolates at the genetic level is simple and accurate (Tarkka*et al*., 1994). 16S rRNA sequences are highly conserved housekeeping genes that are found in nearly all bacteria and are wide enough (1500 bp) for bioinformatics study (Janda and Abbott, 2007). This system involves PCR amplification of 16S rRNA region utilizing universal primers, amplicon sequencing and bioinformatics procedures

utilizing online bacterial sequence databases to create phylogenic relationships. 16S rRNA approach gives greater of 90 % recognition of the genus and lesser 65 to 83 % recognition of species. Isolates that are left undistinguished after the study may range from 1 to 14 % (Drancourt*et al*., 2000; Woo *et al*., 2003).

Characterizing, isolating, and replicating native strains of PGPR from the rhizosphere of major crops that include maize (*Zea mays*), soybean (*Glycine max*), & chilli (*Capsicum annuum*) was the aim of the present investigation, taking into consideration the general conditions. This could be a viable strategy to boost agricultural productivity while reducing the massive usage of artificial fertilizers.

Chapter 2

Review of Literature

In the rhizosphere of plants, PGPR is an essential colony of useful, root-colonizing bacteria. Their synergistic & antagonistic interactions with the soil microbes; lead to a variety of ecologically significant actions. Through the facilitation of biotic and abiotic stress tolerance, they boost the nutrition of host plants and encourage plant growth. The active growth-endorsing activities of PGPR are regarded as an environmentally beneficial substitute for dangerous chemical fertilizers. A biological strategy for the sustainable intensification of agriculture is the application of PGPRs as biofertilizers (Basu *et al*., 2021).

Dhayalan and Sudalaimuthu (2021) address the pressing global concern of rapid human population growth and its consequences, particularly the looming food shortage. They attribute the reasons behind this food shortage to factors like industrialization, urbanization, and modern civilization, which have led to a reduction in available agricultural land and a subsequent decline in food productivity. The authors emphasize the urgency of increasing productivity with limited agricultural resources while highlighting the detrimental effects of excessive chemical fertilizer use on soil ecosystems and human health. Furthermore, the study outlines future trends and research directions in the field of PGPR bio-inoculants, emphasizing their capacity to advance sustainable agriculture.

Oyuela Aguilar *et al*. (2021) performed a comprehensive investigation on rhizosphere-associated microbiome in Argentinian Malbec & Cabernet-Sauvignon vineyards, with a focus on identifying potential applications of the collected bacteria as biological fertilizers and agents for pathogen control. This research aimed to explore the diverse functions of the rhizosphere-associated microbiome and assess its potential benefits for vineyard management. Eleven different genera were represented by the 170 bacterial isolates that were recovered and categorized into three phyla: Firmicutes, Actinobacteria, & Proteobacteria. On the basis of the findings, a sizable portion of the bacterial isolates had one or more PGP traits. Notably, most isolates with PGP activities were found in the genus Pseudomonas, which was followed by

Bacillus, Pantoea, Arthrobacter, & Serratia. The study also emphasized the noteworthy synthesis of hydrolytic enzymes associated with biocontrol actions by *Bacillus* bacterial isolates.

Plant growth as well as development are positively impacted by PGPR, a group of beneficial microbes that invade plant roots (Arshad and Frankenberger, 1996). One of their key mechanisms involves the production of phytohormones, which act as chemical messengers within plants, regulating various physiological processes (Spaepen and Vanderleyden, 2011). This review explores the current understanding of how PGPR-derived phytohormones contribute to enhanced plant growth.

PGPR are known to generate a wide variety of phytohormones, that includes auxins, cytokinins, & gibberellins (Zakir *et al*., 2004). Auxins, like IAA (indole-3-acetic acid), perform a vitalfunction in root development. IAA produced by PGPR stimulates lateral root proliferation & root hair elongation, leading to increased nutrient & water uptake by plants (Spaepen and Vanderleyden, 2011), (Nath *et al*., 2017). Cytokinins, on the other hand, promote division of cells& shoot growth, while gibberellins stimulateelongation of stem &expansion ofleaf (Zakir *et al*., 2004). By influencing the production and distribution of these key phytohormones, PGPR can significantly impact plant architecture and biomass accumulation.

The benefits of PGPR extend beyond direct growth promotion. Some PGPR strains can manipulate plant hormone levels to enhance stress tolerance. For example, certain bacteria possess the enzyme ACC deaminase. This enzyme breaks down the ethylene precursor ACC, thereby reducing stress-inducing ethylene levels in plants (Belimov*et al*., 2009). This can enhancetolerance of theplant to salinity, drought, as well asseveral other environmental stresses.Research on PGPR and their phytohormone production is a rapidly evolving field. Scientists are actively exploring the diversity of phytohormone-producing PGPR strains along with their potential utilization in sustainable agriculture. Identifying & characterizing novel PGPR with potent plant growth-promoting abilities may facilitate development of biofertilizers that are eco-friendly and reduce reliance on chemical fertilizers (Kumar *et al*., 2014).

Auxin:

In Telangana state, India, Damodarachari *et al*. (2018) carried out a comprehensive study with the goal of isolating rhizobacteria from various rhizospheric soils.Mineral nutrient solubilization (zinc, potassium, & phosphorus), IAA production, ACC deaminase activity, EPS (exopolysaccharide) production, biocontrol potential, and tolerance to a variety of abiotic stresses, that include pH, temperature, salt, drought, as well as heavy metals, were among the characteristics that the researchers looked for to screen these isolates for their ability to promote plant growth in vitro. The study identified a total of forty-four Pseudomonas spp. isolates based on cultural, morphological, and biochemical characterization. Among these isolates, twenty-eight exhibited significant plant growth-promoting properties. The researchers also evaluated the isolates' tolerance to various abiotic stresses, revealing distinct responses.

Auxin, a group of plant hormones primarily composed of Indole acetic acid (IAA), perform a central role in various aspects of regulationof growth& development of plants (Davies, 2010). Auxin often referred to as IAA, is a critical phytohormone for root development. Rhizobacteria that produce IAA can significantly influence plant architecture. Studies by Spaepen and Vanderleyden (2011) have shown that PGPR-derived IAA stimulates lateral root proliferation and root hair elongation. This enhanced root system leads to increased nutrient & water uptake by the plant, ultimately promoting growth and yield (Nath *et al*., 2017).

To isolate several PGPR isolates, Singh *et al*. (2020) gathered a total of nine soil samples. Five PGPR isolates out of 56 bacterial isolates were positive for IAA synthesis in nutritional broth without the addition of L-tryptophan. By creating a clear halo zone that is more than 3mm in diameter surrounding the colonies, sixteen bacterial isolates are able to solubilize insoluble phosphate.

23 rhizobacterial isolates with plant growth promotion were isolated by Baliyan *et al*. (2018). These isolates exhibited a variety of functionally advantageous traits and isolates mutually non-inhibitory synergistic interaction in vitro.In the range of 78.6-82.5µg/ml, three isolates demonstrated the capacity to fix nitrogen and release IAA.

Tanveer and Ali (2022) conducted a study to evaluate the effectiveness of Bacillus and Rhizobium strains in producing auxin and enhancing *Vigna radiata* (mung beans) growth under conditions of drought stress. Published in the Pak-Euro Journal of Medical and Life Sciences, their research addresses the critical issue of drough tinduced stress on growth ofcrop & explores potential solutions through microbial interventions. The study involves assessing the ability of Bacillus and Rhizobium strains to produce auxin, a plant growth hormone, in bacterial culture supernatants.

Auxin exerts its influence on plants through a multitude of physiological processes. Here are some key areas it impacts:

- **Root Development**: Auxin is a critical regulator of root development. It promotes lateral root formation, root initiation, as well aselongation of root hair (Perez-Torres *et al*., 2018). This enhanced root system allows plants to acquire more water & nutrients from soil, ultimately leading to improved growth and yield.
- **Stem Elongation**: Auxin stimulates cell elongation in stems, promoting plant height and overall growth (Petrasek *et al*., 2006). This effect is particularly important for some crops where stem length is a key factor in yield, such as asparagus.
- **Apical Dominance**: Auxin generatedatapex ofshoot suppresses bud growth in lateral buds, a phenomenon refers as apical dominance (Sargent *et al*., 1994). This ensures the plant prioritizes growth of the main shoot, leading to a more defined architecture.
- **Vascular Tissue Development**: Auxin is essential for development of xylem & phloem, water, vascular tissues that carry nutrients, as well as products of photosynthetic activity throughout the plant (Teale *et al*., 2006). This efficient transport system is essential for plant health and survival.
- **Fruit Development**: Auxin is involved in fruit set and development. It promotes cell division and fruit growth, contributing to higher yields in fruit-bearing plants (Flaishman*et al*., 2002).

The influence of auxin is mediated by a complex signalling pathway. Auxin binds to specific receptors in plant cells, starting a series of actions that eventually result in modifications to cellular reactions along with gene expression (Santner and Estelle, 2009). These changes

influence various cellular processes, resulting in the diverse physiological effects observed in plants.Understanding auxin's functions allows for targeted manipulation in agricultural practices. Synthetic auxins can be applied to promote root growth in transplants, stimulate fruit sets in certain crops, and even control unwanted shoot development in others. However, a delicate balance is crucial, as excessive auxin application can lead to detrimental effects on plant growth.

Gibberellins: Gibberellins are another class of phytohormones known for their role in stem elongation and leaf expansion. Research by Zakir *et al*. (2004) suggests that some rhizobacteria can contribute to these processes by synthesizing gibberellin-like substances. This hormonal influence by PGPR can lead to taller plants with larger leaves, potentially improving overall biomass and crop yield.The story of gibberellins begins with the work of Kurosawa and Yokota (1926) that identified a fungal metabolite causing excessive stem growth in rice seedlings. This initial observation sparked a journey of unravelling the gibberellin's influence. Subsequent research by Phinney (1958), established the role of gibberellins in promoting seed germination, leading to a better comprehension of their diverse functions. More recently, research by Hedden and Sponsel (2011), highlighted the intricate signaling pathways gibberellins activate within plants, opening doors for targeted manipulation in agriculture.

While stem elongation is a well-known gibberellin effect, the literature reveals a broader spectrum of influences. Studies by Davies (2010), demonstrated GA involvement in the production of key enzymes, while Bewley *et al*. (2012) explored their role in breaking down seed reserves during germination. Interestingly, research by Seo *et al*. (2011) even suggests gibberellin involvement in plant stress response pathways, showcasing their adaptability to environmental challenges. The knowledge gleaned from gibberellin research has translated into practical applications for farmers. As demonstrated by King (2003), gibberellins can enhance seed germination, leading to improved crop yields. Additionally, Perez-Jones *et al*. (2010) documented the use of gibberellins to promote fruit growth in grapes, highlighting their potential to manipulate specific aspects of plant development for economic benefit. The literature emphasizes that gibberellins don't operate in isolation. Studies by Swain and Jones (2007) revealed their intricate interactions with several other plant hormones like cytokinins&auxins. This complex network, as explored by Gupta and Prakash (2013), fine-tunes plant development, ensuring a coordinated response to internal and external cues.

Cytokinins: Cytokinins perform a crucialfunction in cell division and growth of shoot. Rhizobacteria capable of producing cytokinins can significantly impact these processes. As highlighted by Spaepen and Vanderleyden (2011), PGPR-derived cytokinins can stimulate cell division in the shoot, leading to increased shoot branching and leaf production. This translates to a healthier plant canopy and potentially higher yields.The journey of cytokinins began with the work of Miller *et al*. (1954), who identified a cell division-promoting factor in maize endosperm. Since then, research has revealed a multitude of cytokinin functions beyond cell division. Studies by Mok and Mok (1985) highlighted their involvement in bud development, shoot growth, and leaf expansion. Additionally, research by Argueso *et al*. (2009) suggests their role in delaying leaf senescence, allowing plants to maximize photosynthetic potential.

Understanding how cytokinins achieve their diverse effects is key. Research by Zhao *et al*. (2010) shed light on the complex signalling pathways cytokinins activate within plants. These pathways involve specific receptors and histidine kinases, ultimately leading to changes in gene expression that govern various developmental processes. The intricate interplay between cytokinins and other hormones, as explored by Werner *et al*. (2008) further refines plant development. For instance, cytokinin antagonism with auxin helps maintain the balance between shoot and root growth. The knowledge gained from cytokinin research has translated into practical applications for farmers. As demonstrated by Sakamoto *et al*. (2008), cytokinins can be used to promote bud break and lateral shoot development, leading to denser and more productive crops. Additionally, research by Jameson (2000) explores the use of cytokinins to delay fruit senescence, extending shelf life and marketability of harvested produce.

Despite significant progress, our understanding of cytokinins remains incomplete. Current research, exemplified by the work of Kimura *et al*. (2018), delves deeper into the regulation of cytokinin biosynthesis and degradation pathways. This knowledge holds promise for developing more targeted and efficient applications of cytokinins in agriculture. Additionally, research by Nguyen *et al*. (2018) explores the role of cytokinins in plant stress responses, opening doors for developing stress-tolerant crops.

The influence of phytohormone-producing rhizobacteria extends beyond direct growth promotion. Some PGPR strains can manipulate plant hormone levels to enhance stress tolerance.

For example, studies by Belimov *et al*. (2009) demonstrate how certain bacteria possess enzyme ACC deaminase, which breaks down ethylene precursor ACC. This reduction in stress-inducing ethylene levels in plants can improve their tolerance to drought, salinity, and other environmental challenges.

Mitigating Water Stress: The Potential of Osmotolerant Plant Growth-Promoting Rhizobacteria

Water scarcity is a growing concern for agriculture worldwide. Drought conditions not only limit water availability but also create a stressful environment for plants. In this scenario, harnessing the power of beneficial microbes emerges as a promising strategy. Osmotolerant PGPR's potential as inoculants to minimize water stress and improve plant WUE (water use efficiency) is examined in this review. A diverse group of soil bacteria which colonize roots of plants & establish a symbiotic relationship are PGPR. These microbes offer a multitude of benefits to their plant partners, including promoting growth of plants, improving stress tolerance, & enhancing the uptake of nutrients (Mendes *et al*, 2017). While all PGPR offer some level of benefit, osmotolerant PGPR holds particular promise for mitigating water stress. These bacteria possess adaptations that allow them to thrive in environments with low water availability. Their tolerance to osmotic stress enables them to survive and function effectively in rhizosphere, area of soil that encompasses plant roots (Singh *et al*, 2011).

Mechanisms of Water Stress Reduction

Mukhtar *et al*. (2022) conducted a comprehensive study focusing on introduction of heat tolerance in the tomato cultivars using heat tolerant plant growth-promoting bacteria in field conditions. The research aimed to assess the potential of these bacteria in mitigating heat stress in tomato plants through two planned studies: isolation, characterization, and field trials. In the initial stages of the study, various traits linked with promotion of plant growth were evaluated in the isolated bacteria. These traits included IAA production, ammonia production, phosphate solubilization, synthesis of HCN (hydrogen cyanide), siderophores, extracellular enzyme activity (amylase, protease, catalase, & pectinase), ACC-deaminase activity, as well as synthesis of

exopolysaccharide. Presence of these beneficial traits in the selected heat-tolerant isolate is confirmed through the results.

Osmotolerant PGPR employs various mechanisms to alleviate water stress in plants. Some key strategies include:

Phytohormone Production: PGPR can synthesize and secrete plant hormones like auxins and cytokinins (Ali *et al*, 2014). These hormones promote root development, thereby increasing the plant's surface area for water uptake.

Panchami *et al*. (2020) isolated eighty eight isolates; ten were screened as promising based on their performance in growth promoting attributes such as production of indole acetic acid, gibberellic acid, siderophore, hydrogen cyanide, heavy metal tolerance and antibiotic resistance. Genetic analysis was carried out to assess the phylogenetic relationship using 16S rRNA sequencing. The phylogenetic analysis exhibited clear clustering of isolates into three phyla namely Firmicutes, Actinobacteria and γ-proteobacteria. Majority of the isolates were grouped into Bacillus and Pseudomonas at genus level. Three different plant inoculation studies at nursery and field level, *viz*., *Bacillus subtilis* TAUC1, *Bacillus subtilis* TAUC2 and *Pseudomonas putida* TAUC10. The combined inoculation of bioinoculants was superior over individual inoculation with respect to growth, soil and plant nutrient content, biochemical constituents, rhizosphere population, soil enzyme activities and yield.

Alia *et al*. (2018) isolated and characterized the PGPRs of plum (*Prunus domestica*) rhizosphere in Pakistan. A total of ninety five rhizobacteria were isolated, out of which forty strains were selected. The selected isolates were screened for *in vitro* plant growth promoting potential and were subsequently evaluated for host plant growth promotion. The selected isolates demonstrated strong lytic enzymatic activities and were able to produce ammonia, siderophore, Hydrogen cyanide along with capability of phosphate solubilisation. Moreover, the results showed a significant growth suppression of pathogenic *Fusarium oxysporum* and *Rhizoctonia solani* in an *in vitro* assay. The plant microbe interaction study was carried out using 11 most efficient rhizobacterial strains inoculated to roots of plum plants. The inoculated PGPRs significantly augmented the leaves number per shoot, shoot diameter, shoot length and plant

height. The inoculation also significantly increased the chlorophyll contents of leaves, concentration of micro and macro nutrients compared with control.

Nutrient Mobilization: Even under drought situations, some PGPR strains have the ability to solubilize vital minerals like iron and phosphorus, enhancing their availability to plants. Plant growth and stress tolerance are improved by enhanced nutrient uptake.

El-Hamshary *et al*. (2019) conducted an insightful study on the molecular characterization of phosphate-solubilizing microorganisms, highlighting their essential function in transforming insoluble phosphate forms into a more plant-friendly and accessible state. Phosphorus, a vital macronutrient for plants, significantly enhances various biological processes within plant systems. However, the form in which phosphorus often exists in soils is insoluble and unavailable to plants, necessitating the assistance of soil microbes with phosphate-solubilizing capabilities. This study focused on both bacteria and fungi, investigating their potential to convert insoluble phosphorus. Overall, they highlighted the potential of these bacterial and fungal isolates as potent bio-fertilizers for phosphate, offering promising prospects for improving phosphorus availability in agricultural soils and ultimately enhancing plant growth and productivity.

Perez-Perez *et al*. (2021) performed a substantial investigation focusing on characterization of potassium solubilizing bacteria that inhabit the rhizoplane of corn plants. Given the vital role of potassium in development & growth ofplants. Research goalis to isolate as well as identify microorganisms capable of solubilizing these mineral potassium sources. The selected bacteria underwent comprehensive characterization, including an assessment of colony appearance, cell morphology, and identification through partial 16S rDNA sequencing. Furthermore, the study evaluated their capacity to solubilize and release potassium under several environmental conditions, that includessalinity, pH, andtemperature. The study successfully isolated eight strains being a member of the genera *Paenibacillus, Pseudomonas, Arthrobacter, Lysinibacillus, Bacillus* as well as *Stenotrophomonas*.

The research performed by David *et al*. (2023) concentrated on the isolation, molecular characterisation, and application of *Aspergillus niger* & *Penicillium chrysogenum*, which have

the potential to be utilized as biofertilizers to enhance rice development. The research aimed to explore alternatives to chemical fertilizers, as their use has been linked to a decline in soil fertility and overall soil health. In this study, the authors isolated these fungi from the rhizosphere of rice (*Oryza sativa* Linn) plants & employed both cultural as well as molecular methods to identify and characterize the fungal isolates. Various traits associated with mycofertilizer potential, that includesnitrogen fixation,protease synthesis, phosphate solubilization, &cellulose breakdown, were assessed utilizing standard laboratory techniques. The isolates' mycofertilizer potential was evaluated further utilizing a pot experimental approach in an in-situ greenhouse experiment. The researchers selected isolates which demonstrated phosphate solubilization, cellulase production, & protease production for the greenhouse experiment. Among the isolates tested, Aspergillus niger and Penicillium chrysogenum emerged as the most promising candidates. This research contributes to the exploration of sustainable substitutes for chemical fertilizers, which may help improve soil fertility as well as promote healthier agricultural practices.

Shyamili *et al*. (2021) delved into the exploration of endophytic microorganisms, specifically focusing on SVH1 Bacillus sp., and their capacity to encourage the growth of plants, with a particular emphasis on *Hemidesmus indicus*, a medicinal shrub of significance. Subsequently, these bacterial isolates underwent an evaluation of their plant growth-promoting capabilities. Notably, SVH1 demonstrated IAA production at a level of 23.48 µg/ml, surpassing many previous reports in terms of IAA production. The production of IAA was further validated through RP-HPLC analysis. Moreover, SVH1 exhibited a high phosphate solubilization index of 60, signifying its significant phosphate-solubilizing ability. This study underscores the potential of SVH1 Bacillus sp. as a valuable microorganism that may contribute to the enhanced growth and bioactive compound production in *Hemidesmus indicus*. The research expands our understanding of endophytic microorganisms & their possible application in encouraging the development of therapeutic plants, which could have far-reaching implications for traditional medicine and pharmacological industries.

Nadieline *et al*. (2019) addressed the pressing issue of phosphorus deficiency in intertropical soil regions, characterized by high phosphorus-fixing capacity and low phosphorus content. The depletion of rock phosphate resources, traditionally used for phosphate fertilizer

production, and detrimental impact on environment associated with chemical fertilizers have spurred interest in the sustainability of fertilization practices. This investigation aimed to isolate as well as characterize potential PSB (phosphate-solubilizing bacteria) from two phosphorus-deficient agricultural regions in Senegal. The authors identified twelve potential PSB isolates and subsequently subjected them to screening for additional plant growth-promoting traits, that include production of indole-3-acetic acid (auxin) & siderophores. Moreover, the isolates underwent characterization through 16S rDNA sequencing to elucidate their taxonomic affiliations. The results indicated that all isolates exhibited auxin production, while seven of them demonstrated siderophore production. In light of the growing concerns about phosphorus deficiency and the environmental repercussions of conventional fertilizers, this research underscores the importance of exploring alternative approaches, such as harnessing the capabilities of phosphate-solubilizing bacteria as potential biofertilizers. The findings contribute to the ongoing efforts to develop sustainable agricultural practices, particularly in regions grappling with phosphorus deficiency.

Bacterial isolates were isolated from rhizospheric soil by Rai *et al*. (2019) utilizing serial dilutions of 10^{-5}, 10^{-6}, & 10^{-7}. On nutrient agar media, five of the bacterial isolates displayed traits of Pseudomonas species. Zinc & phosphate solubilization is quantitatively investigated via broth & agar assay mrthod. As solid media, Pikoviskaya's Broth & Agar are utilized, correspondingly.Pseudomonas fluorescence isolates in broth determined quantitatively. The amount of zinc in the supernatant was calculated by employing an atomic absorption spectrophotometer at 600 nm for OD. The colony's diameter and the haloes surrounding it were measured. The clear zone turning red when flooded with methyl red solution is a sign that acid is being produced.

Singh *et al*., (2020) collected a total number of 9 rhizospheric soil samples from different crops and isolated a total number of 56 rhizobacterial isolates. Among 56 only 16 strains of rhizobacteriareported to be positive for multiple PGP traits that includeIAA, phosphate solubilization, ammonia & hydrogen sulphide production,siderophore production. Maximum phosphate solubilisation index (3.49) have RKM15 strain, phosphate solubilization (339 mg/L) and siderophore (70.54%). The RKM25 strain have maximum IAA production.

Chen *et al*. (2020) in their study isolated Bacillus aryabhattai (SK1-7) from poplar rhizosphere which solubilized potassium effectively (10.8 µ g/mL with the potassium-solubilizing rate of 32.6 %) owing to the fact that it acidifies the medium and decreased pH of the medium by secreting organic acids which further form complex with silicon and aluminum ions in minerals leading to dissolution of minerals releasing potassium (Sheng *et al*. 2002).

Dubey *et al*. (2021) recorded increased zinc content at different growth stages of rice viz. Maximum tillering (0.64 mg kg^{-1}), Panicle initiation (1.39 mg kg^{-1}), Harvesting stages (1.43 mg kg^{-1}) on application of nano-ZnO in rice leading to supplying sufficient zinc through the nano-fertilizer.

The investigationexecuted by Sulastri *et al*. (2022) investigated colonization ability of 15 indigenous halotolerant PGPB (Plant Growth-Promoting Bacteria) on three different agronomic crops under saline stress conditions. The research highlights the variation in the colonization ability of halotolerant PGPB isolates on the basis of factors for example bacterial strain, plant tissues, plant species, as well as salinity levels. These findings have implications for the practical use of halotolerant PGPB in agriculture, particularly in saline environments, where their ability to colonize specific crops can notably impact growth and health of plant.

Plant Growth-Promoting Rhizobacteria gives Drought Resistance through Root Enhancement

Drought impairs plant growth along with productivity, making it a serious danger to world agriculture. One promising strategy to combat drought stress involves harnessing the power of PGPR. This investigation explores well-established role of PGPR in improving root system morphology and architecture, ultimately enhancing plant drought resistance.A plant's root system serves as its lifeline, anchoring it to the soil and facilitating water and nutrient uptake. A diverse group of soil bacteria are PGPR that forms beneficial partnerships with plants. These microbes colonize plant roots & exert a multitude of positive effects on their host. Notably, PGPR performed a substantial function in influencing root system morphology & architecture (Gupta and Pandey 2014).

Molecular characterization of PGPR

There is significant scope for molecular tools in the identification and monitoring of soil microbes phylogeny. Reports are available on molecular approaches that can be used for details among PGPR and other microorganisms. The ubiquity of 16S rRNA gene sequencing is employed for all prokaryotes and its sequence analysis can be used to assess the relationship between various prokaryotic species. The basis of identification of bacteria depends on the hypervariable 36 region that is conserved sequences and usually interspersed in different bacteria which can be used to distinguish organisms (Sacchi *et al*., 2002).

Four distinct rhizobacterial strains have been identified from mung beans by Kumari *et al*. (2018) employing King's B and nutrient agar media. Based on biochemical examination along with 16S rDNA gene sequencing, they were determined to be *Pseudomonas* sp., *Bacillus* sp., & *Acinetobacter* sp. The production of IAA (ranging from 45.66 µg/ml to 111.93 µg/ml), phosphate solubilization (ranging from 504.9 µg/ml to 1341 µg/ml), ammonia, HCN, siderophores, & antagonistic activity against Rhizoctonia solani, the causative agent of root rot in mung beans, was exhibited by all isolates.

David *et al*. (2023) executed aninvestigation focusing on *Aspergillus niger* and *Penicillium chrysogenum* sisolation, molecular characterization, as well as application along with biofertilizer potential to boostgrowth ofrice. The research aimed to explore alternatives to chemical fertilizers, as their use has been linked to a decline in soil fertility and overall soil health. In this study, the authors isolated these fungi from the rhizosphere of rice (*Oryza sativa* Linn) plants as well as employed both cultural & molecular methods to identify and characterize the fungal isolates.

Adal and Lemma (2023) conducted a study focusing on isolation & characterization of potential biocontrol rhizospheric bacteria with objective of combating white rot (*Sclerotium cepivorum*), a pathogen that severely affects garlic (*Allium sativum* L.) crops. Garlic holds significant importance as a versatile crop used in various aspects, including food, medicine, condiments, and as a cash crop vegetable. The study aimed to identify and evaluate potential antagonists capable of controlling this destructive pathogen. In their research, the authors

screened 23 rhizospheric bacterial isolates for their biocontrol potential against the white rot pathogen.

A noteworthy work on the molecular identification as well as screening of phosphate-solubilizing rhizobacteria from Lombok Island's mangrove ecosystem is presented by Zulkifli *et al*. (2020). In their research published in the Journal of Tropical Biology, the authors explore the potential of these rhizobacteria as an essential part of formulations for biofertilizers, To boost food production on a local & national level while minimizing environmental risks associated with chemical fertilizers. The authors emphasize the importance of phosphate solubilizing rhizobacteria in biofertilizer development. All BRM isolates clustered with bacterial species from the Genus Paenibacillus in the phylogenetic tree, in accordance with the molecular analysis, which was carried out by comparing the isolates' 16S rRNA gene sequences with the GenBank database.

With the possible application of these strains as bioinoculants,Tzec-Gamboa *et al*. (2020) provide an interesting study centred on the biochemical along with molecular characterization of native rhizobia nodulating Leucaena leucocephala.Effective symbiotic relationships in soil necessitate inoculation with suitable rhizobial strains, making this study particularly relevant for agricultural biotechnology. The authors begin by emphasizing the importance of the legume-rhizobium association as a means to enhance crop productivity through N2-fixation. They highlight the necessity of selecting appropriate strains for effective symbiosis establishment in agricultural soils. Additionally, they introduce the concept of PGPR, which has the potential to enhance plant health and soil fertility, thereby attracting biotechnological interest.

El-Hamshary*et al*. (2019) conducted an insightful study on the molecular characterization of phosphate-solubilizing microorganisms, shedding light on their pivotal role in transforming insoluble phosphate forms into a more plant-friendly and accessible state. This study focused on both bacteria and fungi, investigating their potential to convert insoluble phosphorus. Isolates were obtained from the rhizospheres of Curcuma, Ocimum, and Eruca plants. The researchers conducted comprehensive screening, identification, and intrinsic antibiotic resistance testing to evaluate the suitability of these microorganisms for use as bio-fertilizers.Nine bacterial strains (R1B2, R2W22, R3B28, R4B31, R5C33, R6B34, R7C35, R8B40, and C15) among 100 bacterial

isolates tested for phosphate solubilization demonstrated essential phosphate solubilization abilities, as evidenced by clear halo zones in PVK (Pikovaskya) & NBRIP (National Botanical Research Institute Phosphate) media.

Employing 16S rDNA sequencing, Gamit and Tank (2014) isolated bacteria from *Cajanus cajan*rhizospheric soil and found that they were most comparable to *Pseudomonas pseudoalcaligenes*. When evaluated in a pot experiment with *Cajanuncajan*, these isolates demonstrated a substantial rise in biomass, shoot length, along root length.

Effect of PGPR on vegetable crops

Hyder *et al*. (2020) studied anti-fungal activity of PGPR under *in vitro* condition, colonizing around chilli rhizosphere, against virulent strains of *Phytophthora capsici* which cause damping-off in cultivated chilli pepper. Eight of the fifteen bacterial strains that they isolated from the chilli rhizosphere were shown to potentially be hostile to *P. capsiciin vitro*. Additionally, biochemical and molecular analyses were performed on bacterial strains that showed potent antifungal activity. While siderophore production ranged from 12.5% to 33.5%, all investigated bacterial strains produced HCN (hydrogen cyanide), catalase, as well as IAA in positive amounts (varing from 6.10 to 56.23 μg ml^{-1}). The examined bacterial strains' 16S rRNA sequence analysis revealed 98–100% similarity with *Bacillus subtilis*, *B. cereus*, *B. megaterium*, *Pseudomonas putida*, *P. aeruginosa*, as well as *P. libanensis*. All of the investigated bacterial strains considerably reduced *P. capsici* infections (52.3–63%) & boosted the plant growth characteristics in chilli peppers, as determined by greenhouse assessments.

Naqqash *et al*. (2016) performed aninvestigation focusing on potential of PGPR to boostgrowth of potatoes while reducing need for nitrogen and phosphorus fertilizers. Five different bacteria isolated byresearchers, Agrobacterium sp. TN14, Azospirillum sp. TN10, Enterobacter sp. TN38, Rhizobium sp. TN42, & Pseudomonas sp. TN36, from potato rhizosphere, as well as identified them on the basis of their 16S rRNA gene sequences.Research emphasizes the bacterium's multifaceted mechanisms of antagonism against soil-borne fungi, underlining its significance in sustainable agricultural practices.

A thorough investigation into the application of heat-tolerant PGPB in the field to induce heat tolerance in tomato cultivars was carried out by Mukhtar *et al*. in 2022. The isolate was then identified by 16S rRNA gene sequencing, and the successful amplification of the Acds gene revealed information on its genetic characteristics. The study's findings underscore PGPB'svaluable function in alleviating heat stress, which has implications for sustainable agriculture in regions prone to high temperatures.

Effect of PGPR on Cereals crops

Modi *et al*. (2022) conducted a study focused on isolating and characterizing Bacillus consortia with the potential for promoting plant growth in rice (*Oryza sativa* L.). In fifteen distinct sites in Gujarat, India, the researchers gathered soil samples from the rhizosphere of uncultivated weeds. Following primary screening, the researchers selected 15 KMB (potassium-mobilizing), 27 PSB (phosphate-solubilizing), as well as 20 NFB (nitrogen-fixing) isolates. Following molecular identification, only Bacillus isolates were further characterized. 3 superior Bacillus isolates were selected through secondary screening from each category. All isolates, regardless of category, exhibited compatibility and demonstrated significant plant growth-promoting traits, including production ofammonia, ARA (acetylene reduction activity), phosphate solubilization, potassium mobilization, siderophore production, Indole-3-acetic acid (IAA) production, & production of organic acid.

Ramesh *et al*. (2014) isolated *Bacillus aryabhattai* strain MDSR14 and used as PGPR which enhanced MBC in rhizospheric soil of wheat (241mg C kg^{-1} soil) and soybean (263mg C kg^{-1} soil) and Dehydrogenase activity in wheat (137 µg triphenyl $formazane^{-1}$ soil $24h^{-1}$) and soybean (161 mg/µg triphenyl $formazane^{-1}$ soil $24h^{-1}$) rhizosphere.

Suleman *et al*. (2018) in a pot experiment used inoculation of *Enterobacter* sp. (MS32) in wheat to improve plant biomass compared to non-inoculated control. *Enterobacter* sp. Isolated from potato rhizosphere was used as PGPR in maize crop which increased root- shoot length and biomass of maize (Verma *et al*. 2018).

Effect of PGPR on Pulses crops

Ten bacterial strains were identified by Jain *et al*. (2016), who then tested them in vitro for qualities that would promote plant growth. Of the ten isolates, nine have been demonstrated to have the ability to solubilize phosphate, five to produce IAA, and three to fix nitrogen. Microbial strains SJ-5 demonstrated positive outcomes for every trait that promotes plant growth, and they were further described utilizing molecular & biochemical techniques.These were identified as Bacillus sp. by means of biochemical as well as 16S rRNA gene sequence analysis. Investigations on plant inoculation demonstrated that this strain of bacteria that promotes plant growth substantially boosted biomass & shoot and root length. Compared to the uninoculated control, a noticeable rise in the quantity of lateral roots was noted. Their research revealed that this PGPB might be employed to produce inoculums or biofertilizers to improve soybean growth and nutritional content.

Jida and Assefa (2011) isolated a total number of 30 bacterial isolates from lentil rhizosphere. They showed IAA production (36%) and inorganic phosphate solubilization (16.7%). All isolates are characterized as symbiotic nitrogen fixation.

Only two of the eight bacterial isolates that Srivastava *et al*. (2020) recovered from the lentil rhizosphere were identified as gram-positive, rod-shaped, endospore-former colonies with white, dry, & irregular edges on plates that included nutritional agar media. Every one of the 20 strains underwent antagonistic activity screening. Of the 20 bacterial isolates, only eight exhibited antagonistic activity against *Fusarium oxysporum* f. sp. lentis. The strains B4, B-5, as well as B-8 exhibited the greatest inhibition (%) in radial growth of *Fusarium oxysporum* f. sp. lentis.Strains B-1 and B-8 exhibited maximal phosphate solubilization efficiency ranging from 60% - 40%. Similarly all the 8 bacterial isolates showed IAA production.

Azotobacter chroococcum (AU-1), *Bacillus subtilis* (AU-2), *Pseudomonas aeruginos* (AU-3), as well as *Bacillus pumilis* (AU-4) are the four bacterial isolates from the Cicerarietinum (chickpea) rhizosphere that Pandey *et al*. (2019) isolated as PGPR. The ACC degrading potential of all four strains ranged from 600-700nmol αketobutyrate per mg of cellular protein per hour. All 4 rhizobacterial strains produce IAA of approximately 20-35.34 µg/ml. and all of these PGPR are capable of ammonia production.

A total of 39 rhizobacteria strains were recovered by Kumari *et al*. (2018) from the mung bean plant's rhizospheric soil. The pathogen Rhizoctoniasolani was tested for antagonistic activity in vitro by each isolate. The antagonistic ability of all four strains against Rhizoctoniasolani was confirmed. *Pseudomonas*, *Bacillus*, along *Acinetobacter* were the isolated cultural, morphological, & biochemical characteristics.

On Pikovskaya medium, Yousef (2018) identified an overall of 15 bacterial isolates from Helba (*Trigonella foenumgraecum*), wheat (*Triticum aesativum*), along with faba beans (*Vicia faba*). When six of these isolates reacted with Salkowski's reagent, they produced IAA by turning pink. In a tryptone broth medium with 0.3% NaCl, six of the fifteen bacterial isolates were able to produce substantial concentrations of IAA & antioxidants. IAA production by these bacterial strains varied, ranging from 13.0 to 25.5mg/L.

Overall 63 bacterial isolates were obtained from the lentil rhizosphere by Caamano *et al*. (2017), who then screened them for PGP activity. The 62 strains (57 lentil strains as well as 5 Adesmia strains) were able to produce IAA with concentrations ranging from 0.111 to 16.5µg/ml, whereas 17 strains exhibited ACC deaminase activity.Thirty eight were compatible with rhizobia. 16S rRNA sequencing was done to identify the 10 selected strains resulting as *Pseudomonas* sp.

Geetha *et al*. (2014) isolated 140 rhizobacterial strains from the green gram rhizosphere as well as tested them for antifungal activity towards *Macrophominaphaseolina*, *Colletotrichum capscici*, *Rhizoctonia solani*, as well as *Fusarium oxysporum*. Antagonistic activity demonstrating 20 isolates examined for their capacity to promote plant growth, produce extracellular enzymes, germinate seeds, and withstand salt and temperature. Ammonia, phosphate solubilization, IAA, HCN, & antifungal activity against phytopathogenic fungi were all produced by six isolates.

Twenty of the 40 chickpea rhizobacterial isolates that Kumari and Khanna (2014) isolated from Kings B medium had yellowish green pigmentation, as well as two of them had blue green pigmentation, which is typical of Pseudomonas sp. Sixteen isolates from NA medium had typical colony morphology, which was primarily off-white to creamish in color, typical of the genus Bacillus. Two of the isolates produced pink color pigmentation with the entire margin,

which was indicative of Serratia. In an in vitro dual culture test against *Fusarium osysporum* f. sp. *ciceris*, 15 isolates of *Pseudomonas* sp. 11 of *Bacillus* sp. and both Serratia isolates demonstrated antagonistic activity against *Fusarium oxysporum*.

For the purpose of further characterising the biochemical as well as morphological characteristics of pigeon pea, Rani *et al*. (2012) isolated an overall of 65 rhizobacterial isolates. Out of 65, 36 underwent screening for colony features, motility, & Gram nature. Six isolates were found to exhibit maximum phosphate solubilization, five demonstrated HCN production, eight separate isolates for enzyme productions that included chitinase, β-1.3 gluconase, along with IAA production, and seven of the 36 bacterial isolates tested for biochemical traits were selected for the PGPR trait analysis.

Ahmed *et al*. (2019) used PGPR Bacillus aryabhattai (S10) as an inoculation in mungbean and maize crops and recorded and improved K, P & Ncontents in mungbean and maize shoots over uninoculated control being a virtuous nutrient solubilizer.

Effect of PGPR on Disease Resistance

PTR-3 (*Pseudomonas aerugenosa*) has been demonstrated to be a more efficient biocontrol agent against *R. solani*, which causes rice sheath blight disease, by Kamei *et al*. (2014). P-solubilizing activity was best in the PTR-3 isolate (17.3 mg/50ml). The greatest concentrations of siderophore (3.9 μmol benzoic acid/ml), HCN, and salicylic acid (0.54 mg/ml) had been found in PTR-1. PTR-3 had the highest phosphate solubility.

The biocontrol activity of PGPR against four phytopathogens of Jatropha curcos, *Aspergillus verscolor*, *Fusarium oxysporium*, *Aspergillus nidulance*, & *Macrophominaphasiolina* was examined by Saraf *et al*. (2013). 10 PGPR isolates were found in agricultural fields. Following a 5 day incubation period, Jatropha seeds of *P. putida* and *P. pseudoalcaligens* diminished *M. phasiolina* growth by 90% & 45%, correspondingly.

After screening bacteria from the alfalfa plant's rhizosphere, Bharucha *et al*. (2013) determined that the bacteria were *Bacillus* species based on morphological as well as biochemical testing. *Aspergillus niger* & *Fusarium oxysporum* were utilized to evaluate the

antagonistic activity of *Bacillus species*. 200 mg/ml of siderophores have been extracted employing ethylene acetate.

Eighty isolates were separated and evaluated by Kumar *et al*. (2011) for features that promote plant growth as well as antagonistic activity against *Sclerotium rolfsii* & *Colletotrichum capsici*. The findings showed that the mycelial growth of *S. rolfsii* as well as *C. capsici* was statistically inhibited in 15.0 along with 43.7% of isolates, respectively. Furthermore, 48.7% of isolates produced siderophores, 57.5% solubilized phosphate, & 21.1% produced IAA in excess of 20lg/mL. Only three isolates, nevertheless, tested positive for every property. The Microbial Identification System (BIOLOG) was employed to identify possible bacterial isolates, and 16S rDNA sequencing of isolates demonstrated that Bacillus species predominated in India's farmed vegetable rhizosphere soil.

Singh, Kapoor, and Kaur (2022) offer a thorough and insightful exploration of enzyme-based biocontrol methods in the context of plant disease management. Their article highlights these strategies'potential to support environmentally friendly & sustainable approaches in agriculture.

Khan and Irfan (2022) present a valuable study focused on the isolation as well as identification of antifungal bacteria from citrus field's rhizosphere. Their research, published in the Punjab University Journal of Zoology, addresses a significant global issue: fruit rotting, primarily caused by fungal pathogens. The authors embark on their investigation by isolating overall 68 strains of bacteria from the citrus fields soil, aiming to assess their potential for antifungal activity. Utilizing the streak plate method, they rigorously screen these strains and identify one bacterium with notable antagonistic properties against the pathogenic fungus.

Awadh Ram *et al*. (2019) executed aninvestigation focusing on isolation & characterization of PGPR from organic preparations. In this research, bacteria were isolated from Amritpani and Panchagavya, organic preparations, and subjected to thorough analysis, including biochemical characterization and molecular confirmation using molecular tools. The study revealed that while the majority of the tested strains exhibited similar substrate utilization patterns, some isolates displayed variations.

Dasgupta *et al*. (2015) executeda research focused on screening PGPR from rhizospheric soil of Dhaincha (*Sesbania bispinosa*), with aim of identifying efficient PGPR strains that could potentially serve as alternatives to chemical fertilizers & insecticides in modern agriculture. PGPRis a group of advantageous microorganisms known for their capability to colonize the plant rhizosphere & encourage growth of plants through various mechanisms. Their utilization in agriculture has been gaining momentum as they offer sustainable and eco-friendly alternatives to conventional agricultural inputs.

Adal and Lemma (2023) conducted a study focusing on isolation & characterization of potential biocontrol rhizospheric bacteria with objective of combating white rot (*Sclerotium cepivorum*), a pathogen that severely affects garlic (*Allium sativum* L.) crops. Garlic holds significant importance as a versatile crop used in various aspects, including food, medicine, condiments, and as a cash crop vegetable. The study aimed to identify and evaluate potential antagonists capable of controlling this destructive pathogen. In their research, the authors screened 23 rhizospheric bacterial isolates for their biocontrol potential against the white rot pathogen. They conducted comprehensive laboratory and greenhouse experiments using microbiological techniques. Out of the 23 isolates, 11 (47.8%) exhibited promising results by inhibiting the radial pathogen's growth, achieving a growth inhibition zone ranging from 60 to 88%.

Jiao *et al*., (2021) evaluated PGPR as environment friendly way of controlling plant disease as well as improving promotion of plant growth. PGPR suppress disease by synthesizing pathogen-antagonistic compounds, as well as triggering plant immune response.

Chouyia *et al*., (2020) isolated overall 16 phosphate solubilizing bacteria from Moroccan oat rhizosphere. *Streptomyces roseocinereus* as well as *Streptomyces batalensis* were identified as the 2 strains MS1B15 & MS1B13. They had the greatest phosphate solubilization index (PSI=1.75 & PSI=1.63) and were further tested for PGP activities, for example ACC deaminase activity, siderophores, nitrogen fixation, IAA, along with antimicrobial activity against plant pathogens.

Bambhaniya *et al*., (2014) isolated two rhizobacterial isolates and evaluated the plant growth promotion and antagonistic activity against *Fusarium oxysporum* (MTCC6659) &

Alternaria solani (MTCC4633) which cause wilt and blight disease of root rot. Both these two bacterial isolates solubilize phosphate; produce IAA, ammonia and HCN.

Pseudomonas alcaligenes, *Bacillus pumilus*, *Rhizobium* sp., as well as *Pseudomonas alcaligees*have been evaluated by Akhtar *et al*. (2010) for their impact on wilt disease caused by *Fusarium oxysporum* f. sp. lentis. Reduction in growth of plant, number of pods & nodulation observed in significant wilting caused by *Fusarium oxysporum*. Inoculation with *Rhizobacterium* sp. increased growth of plant, nodulation, number of pods& root colonization by the bacteria diminished the wilting in plants inoculated with Fusarium.

Azizpour and Rouhrazi (2016) isolated a total number of 10 rhizospheric bacterial isolates from chickpea rhizosphere and estimated *in vitro* as a possible fungal pathogen antagonist. Three *Pseudomonas fluorescens* strains, three *Pseudomonas putida* strains, three *Bulkholderia multivorans* strains, & 1 *Mezorhizobium ciceri* strain are antagonistic strains; these strains were all sequenced utilizing 16S rDNA and compared to the Gene Bank database.

Research gap identification:

Agricultural development & food security are dependent on characteristics of soil. Demand for agriculture production has risen hugely with the increasing population. This contributes to large scale production and synthetic fertilizers utilization. Therefore, the extension of agricultural land along with productive soil is nearly impossible. Soil health and productivity are continuously degrading by fertilizers & pesticides utilization in fields of agriculture. So scientists are trying to change their focus for a more effective & reliable resources of agricultural practices. PGPR has symbiotic along with antagonistic interactions with soil & bacteria. PGPR shows a co-evolution between plants and microorganisms. Plant growth promoters obtained through indirect & direct ways for example rhizo-remediation, bio-fertilization, disease protection *etc*. lead to microbial revitalization (Gouda *et al*., 2018). PGPR-based biofertilizers are rapidly becoming more prevalent in India, in popularity & demand (Raj *et al*., 2005).

Although PGPR and its derivatives come in a variety of forms, their applications and relevance in sustainable agriculture are still debatable and restricted. Several environmental conditions impact the growth and proliferation of plants as well as the effectiveness of PGPR (Gouda *et al*., 2018). There is a continuous search for additional PGPR strains since the potential

of PGPR to promote plant growth & development differs depending on the strain. This anticipates gathering, identifying, and employing the PGPR strains for evaluation. PGPR is identified by employing molecular, biochemical, as well as morphological techniques. Each amplicon is a distinct sequence because the molecular mechanism amplifies the 16S parts of the conserved bacterial DNA and is specific to each microbe. To create a phylogenetic tree and identify the bacterial strain, these amplicons are sequenced & put through bioinformatics analyses (Janda and Abbott, 2007).

These gaps and limitations possibly focused on the use of new approaches which integrate utilization in nanotechnology, biotechnology, agro biotechnology & unite diverse environmental as well as biological purposes to add different formulations & opportunities with massive possibilities.

Objectives:

Taking the above mentioned views into due consideration the present investigation has been undertaken with the following objectives:

1. To collect soil samples from different regions of Maharashtra and isolation of bacteria.
2. To identify and multiply bacterial isolates based on morphological, biochemical and molecular characteristics.
3. To standardize media and evaluation of growth parameters of rhizobacteria.
4. To screen various bacterial isolates for their plant growth promoting activities under *in vitro* conditions.
5. To study the effect of Plant Growth Promoting Rhizobacteria (PGPR) in selected regional crops.

Chapter 3

Materials and Methods

1. Collection of soil samples from different regions of Maharashtra and isolation of rhizobacteria

Site description and soil sample collection:

The sampling site's dry matter containing topsoil was cleared away. Four locations in Maharashtra were utilized to gather soil samples from the rhizospheres of vegetable, pulse, & cereal crops (maize, soybean, & chilli, correspondingly) (Table3.1). Utilizing a hand trowel, soil samples were taken up to 15 cm below the soil's surface and stored in polythene bags. appropriately labeled, sent to the lab for additional research, and utilized to isolate suspected bacteria (Lamsal *et al*, 2012).

Table 3.1 Different regions of Maharashtra for collecting soil samples for experimental studies.

Sr. No.	Regions of Maharashtra
1	Marathwada
2	Western Maharashtra
3	Vidarbha
4	Konkan

Isolation of rhizobacteria:

Rhizospheric soil (10 g) had been suspended in 90 ml of sterile water & 30 mins agitation was done at 120 rpm for 25-30 mins at 28±2°C on rotatory shaker to isolate rhizobacteria. After diluting 1 ml of this stock to 10^{-8}, 0.1 ml of diluted sample has been spread out on NA plates (Appendix I A). Following incubation period, number of bacterial colonies & their morphological characteristics were evaluated on NA plates. According to the process described in Bergey's manual of determinative bacteriology (Kreig and Holt 1984; Avis 2008), each isolate had beencharacterized morphologically (Armada *et al*. 2014). The isolated strains were distinguished by their morphological, cultural, as well as staining traits. Color, size, form, edge,

elevation, opaqueness, & the bacterial colony were among the morphological characteristics that were observed (Somasegaran and Hoben 2012). Bacterial colonies were chosen as well as subcultured on the basis of their shape & traits (Aneja 2003).

Colonies that displayed rapid growth and exhibited distinct characteristics were chosen for further analysis. Bacterial cultures were regularly placed on fresh NA Medium plates to obtain pure, individual colonies. These isolates had been examined for their PGP (Plant Growth Promoting) traits. Once their PGP traits were confirmed, they were preserved in glycerol stocks at -20°C for future application.

2. Identification and multiplication of bacterial isolates based on morphological, biochemical and molecular characteristics

Morphological and Biochemical characterization:

Rhizobacterial isolates will be preliminarily identified utilizing the methods described in Bergey's Manual of Systematic Bacteriology (Kreig and Holt, 1984).The isolated strains will be differentiated on the basis of their cultural, morphological, as well as staining characteristics.

Molecular characterization using 16S rRNA technique:

- For molecular characterization Partial 16S rRNA gene sequencing was carried out. The 16S rRNA technique is a molecular characterization method that uses PCR (polymerase chain reaction) to amplify and sequence the 16S rRNA gene for identification and classificationof bacteria.
- DNA from selected bacterial isolates was extracted using protocol provided by the Bacterial Genomic DNA Extraction Kit (Hi-Media).
- PCR was performed to generate 16SrDNA amplicons with the universal forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1492R (5'-CGGCTACCTTGTTACGACTT-3').
- An automated thermal cycler (Gene Amp PCR system 9700) was used.
- (program of 35 cycles: initial denaturation at 94°C for 4min, subsequent to 35 cycles of 30sec at 94°C, annealing at 55°C for 30sec, as well as extension at 72°C for 15min with a final extension of 10min at 72°C).

- Purified PCR products sequenced by Sanger sequencing method at Eurofins Genomics India Pvt. Ltd., Bengaluru, India.
- SeqMan software was employed to assemble acquired 16S rDNA sequences.
- To identify individual strains and retrieve their complete sequences,BLAST(Basic Local Alignment Search Tool) supplied by NCBI (National Center for Biotechnology Information) was employed. BLAST compares sequence data to a huge database of sequences that are known to identify similarities and matches.
- Aligned sequence was saved in FASTA format. Phylogenetic testing was carried out utilizing bootstrap analysis with 1000 replicates utilizing MEGA 11 software, as well as a neighbourhood joining bootstrap method was employed to build the tree among the various isolates.

3. Standardization of media composition for multiplication of selected Potential PGPR

The synthetic medium (Appendix I N) of Aragno and Schlegel (1991) with minor modifications was used to standardize physical and nutritional parameters for higher growth of selected PGPR isolates. A suitable volume of inoculums from actively growing culture of the PGPR isolate was transferred to 100 mL medium in a 500 ml conical flask such that the initial OD in the 500 mL flask is about 0.1. The volume of inoculum was calculated by the flowing equation:

$$\text{Inoculum volume} = \frac{(\text{Initial OD in 500 mL flask}) \times (\text{Volume of medium in 500}}{(\text{OD of inoculum})}$$

Thus for example if inoculum OD is 4, Inoculum volume required to be transferred into 500 mL flask would be =0.1×100/4 i.e., 2.5 mL. In orbital shaker,the flasks were kept at 120 rpm under specified temperature for 32h. A 2 mL sample of the culture broth was taken to analyze OD, CFU and pH at 4h intervals.

Effect of carbon source on cell growth:

In Schlegel's medium, different sugars such as glucose, maltose and sucrose were used separately in the medium to examine the impact of various carbon sources on bacterial isolates' cell growth.

Effect of nitrogen source on cell growth:

Various sources of nitrogen like Ammonium sulphate [$(NH_4)_2SO_4$], NH_4Cl (Ammonium chloride), & Potassium nitrate (KNO_3) were used separately along with best carbon source in the medium to determine the impact of various nitrogen sources on bacterial isolate's cell growth.

Effect of pH on cell growth:

Initial pH was varied from 5, 7 and 8 in various flasks and the cell growth of isolates had been examined in existence of best carbon & nitrogen sources. Employing 1N HCl or else 1N NaOH pH of medium had been adjusted.

Effect of temperature on cell growth:

Impact of various temperatures on growth ofcell of the PGPR isolates was studied by growing the bacterial culture at the optimum initial pH and the best carbon and nitrogen sources at various temperatures 28°C, 33°C, 37°C & 40°C to govern optimum temperature.

Effect of NaCl concentration on cell growth:

Under optimized conditions of pH, temperature, best carbon and nitrogen source, impact of salt concentration on bacterial isolates'cell growth was studied. The sodium chloride (NaCl) was added at 2.5 % and 5 % concentration to the medium.

4. **Screening of various bacterial isolates for their plant growth promoting activities under *in vitro* conditions:**

Bacterial isolates underwent initial screening to evaluate the PGP traits, including potassium zinc, & phosphate solubilization as well as IAA synthesis & siderophore, amylase production.

Phosphate solubilization

Efficiency of phosphate solubilization (PSE) in the isolated bacterial strains had been assessed through spotting rhizobacterial cultures on the Pikovskaya's Agar plates (as described by Pikovskaya, 1948) then incubating them at 28±2°C, following the protocol outlined (Vazquez *et al*., in 2000. These plates were examined on the 7th Day After Incubation (DAI) for appearance of halo zone around colonies, indicating inorganic phosphate solubilization by the bacteria. The PSI had beencomputed by measuring total diameter (horizontal and vertical dimensions of colony+halo zone) and bacterial colony, following method described (Edi-Premono*et al*., 1996).

Potassium solubilization

The Aleksandrov agar medium was employed to culture the PGPR isolates, which included potassium alumino silicate, to assess their ability to solubilize potassium. In this method, bacterial culture was placed as a spot on the medium and then incubated at 28±2°C. On the 7thDAI (Day After Incubation), plates were observed. The appearance of halo zone indicated potential for potassium solubilization. The Potassium Solubilization Index (KSI) was calculated by measuring the whole diameter, which included both horizontal and vertical measurements of the colony and the halo zone, in addition to the bacterial colony itself (Sood *et al*., 2023).

Zinc solubilization

The microbial isolates were examined for potential to solubilize zinc with modified Pikovskaya agar media containing insoluble zinc oxide (ZnO). A bacterial culture was placed as a spot on the medium, followed by incubation (28±2°C). Observations were recorded on 7th Day After Incubation (DAI). The clear zone formation around bacterial spot specified the isolate's potential for zinc solubilization. The Zinc Solubilization Index (ZSI) was determined by measuring total diameter, including both horizontal and vertical dimensions of the colony and the halo zone, along with the bacterial colony (Sharma *et al*., 2012).

Production of siderophore

The potential of bacterial isolates to produce siderophore was performed with CAS (Chrome Azurol S) agar medium (Appendix III B) plate (Schwyn and Neilands, 1987). Bacterial isolates had been grown in NB at 28±2°C for 24h on orbital shaker at 120 rpm. On CAS agar

plates, bacterial culture had been spotted and incubated for 7days (28±2°C). The bacterial spot's surrounding yellow-orange halo zone suggested the development of siderophores.

Amylase production

The isolates had been streaked on plates with Starch Agar Medium, incubated for 72 h (28±2 ºC). Iodine solution was flooded on plates, allowed to react for 1-2 min and formation of clear halo zone was observed (Cappuccino, 1983).

Production of Indole-3-acetic acid (IAA)

The isolated bacteria were introduced into nutrient broth containing L-tryptophan (2 mg mL), a precursor for Indole-3-acetic acid (IAA) production. The culture was then incubated (28±2°C) with continuous shaking for 48 hours, at 125 rpm, following method outlined (Rahman *et al*., 2010). After incubation, 2 mL of the culture solution had been centrifuged at 10000 rpm & 4°C for 10 minutes, as per Gordon and Weber's protocols.

To detect Indole acetic acid production, Salkowski's reagent- 2 mL (consisting of 0.5 M $FeCl_3$, 1 mL, in 35% $HClO_4$, 50 mL) had been added to 1 mL of supernatant. This mixture was then kept for 30 minutes in darkness at room temperature for incubation. The synthesis of IAA was detected by the emergence of a pink color, which was measured by measuring absorbance at 530 nm utilizing a UV-Vis spectrophotometer (Loper and Scroth, 1986). The appearance of a pink-red color indicates positive IAA production. A graph was generated using standard IAA solutions (ranging from 0 to 40 µg/ml) from which IAA concentration of samples was calculated, as illustrated in Fig. 7.

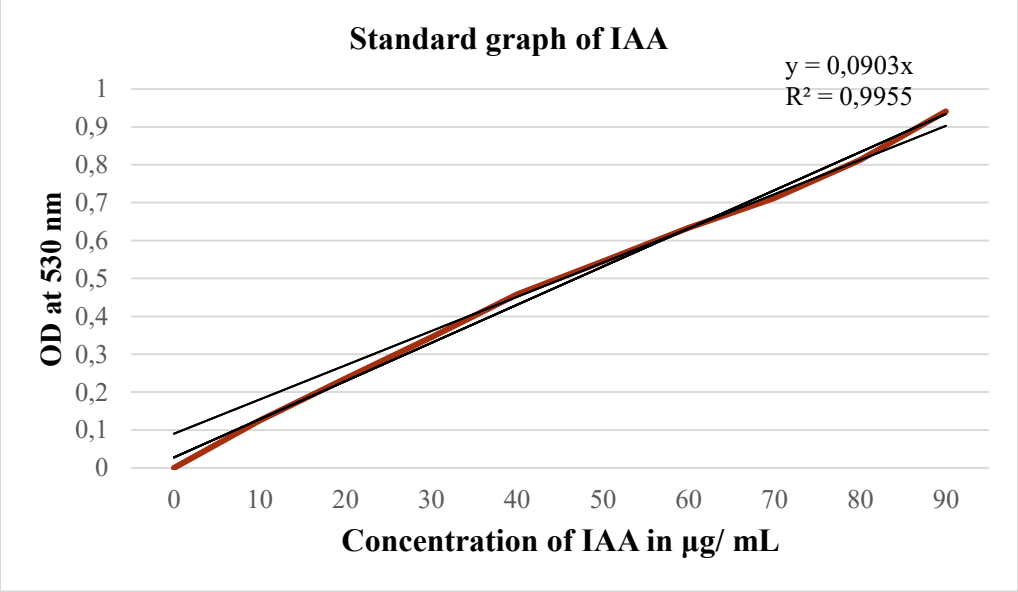
Standard stock solution of Indole-3-acetic acid (IAA)

Indole acetic acid was produced as a standard stock solution at a concentration of 0.1 mg mL in 50% ethanol. IAA (10 mg) first dissolved in small quantity of 50 % ethanol and then volume made up to 100 mL with distilled water.

Preparation of the standard curve

Standard curve was prepared by measuring out 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.8 & 1mL of IAA standard stock solution into test tubes. To the IAA solutions 1-2 drops of

orthophosphoric acid was added followed by 2 mL of Salkowski reagent & up to 3 mL final volume was made with nutrient broth. A blank was prepared by adding 1-2 drops of ortho-phosphoric acid to 1 mL of nutrient broth followed by adding 2 mL Salkowski reagent in test tube and omitting the IAA stock solution. In dark both mixtures had been incubated for 30 min at room temperature as well as absorbance had been taken at 530 nm. Standard curve had beenset by plotting concentration of IAA solution on X-axis against absorbance at 530 nm on Y-axis (Appendix IV).



Graph 1. Standard graph for estimation of Indole-3-acetic acid (IAA) production

Catalase activity

To assess catalase activity, a few drops were dispensed from 3% H_2O_2 (hydrogen peroxide) onto a clean glass-slide. With the help of sterile toothpick, an isolated colony was picked up and gently introduced into the hydrogen peroxide drop. The sample was observed for the bubbles formation which indicates the presence of positive catalase activity (Graham and Parker, 1964).

Oxidase test

A toothpick was employed to gently apply a tiny amount of the test bacterial strain onto a piece of filter paper that had been sterilized. In oxidase reagent, filter paper had been previously immersed. Subsequently, filter paper had been observed for any changes in coloration (Kovacs, 1956).

Urease test

In this procedure, 5 mL of bacterial cultures were combined with a urea-buffer solution consisting of 1 percent urea at pH 6 and 0.00025 percent phenol red. The mixture had been placed in tubes & incubation was doneon an orbital shaker at 37°C for 3-5 days at 120 rpm. The urea breakdown by the bacteria was revealed by the red color formation (Lindstrom and Lehtomaki, 1988).

5. The effect of Plant Growth Promoting Rhizobacteria (PGPR) in selected regional crops.

On the basis of *in vitro* screening and evaluation of PGP traits of isolates, total six best PGPR isolates were selected to evaluate their efficacy on plant growth promoting activity and growth attributes of Chilli, Soybean and Maize in pots and Mungbean in field trial.

Experiment to evaluate PGPR growth potential

(a) Physico-chemical analysis of soil

The soil samples had been collected by randomized design with the help of hand trowel for nutrient analysis before sowing the PGPR treated seeds. The soil samples were transferred into polythene bags and brought to laboratory and dried in shade. Utilizing the techniques outlined in Table 3.2, dried soil samples were examined for physicochemical characteristics that include pH, conductivity, total organic carbon, available nitrogen, phosphorous, & potassium.

Table 3.2 List of physico-chemical parameters and methods utilized for the analysis of soil samples.

S.No.	Parameters	Methods Employed
1	pH	Digital pH meter (Jackson, 1973)
2	EC (mS/cm)	Electrical conductivity meter (Jackson, 1973)
3	Organic Carbon (%)	Titration method (Walkley and Black, 1934)
4	Available Nitrogen (Kg ha^{-1})	Alkaline potassium permanganate method (Subbiah and Asija, 1956)
5	Available Phosphorous (Kg ha^{-1})	Olsen spectrophotometer method (Olsen *et al*., 1954)
6	Available Potassium (Kg ha^{-1})	Flame Photometer (Jackson, 1973)

(b) Seed treatments

Six rhizobacterial isolates with diverse growth promoting features were selected toexamine beneficial effects of these PGPR isolates on maize, soybean, and chilli crops'growth. Seed germination test and growth study were performed using selected isolates. The healthy and fresh seeds of maize, soybean, chilli and mungbean were procured from Mahatma Phule Krishi Vidyapeeth, Jalgaon, Maharashtra. The seeds were rinsed six times with sterile distilled water after being surface sterilized for two minutes with 0.1% HgCl2. The PGPR isolates that were chosen were cultured in NB for 24 hours at 28±2°C in a shaking incubator (120rpm). They were then extracted by centrifugation at 6,000rpm for 10 minutes at 4°C. The pellet was suspended in SDW (sterile distilled water) after being washed twice to achieve a rhizobacterial density of ≅107CFUmL^{-1}. Plates with rhizobacterial suspensions were filled with sterilized seeds. The control seeds were those that were not steeped in rhizobacterial isolate.

Table 3.3 PGPR isolates used to treat seeds of selected crops *i.e*. maize, soybean, and chilli.

S.No.	Treatments	PGPR strains	Microorganisms with Accession No.
1	Treatment 1	Control	Distilled Water
2	Treatment 2	CA2	Pseudomonas aeruginosa_PP754221 (Marathwada)
3	Treatment 3	CA1	Delftia tsuruhatensis_PP75424 (Western MH)
4	Treatment 4	GM2	GM2_Pseudomonas sp._PP754223 (Western MH)
5	Treatment 5	ZM3	Acinetobacter sp._PP754225 (Vidarbha)
6	Treatment 6	GM3	Stenotrophomonas maltophilia_PP754226 (Vidarbha)
7	Treatment 7	ZM4	Aeromonas caviae_PP754228 (Konkan)

A. Pot Experiment

Three replicates were employed in the randomized block design of the pot trials. Six PGPR isolates were selected for treatment and untreated seeds served as control (Table 3.3). The plastic bags (26cm top diameter, 16cm bottom diameter and 24cm height) were filled with soil (7-8kg). In this experiment growth parameters for example number of leaves per plant,root & shoot length, fresh weight & dry weight *etc* as well as biochemical parameter such as total chlorophyll contents of plant were estimated.

1. Effect of selected PGPR on Chilli Crop

In a seed germination tray, chilli seeds were sowed separately and kept up to date. The seedlings were carefully pulled 21 days after they were sown. After that, the seedlings were put in pots & given 45 days to grow, during which time their growth metrics were recorded.

2. Effect of selected PGPR on Soybean Crop

Soybean seeds were treated with PGPR treatments and sown in pots and growth parameters were recorded

3. Effect of selected PGPR on Maize Crop

Similarly, the maize seed was treated with different PGPR solutions and sowing of the treated maize seed was done individually in each pot. All the pots were watered regularly and the observations on germination were recorded after 7 days of sowing. The subsequent observations on shoot length, fresh weight and dry weight, the root length, etchad been documented at 75days of growth. The soil sample from each treatment were taken and given for analysis in the government laboratory.

Five plants from each treatment were selected on average growth parameter basis of each plots and tagged for observations. Mean value of each treatment was used for further analysis. Shoot and root length of plants were recorded after the plant attained maturity. The plants were carefully harvested with the roots from each treatment and both shoot and root length of plant were recorded. The number of leaves was counted at the flowering stage. The mean values were taken for statistical analysis. To get rid of soil particles, plants were cleaned with tap water.

Employing blotting paper, water that had adhered to the roots was absorbed, and new weights of the shoot & root had been noted. To determine the shoot and root dry weights, the plants' shoots and roots have been dried for 72h at 80°C in a hot air oven. Biochemical parameter total chlorophyll contents that were estimated before flowering. Nitrogen, phosphorus and potassium content of plant part were analyzed at maturity stage.

B. Field trial of the selected PGPR on Mungbean crop

This experiment was conducted in randomized complete block design in a field in SDMVM's College Agriculture farm. Standard practices of field preparation of plouging, harrowing and leveling were followed and no fertilizer dose was applied to the experiment field. In field experiment total 21 plots were prepared and PGPR seeds were sown in each plot with a spacing of 30 cm row to row and 10 cm plant to plant and maintained 15 plants per plot. Total three replicates were put for each PGPR isolates treatment.

In this experiment physical growth parameters as root and shoot length, biochemical parameters such as total chlorophyll contents and yield parameter such as pods per plant, number of seeds per pod and 100 seed weight were estimated.

Total chlorophyll contents

Chlorophyll was estimated in leaves adopting method of Mackinney (1941) & MaClachlan & Zalik (1963), correspondingly. 100 mg of green healthy leaves were weighed and ground with 10mL chilled 80 percent aqueous acetone utilizing a pre chilled mortar & pestle. Whatman no. 1 was employed to filter the supernatant into a tube and made up the volume to 10 mL using 80 % aqueous acetone. Colour intensity of solution had been read at 645 & 663 nm for estimation of chlorophyll using a UV-VIS Spectrophotometer.

The chlorophyll content (mg g^{-1}FW) was estimated using the following formula:

$$\text{Total chlorophyll content} = 20.2\,(\text{OD } 645) + 8.02\,(\text{OD } 663)\ \frac{V}{W \times 1000}\ \text{mg/g FW}$$

Where, OD = Optical density of the extract at the given wavelengths (645, 663 nm for chlorophyll

V = Final volume of chlorophyll extract in 80 % aqueous acetone

W (FW) = Fresh weight of leaf tissue (g)

d = Length of light path = 1 cm

Statistical analysis

Results were expressed as the mean values with standard error. The analysis of variance of the treatment effect on measured data was performed by using Statistical Package for Social Sciences (SPSS, version 17). Experiments were analyzed using Standard Analysis of Variance (ANOVA). Means were separated by least significant differences (LSD) test at $p \leq 0.05$ significance levels.

Chapter 4

Results and Discussions

1. Collection of soil samples from different regions of Maharashtra and isolation of rhizobacteria

Site description and soil sample collection:

As demonstrated by Fig. 1, the rhizobacteria linked to the cereal, pulse, & vegetable crop plants from several Maharashtra regions were described in this work. Overall 3 samples of soil, each corresponding to one of the mentioned crops, were collected, as outlined in Table 4.4. Essential soil characteristics including moisture content, temperature, and pH were meticulously analyzed using standard laboratory techniques and the results were recorded, as summarized in Table 4.5.

Sr. No.	Regions of Maharashtra	No. of soil samples
1	Marathwada	3
2	Western Maharashtra	3
3	Vidarbha	3
4	Konkan	3
Total No. of Samples		**12**

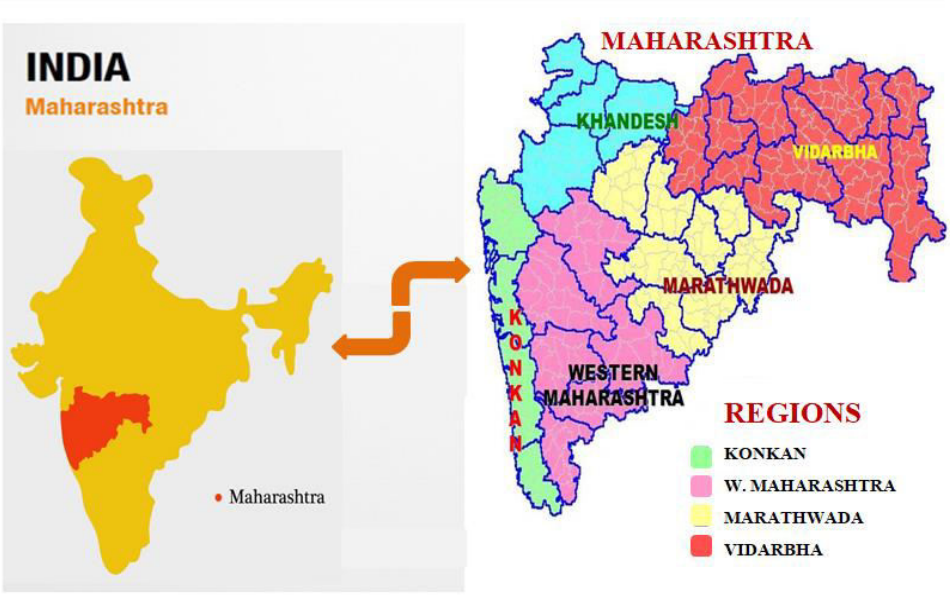


Fig.1. Different regions, Maharashtra, INDIA.

Fig.2. Soil sample collection from different regions of Maharashtra, INDIA.

Table 4.4 Details of collected site-specific soil samples.

Sr. No.	Rhizospheric soil samples	Sample Code	Date of Collection	GPS Coordinate		Places
				Latitude	Longitude	
Samples from Marathwada						
1	Maize (*Zea mays*)	ZM1	28/08/2022	20.237554°"N	76.090415°"E	Jalna
2	Soybean (*Glycine max*)	GM1	28/08/2022	20.368706°"N	76.088649°"E	Parbhani
3	Chilli (*Capsicum annuum*)	CA1	29/08/2022	19.79236°"N	75.279881°"E	Aurangabad
Samples from Western Maharashtra						
4	Maize (*Zea mays*)	ZM2	29/08/2022	19.792626°"N	75.278649°"E	Ahmadnagar
5	Soybean (*Glycine max*)	GM2	29/08/2022	19.793052°"N	75.280174°"E	Rahuri
6	Chilli (*Capsicum annuum*)	CA2	29/08/2022	19.793072°"N	75.279916°"E	Shrirampur
Samples from Vidarbha						
7	Maize (*Zea mays*)	ZM3	21/08/2022	19.586086°"N	74.824788°"E	Washim
8	Soybean (*Glycine max*)	GM3	21/08/2022	19.602756°"N	74.80459°"E	Akola
9	Chilli (*Capsicum annuum*)	CA3	21/08/2022	19.602697°"N	74.804629°"E	Kondala
Samples from Konkan						
10	Maize (*Zea mays*)	ZM4	04/09/2022	20.182633°"N	77.141737°"E	Dapoli
11	Soybean (*Glycine max*)	GM4	04/09/2022	20.182412°"N	77.141771°"E	Sangulwadi
12	Chilli (*Capsicum annuum*)	CA4	04/09/2022	20.182832°"N	77.140519°"E	Saralgaon

Table 4.5 Analysis of the physical parameters of collected soil samples.

Soil samples from different places	Soil Parameters				
	Sr.No.	**Sample code**	***Temperature (°C)**	***Ph**	***Moisture Content**
	1	ZM1	15.00±0.30	6.80±0.03	65.00±0.25
	2	GM1	18.00±0.40	6.50±0.03	58.00±0.40
	3	CA1	22.00±0.45	6.50±0.03	45.00±0.28
	4	ZM2	28.00±0.10	6.20±0.07	40.00±0.13
	5	GM2	35.00±0.27	6.80±0.05	35.00±0.18
	6	CA2	32.00±0.33	6.40±0.03	65.00±0.20
	7	ZM3	29.00±0.37	7.00±0.03	68.00±0.27
	8	GM3	27.00±0.13	6.50±0.04	56.00±0.30
	9	CA3	30.00±0.45	6.50±0.03	55.00±0.21
	10	ZM4	26.00±0.22	6.10±0.03	60.00±0.93
	11	GM4	24.00±0.15	6.80±0.05	62.00±0.67
	12	CA4	20.00±0.18	7.20±0.05	65.00±0.76

*P<0.05, level of significance; ±, standard deviation

Isolation of PGPR:

Total of 1174 microbial colonies were isolated after two days of incubation from the soil samples collected from rhizosphere of various crops as mentioned in Table 4.6. Dilution plate count technique was used to obtain pure bacterial strains from the soil samples. Biochemical, molecular and other PGP characteristics of bacteria require pure cultures. Therefore, utmost care was taken to get single colonies (Armada *et al*., 2014).

Table 4.6 Number of bacterial colonies after two days of incubation.

Region	Sr. No.	Strain code	Number of colonies	CFU/ml
Marathwada	1	ZM1	72	7.2×10^{7} (72,000,000)
	2	GM1	48	4.8×10^{7} (48,000,000)
	3	CA1	240	2.4×10^{8} (240,000,000)
Western Maharashtra	4	ZM2	63	6.3×10^{7} (63,000,000)
	5	GM2	146	14.6×10^{7} (146,000,000)
	6	CA2	33	3.3×10^{7} (33,000,000)
Vidarbha	7	ZM3	97	9.7×10^{7} (97,000,000)
	8	GM3	54	5.4×10^{7} (54,000,000)
	9	CA3	110	1.1×10^{8} (11,00,00,000)
Konkan	10	ZM4	68	6.8×10^{7} (68,000,000)
	11	GM4	158	15.8×10^{7} (158,000,000)
	12	CA4	85	8.5×10^{7} (85,000,000)

CFU/ ml = (Number of colonies * Dilution factor) / Volume of culture plated

*Volume plated =0.1ml, ** 10^{-5} (Ignacio *et al*., 2021)

The isolated strains were distinguished from one another on the basis of their physical & cultural traits. Following two days of inoculation, the bacterial colony's colour, size, form, border, and opaqueness were among the morphological characteristics that were noted (Table 4.7). Bacterial colonies were chosen and subculturedon the basis of their characteristics&colony morphology.

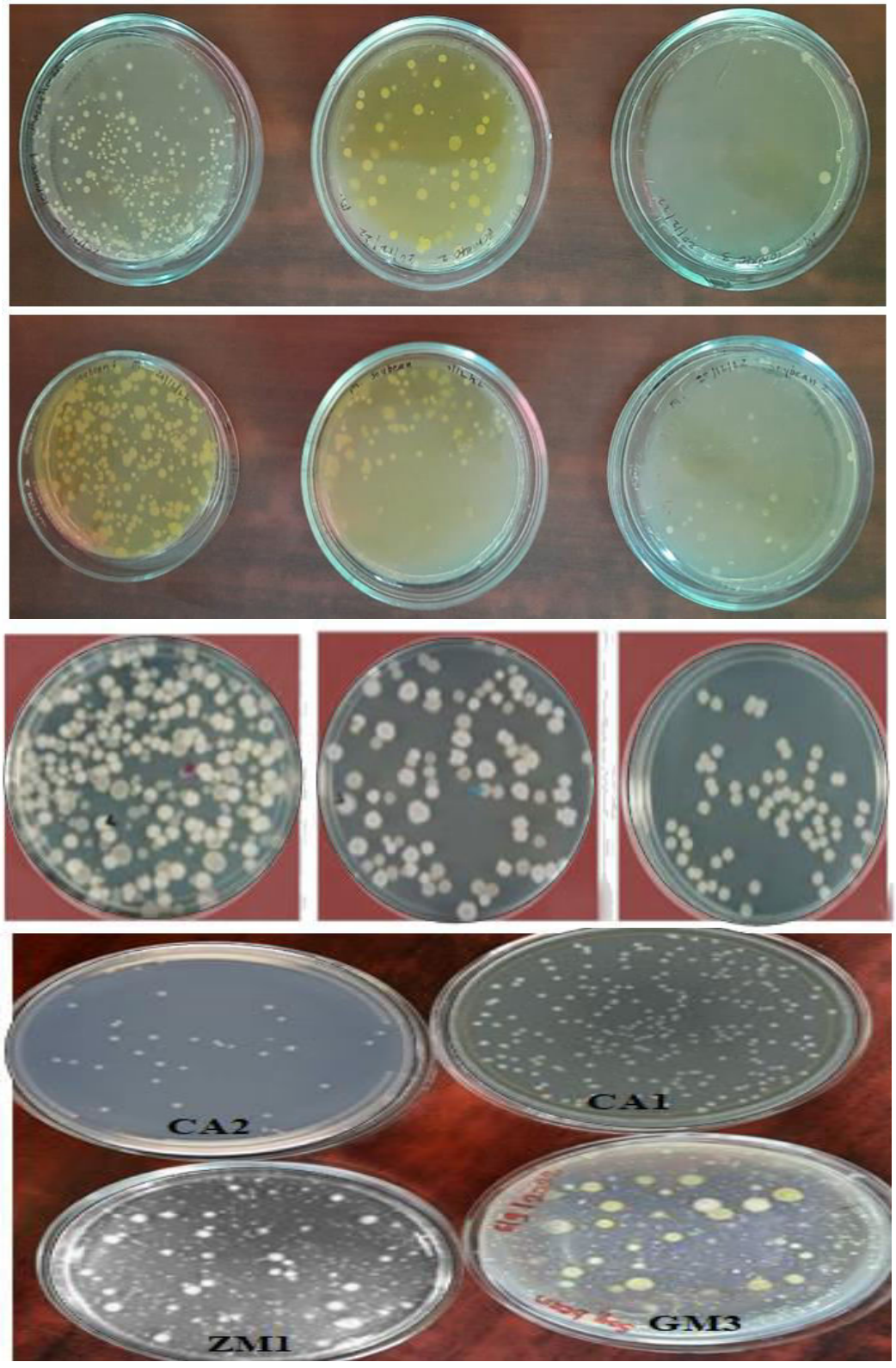


Fig. 3 Isolation of bacteria from different rhizospheric samples.

Bacterial colonies are randomly selected based on morphological features. The morphological screening recorded different characteristics of colonies after two days of incubation such as most bacterial colonies were milky white in color with circular in shape and smooth margin (Somasegaran and Hoben, 2012). Bacterial colonies were chosen and subcultured on the basis of their colony morphology along with characteristics (Aneja, 2003). For over four biochemical assays, the majority of the tested isolates exhibited positive findings.

Table 4.7 Morphological and growth features of PGPR isolates of two days old bacterial colonies.

Sr.No.	Isolates	Color	Size	Shape	Margin	Elevation	Gram's Staining
1	ZM1	Milky	0.5mm	Circular	Smooth	Convex	Gram-negative
2	GM1	Milky	0.9mm	Irregular	Rough	Raised	Gram-negative
3	CA1	Whitish	0.3mm	Circular	Smooth	Flat	Gram-negative
4	ZM2	Off-white	1.2mm	Circular	Smooth	Raised	Gram-negative
5	GM2	Yellowish	1.4mm	Circular	Smooth	Convex	Gram-negative
6	CA2	Milky	0.3mm	Irregular	Smooth	Convex	Gram-negative
7	ZM3	Whitish	0.4mm	Circular	Smooth	Flat	Gram-negative
8	GM3	Off-white	0.8mm	Circular	Smooth	Flat	Gram-negative
9	CA3	Whitish	0.4mm	Irregular	Smooth	Convex	Gram-negative
10	ZM4	Milky	0.7mm	Circular	Smooth	Flat	Gram-negative
11	GM4	Yellowish	0.3mm	Circular	Rough	Flat	Gram-negative
12	CA4	Whitish	0.6mm	Circular	Smooth	Flat	Gram-negative

Screening of the bacterial isolates

Microbial colonies were obtained from the collected rhizospheric soils samples of specific crops. Morphological and Biochemical observations were made for the bacterial isolates, and nine isolates were initially selected. Among these, three isolates were chosen for additional screening as per the effectiveness and amount of their crop-boosting characteristics.

The following sections offer detailed explanations of the plant growth enhancing attributes of the particular isolates, determined through *in vitro* biochemical examinations. All of the selected isolates' biochemical characterisation outcomes are displayed in Table 4.8.

In this study, PGPR isolates were extensively examined, and the severaltests revealed positive outcomes. This underscores consequence of the PGPR possessing multifaceted traits, as opposed to singular attributes, aligning with the previous findings (Imran *et al*., 2014).

Table 4.8 Screening of PGPR for their several Plant Growth Promoting features.

Strain Code	Biochemical tests of PGPR								
	Phosphate Solubilization	IAA production	Zinc solubilization	Siderophores production	Potassium solubilization	Amylase	Catalase	Urease	Oxidase
ZM1	++	++++	+++	++++	++++	+++	++++	++	++
GM1	+++	+++	+++	++++	++	++	+++	++	++++
CA1	++	+++	+++	++++	++	+	++++	++	++
ZM2	++++	++++	++++	+++	+++	++++	++	++	++
GM2	++++	++++	++++	++++	+++	++++	++++	+++	++
CA2	+++	++	++++	+++	++++	+++	+++	++++	++
ZM3	++++	++	++++	++	++++	++	++++	++	++
GM3	+++	+++	++++	+++	+++	+++	++++	++	++
CA3	++++	++	+++	++++	+++	++++	++	+++	++++
ZM4	++++	++++	++++	+++	++++	++++	++++	++	++
GM4	++++	++++	+++	+++	+++	++++	++++	++	++
CA4	+++	+++	+++	++++	++++	++++	++++	++	++

Phosphate solubilization

In general, PGPR are known for its significant role in improving plant growth, health and crop yield. Inorganic phosphate solubilization is one of the major mechanisms of plant growth promotion by PGPR. Phosphate solubilization by plant growth-promoting rhizobacteria (PGPR) is a process that helps plants access phosphorus in the soil.

Remarkably, in the bacterial isolates obtained in this study, CA4a exhibited the highest Phosphate Solubilization Index (PSI) *i. e*. 13.50 (Table 4.9) while the remaining isolates also displayed the ability to solubilize phosphate (Fig. 4). It is well established that Pikovskaya medium in existence of PSB performedvital role in solubilization of insoluble tricalcium phosphate (Chen *et al*., 2006).

All three isolates associated with rhizospheric soil of *Capsicum annuum* crops, indicated better results for Phosphate solobilization as compared to *Zea mays*. The majority of phosphate solubilizing bacteria was obtained from rhizospheric soil rather than non-rhizospheric soil, aligning with previous findings (Reyes *et al*., 2006). Numerous species of PGPR have demonstrated proficient phosphate solubilization abilities (Castanheira *et al*., 2016). For instance, *Burkholderia cepacia* (Pande *et al*., 2017, 2019), B. *tropica, B. unamae*, and *B. cepacia* (Ghosh *et al*., 2016) have been identified as effective phosphate solubilizers.

Isolation and screening of PSB strains from different soil resources are the bases for further research and application of phosphate solubilizing bacteria as biofertilizers or biocontrol agents. Enhanced plant growth and phosphate uptake have been observed in various crop species due to PSB inoculants. For instance, *Pseudomonas* sp. has been reported to promote growth in rice (Gusain *et al*., 2015) and soya bean (Fankem *et al*., 2015). Similarly, Hussain *et al*. (2013) evaluated five promising PSB strains—PS-01 (*Burkholderia* sp.), PS-12 (*Bacillus* sp.), PS-32 (*Pseudomonas* sp.), PS-41 (*Flavobacterium* sp.), and PS-51 (*Pseudomonas* sp.)—and found significant increases in plant height, root length, shoot dry weight, root dry weight, and grain yield by 16%, 11%, 42%, 29%, and 33%, respectively, compared to un-inoculated controls. Additionally, Surapat *et al*. (2013) reported that inoculating chili plants (*Capsicum frutescens* L.) led to a significant improvement in plant growth and phosphate uptake relative to untreated plants.

Several researchers found potent phosphate solubilizers as Acinetobacter (Gulati *et al*., 2009; Marwa *et al*., 2019), Advenella (Singh *et al*., 2014), Klebsiella (Chaiharn and Lumyong, 2011), Kosakonia (Kamran *et al*., 2017), Pantoea and Enterococcus (Panwar *et al*., 2016), Pantoea and Enterobacter (Mussa *et al*., 2018), Pseudomonas (Misra *et al*., 2012; Kumari *et al*., 2018a) & Ralstonia (Paul and Datta, 2016).

Table 4.9 Phosphate solubilization ability of PGPR isolates.

	Sr. No.	PGPR Isolates	[a]Diameter of Colony (mm)	[a]Diameter of Halo Zone (mm)	[a]PSI
Marathwada Region	1	ZM1a	3.33±0.33	6.00±0.23	3.00±0.08
	2	ZM1b	2.20±0.23	4.10 ±0.66	3.05±0.11
	3	ZM1c	5.30±0.33	9.00±0.67	2.80 ±0.16
	4	GM1a	5.00±0.00	6.10 ±0.33	2.22±0.06
	5	GM1b	7.10±0.33	10.00 ±0.57	2.42 ±0.20
	6	GM1c	4.80±0.33	27.00±0.00	2.48 ±0.18
	7	CA1a	5.50±0.18	6.10±0.33	2.37 ±0.24
	8	CA1b	4.00±0.33	7.00±0.00	2.45±0.13
	9	CA1c	4.10±0.00	5.80±0.67	2.46±0.12
Western Maharashtra Region	10	ZM2a	7.00±0.20	32.00±0.11	5.57±0.23
	11	ZM2b	6.20±0.10	30.00±0.18	5.83±0.23
	12	ZM2c	8.00±0.18	31.10±0.20	4.88±0.45
	13	GM2a	8.10 ±0.45	30.10±0.22	4.71±0.67
	14	GM2b	8.00±0.67	31.00±0.67	4.87±0.20
	15	GM2c	7.90±0.22	33.00±0.55	5.17±0.18
	16	CA2a	2.00±0.42	6.00±0.42	4.00±0.22
	17	CA2b	2.00±0.80	5.80±0.33	3.90±0.33
	18	CA2c	2.10±0.55	5.50±0.22	3.61±0.45
Vidarbha Region	19	ZM3a	7.00±0.42	28.10±0.10	5.01±0.48
	20	ZM3b	7.20±0.22	31.00 ±0.67	5.30±0.78
	21	ZM3c	7.30 ±0.67	32.00 ±0.55	5.38 ±0.33
	22	GM3a	5.00 ±0.44	27.20 ±0.80	6.44±0.22
	23	GM3b	6.10 ±0.58	31.10 ±0.33	6.09 ±0.10
	24	GM3c	5.80 ±0.15	30.00 ±0.12	6.17 ±0.18
	25	CA3a	4.00 ±0.20	28.00 ±0.20	8.00 ±0.35
	26	CA3b	5.00 ±0.64	29.10 ±0.33	6.82±0.27
	27	CA3c	4.10 ±0.33	30.00 ±0.45	8.31±0.22
Konkan Region	28	ZM4a	4.00±0.20	30.10±0.67	8.52 ±0.18
	29	ZM4b	5.20±0.10	32.00±0.33	7.15 ±0.33
	30	ZM4c	5.30±0.15	34.00±0.20	7.41 ±0.67
	31	GM4a	7.00±0.33	26.20±0.22	4.74 ±0.78
	32	GM4b	7.10±0.52	29.10±0.18	5.09 ±0.33
	33	GM4c	6.80±0.65	27.00±0.42	4.97 ±0.20
	34	CA4a	2.00±0.18	25.00±0.67	13.50 ±0.18
	35	CA4b	3.00±0.33	29.10±0.78	10.70 ±0.44
	36	CA4c	3.10±0.25	28.00±0.66	10.03 ±0.65

[a]Datais the mean values of three replicates. Means±SE ($p \leq 0.05$).

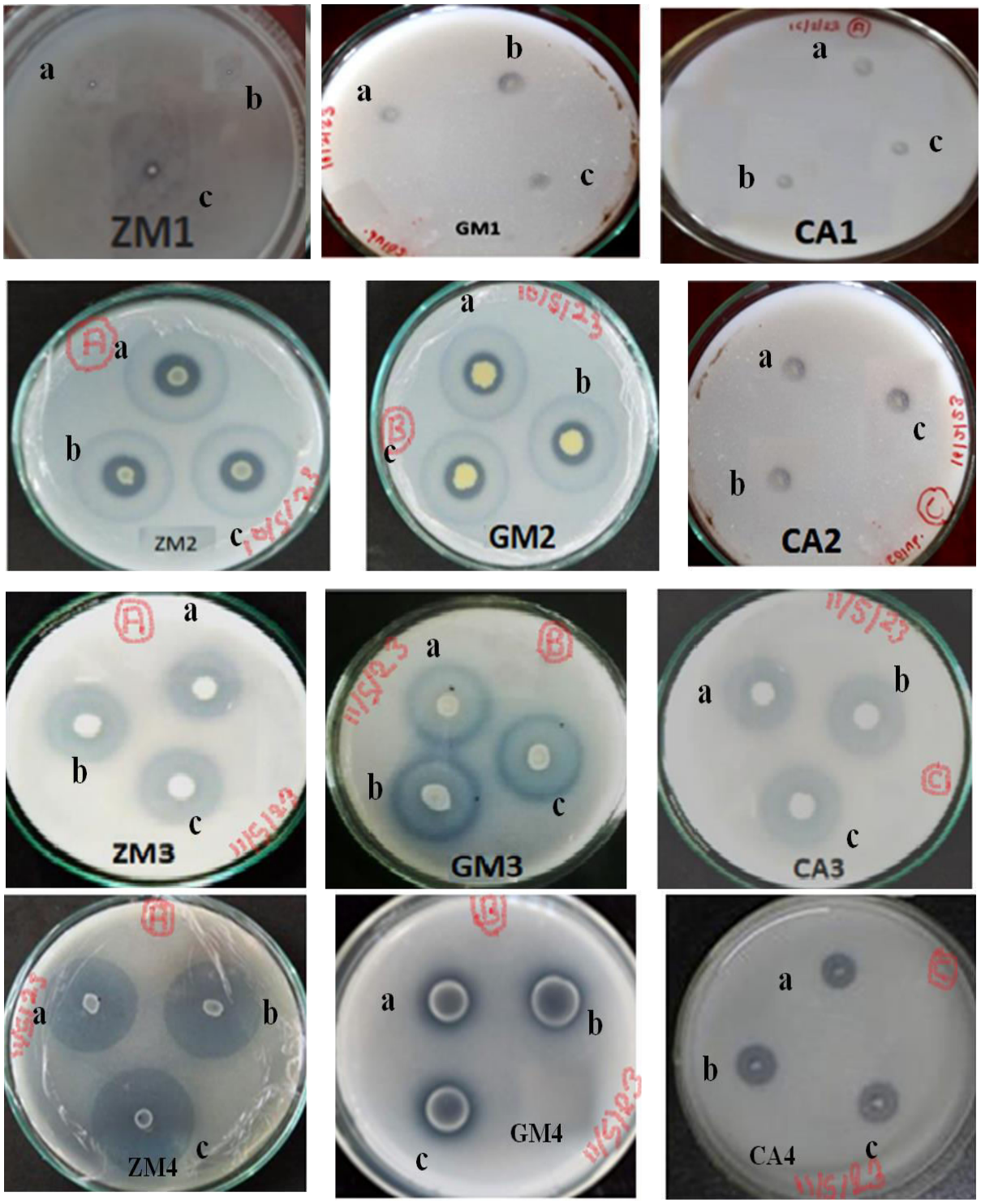


Fig. 4 Solubilization of tricalcium phosphate on Pikovskaya agar medium by selected PGPR isolates after 7 days of incubation at 28±2°C**.**

Potassium solubilization

Numerous studies have demonstrated the presence of diverse potassium-solubilizing bacteria (KSB) in soil. For instance, *Klebsiella variicola* and *Enterobacter cloacae* have been successfully isolated from the tobacco rhizosphere (Zhang and Kong, 2014). Additionally, efficient KSB strains, including *Pseudomonas* spp., *Bacillus* spp., and *Burkholderia* spp., have been identified in tea plantation soil (Bagyalakshmi *et al*., 2017).

Bacterial isolates were found in the present work that could solubilize potassium alumino silicate, an insoluble potassium source, present in solid Aleksandrov agar medium. In the present study bacterial isolate GM4b showed highest potassium solubilization index (12) from insoluble potassium alumino silicate (Table 4.10). The result of other tested isolates which demonstrated ability to solubilize potassium is recorded (Fig. 5).

In the present study two isolates from rhizospheric soil of Soybean (*Glycine max*) from Konkan region represented maximum potassium solubilization as compared to other isolates. Variety of bacteria have been identified from crop rhizosphere that could solubilize potassium from minerals (Zeng *et al*., 2012). A similar study (Parmar and Sindhu, 2013) showed that several bacterial isolates have the ability to solubilize potassium-containing minerals.

In previous study, Bagyalakshmi *et al*., (2017) isolated 30 bacterial strains and one isolate VKSB12 could solubilize potassium. However, the ability of these bacterial strains to solubilize potassium from more insoluble forms, such as sulphate of potash (SOP) and Montmorillonite was quite low (28.97mg L^{-1}& 24.81mg L^{-1}, correspondingly) and concomitant reduction in pH was also not much (4.5, 4.8 and 4.9 pH, respectively).

Similarly, another study indicated that invasive plants were linked to increased soil potassium availability (Sardans *et al*., 2017). These findings confirm that KSB can enhance plant growth by solubilizing potassium, fixing nitrogen, solubilizing phosphate, and producing auxins (Ghadam Khani *et al*., 2019).

Table 4.10 Potassium solubilization ability of bacterial isolates using plate assay.

	Sr. No.	PGPR Isolates	[a]Diameter of Colony (mm)	[a]Diameter of Halo Zone (mm)	[a]KSI
Marathwada Region	1	ZM1a	5.10 ±0.11	25.00 ±0.44	5.90 ±0.78
	2	ZM1b	5.90 ±0.13	23.20 ±0.24	4.93 ±0.56
	3	ZM1c	5.10 ±0.81	22.10 ±0.16	5.33 ±0.33
	4	GM1a	7.30 ±0.33	13.30 ±0.18	2.82 ±0.23
	5	GM1b	5.80 ±0.45	12.80 ±0.22	3.20 ±0.89
	6	GM1c	6.20 ±0.33	13.00 ±0.67	3.09 ±0.67
	7	CA1a	9.00 ±0.56	13.00 ±0.24	2.44 ±0.44
	8	CA1b	8.00 ±0.78	14.80 ±0.78	2.85 ±0.24
	9	CA1c	09.10 ±0.89	18.00 ±0.33	2.97 ±0.20
Western Maharashtra Region	10	ZM2a	5.10 ±0.73	27.00 ±0.56	6.29 ±0.22
	11	ZM2b	4.00 ±0.22	29.10 ±0.33	8.27 ±0.67
	12	ZM2c	5.00 ±0.11	28.00 ±0.44	6.60 ±0.48
	13	GM2a	4.20 ±0.10	16.00 ±0.22	4.80 ±0.53
	14	GM2b	4.10 ±0.44	17.10 ±0.12	5.17 ±0.78
	15	GM2c	4.00 ±0.12	15.00 ±0.46	4.75 ±0.10
	16	CA2a	5.10 ±0.67	26.00 ±0.25	6.09 ±0.20
	17	CA2b	5.00 ±0.44	25.10 ±0.56	6.02 ±0.33
	18	CA2c	5.00 ±0.24	23.00 ±0.78	5.60 ±0.44
Vidarbha Region	19	ZM3a	4.10 ±0.18	21.00 ±0.12	6.12 ±0.56
	20	ZM3b	4.00 ±0.20	18.00 ±0.67	5.50 ±0.12
	21	ZM3c	3.00 ±0.76	19.00 ±0.18	7.33 ±0.33
	22	GM3a	5.00 ±0.34	18.00 ±0.24	4.60 ±0.67
	23	GM3b	9.00 ±0.67	33.00 ±0.33	4.66 ±0.25
	24	GM3c	6.00 ±0.22	21.00 ±0.55	4.50 ±0.42
	25	CA3a	2.00 ±0.36	14.20 ±0.45	8.10 ±0.66
	26	CA3b	3.00 ±0.66	15.00 ±0.78	6.00 ±0.20
	27	CA3c	3.00 ±0.78	16.00 ±0.67	6.33 ±0.33
Konkan Region	28	ZM4a	3.10 ±0.24	21.00 ±0.22	7.77 ±0.89
	29	ZM4b	3.00 ±0.11	18.00 ±0.18	7.00 ±0.55
	30	ZM4c	4.00 ±0.44	19.00 ±0.33	5.75 ±0.46
	31	GM4a	2.00 ±0.52	18.00 ±0.12	10.00±0.22
	32	GM4b	3.00 ±0.88	33.00 ±0.67	12.00 ±0.56
	33	GM4c	4.00 ±0.10	21.00 ±0.56	6.25 ±0.33
	34	CA4a	3.00 ±0.24	25.20 ±0.23	9.40 ±0.18
	35	CA4b	4.00 ±0.33	26.00 ±0.33	7.50 ±0.22
	36	CA4c	4.00 ±0.37	29.00 ±0.44	8.25 ±0.38

[a]Datais the mean value of three replicates. Means±SE ($p \leq 0.05$).

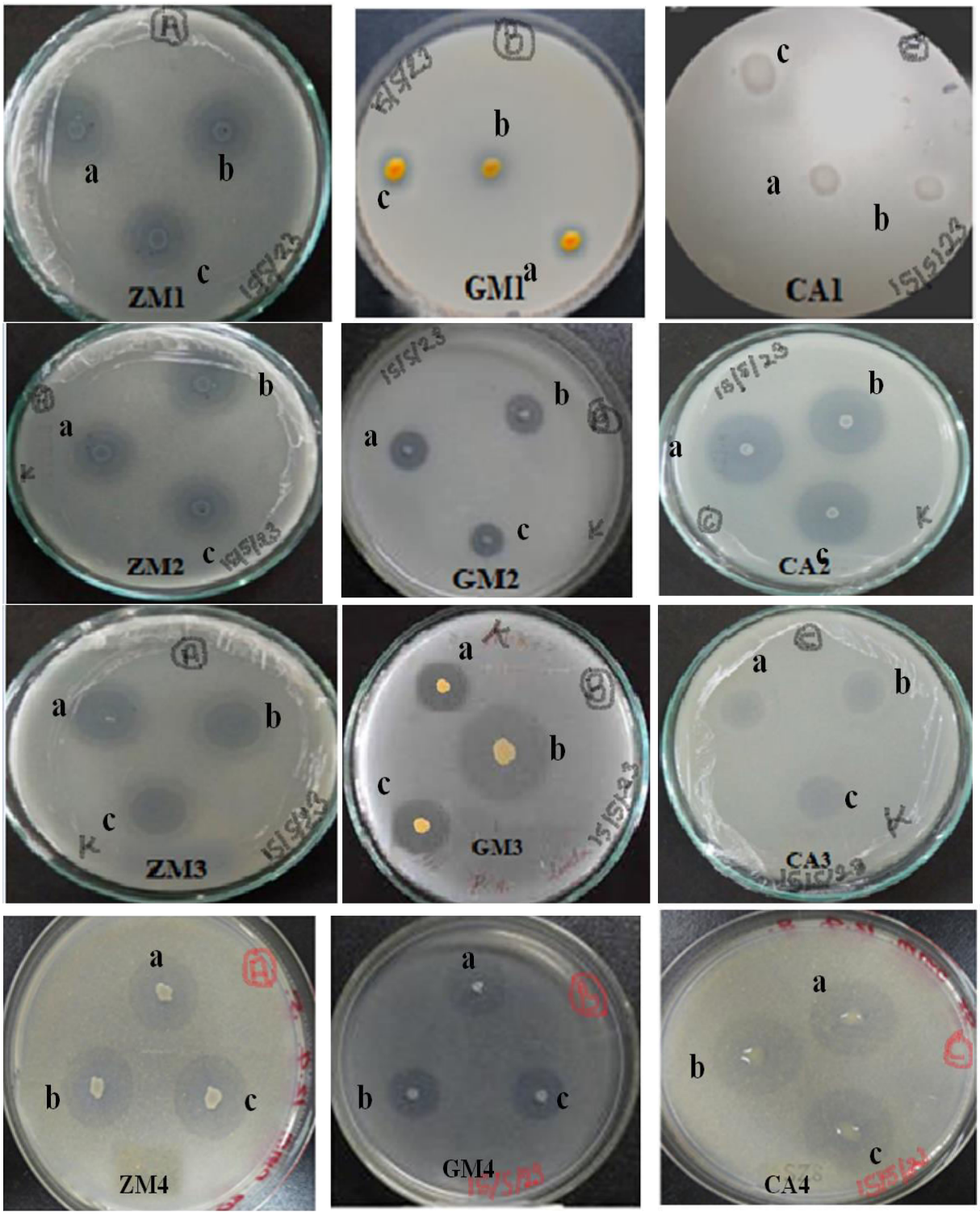


Fig. 5 Solubilization of potassium alumino silicate by selected PGPR isolates after 7 days of incubation at 28±2 °C.

Zinc solubilization

Rhizosphere bacteria solubilize zinc by making it more accessible to plants. In this current research, 36 bacterial strains were meticulously chosen for their high efficiency in solubilizing insoluble zinc oxide. Among these, two isolates, namely CA2c and GM4a exhibited the most substantial Zinc Solubilization Index (ZSI) of 11.20 and 10.40, respectively (Table 4.11). Zinc solubilization from insoluble ZnO by these isolates resulted in a halo zone 51.00 and 47.00 mm, size at and 7^{th} DAI, respectively (Fig. 6).

Zinc, a vital micronutrient, is essential for various plant metabolic processes and acts as a cofactor for several crucial enzymes. However, when applied to soils, zinc often becomes unavailable to plants due to its tendency to form insoluble complexes, leading to significant yield losses (Goteti, 2013). Previous studies have demonstrated that rhizobacteria isolated from fields have the potential to enhance zinc availability by solubilizing it from insoluble sources, thereby contributing to improved crop yields (Hussain *et al*., 2015). In current examination bacterial isolates from rhizophere of Chilli (*Capsicum annuum*) associated with the Western Maharashtra region and Soybean (*Glycine max*) from Konkan region, recorded better result as compared to other isolates.

Fasim *et al*. (2002), observed a clear halo zone around spotted *Pseudomonas aeruginosa* bacteria on a medium having ZnO as an insoluble Zn source. Similarly, Bacillus sp. was documented to solubilize zinc from insoluble ZnO (Hussain *et al*., 2015; Mumtaz *et al*., 2017). Pawar *et al*. (2015) isolated *Burkholderia cenocepacia, Pseudomonas aeruginosa and Pseudomonas striata* that could solubilize zinc compound like ZnO, ZnCO3 and [Zn3 (PO4)2].

In previous studies, zinc solubilization efficiency varied among bacterial strains (Bhatt & Maheshwari, 2020; Kushwaha *et al*., 2021). differences in zinc solubilization efficacy among zinc compounds might be attributed to variations in the environments in which they were isolated. In addition to zinc solubilization, these zinc-solubilizing bacterial isolates exhibit other plant growth promoting traits, such as indole-3-acetic acid (IAA) and potassium solubilization. Therefore, these ZSB isolates could support plant growth.

Table 4.11 Zinc solubilization ability of bacterial isolates using plate assay**.**

	Sr. No.	PGPR Isolates	[a]Diameter of Colony (mm)	[a]Diameter of Halo Zone (mm)	[a]ZSI
Marathwada Region	1	ZM1a	7.00 ±0.44	29.00 ±0.67	5.14±0.33
	2	ZM1b	6.00 ±0.67	26.00 ±0.22	5.33 ±0.25
	3	ZM1c	6.10 ±0.22	31.00 ±0.13	6.08 ±0.18
	4	GM1a	3.00 ±0.12	7.00 ±0.10	3.33 ±0.22
	5	GM1b	6.00 ±0.18	11.00 ±0.24	2.83 ±0.55
	6	GM1c	5.00 ±0.33	9.10 ±0.56	2.82±0.78
	7	CA1a	5.10 ±0.24	28.00 ±0.76	6.49 ±0.24
	8	CA1b	5.00 ±0.67	30.00 ±0.33	7.00 ±0.33
	9	CA1c	5.00 ±0.18	29.10 ±0.89	6.82 ±0.42
Western Maharashtra Region	10	ZM2a	6.00 ±0.89	49.00 ±0.67	9.16 ±0.56
	11	ZM2b	5.00 ±0.78	46.00 ±0.22	10.20 ±0.20
	12	ZM2c	6.00 ±0.66	48.00 ±0.44	9.00 ±0.66
	13	GM2a	5.20 ±0.55	18.00 ±0.25	4.46 ±0.52
	14	GM2b	4.00 ±0.24	20.10 ±0.22	6.02 ±0.42
	15	GM2c	6.00 ±0.34	28.10 ±0.46	5.68 ±0.18
	16	CA2a	6.00 ±0.67	50.00 ±0.78	9.33 ±0.23
	17	CA2b	6.00 ±0.55	52.00 ±0.89	9.66 ±0.17
	18	CA2c	5.00 ±0.45	51.00 ±0.47	11.20 ±0.33
Vidarbha Region	19	ZM3a	7.00 ±0.34	40.00 ±0.82	6.71 ±0.67
	20	ZM3b	9.00 ±0.89	40.00 ±0.33	5.44 ±0.78
	21	ZM3c	9.00 ±0.78	41.00 ±0.55	5.55 ±0.89
	22	GM3a	6.00 ±0.56	45.00 ±0.86	8.50 ±0.22
	23	GM3b	6.00 ±0.44	48.00 ±0.12	9.00 ±0.13
	24	GM3c	5.00 ±0.18	46.00 ±0.18	10.20 ±0.67
	25	CA3a	5.00 ±0.22	26.00 ±0.33	6.20 ±0.23
	26	CA3b	6.00 ±0.23	28.00 ±0.62	5.66 ±0.45
	27	CA3c	5.10 ±0.44	29.00 ±0.66	6.68 ±0.21
Konkan Region	28	ZM4a	3.00 ±0.67	20.00 ±0.33	7.66 ±0.78
	29	ZM4b	3.00 ±0.56	19.00 ±0.81	7.33 ±0.89
	30	ZM4c	6.00 ±0.45	24.00 ±0.20	5.00 ±0.25
	31	GM4a	5.00 ±0.12	47.00 ±0.33	10.40 ±0.67
	32	GM4b	6.00 ±0.28	43.00 ±0.55	8.16 ±0.33
	33	GM4c	6.00 ±0.78	48.00 ±0.72	9.00 ±0.55
	34	CA4a	5.00 ±0.33	29.00 ±0.52	6.80 ±0.67
	35	CA4b	5.00 ±0.21	28.00 ±0.33	6.60 ±0.44
	36	CA4c	4.00 ±0.67	30.00 ±0.28	8.50 ±0.52

[a]Datais the mean values of three replicates. Means±SE (p≤0.05).

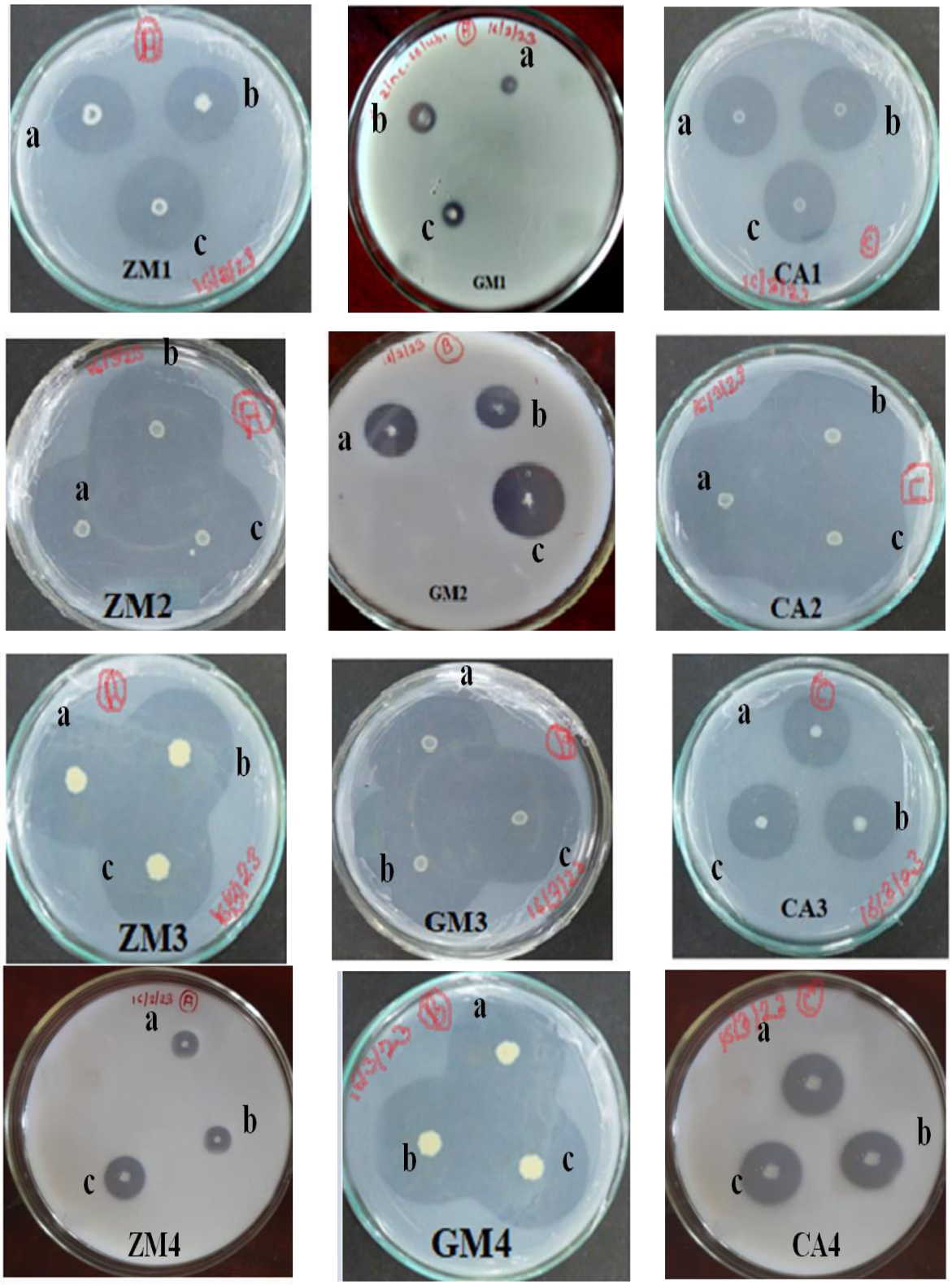


Fig. 6 Solubilization of insoluble zinc oxide on modified Pikovskaya agar medium by selected PGPR isolates after 7 days of incubation at 28±2°C.

Production of siderophore

Siderophilic bacteria, a kind of plant growth-promoting bacteria, have attracted much attention and been investigated for decades. These bacteria can promote plant growth and regulate the soil microenvironment by secreting siderophores. Out of a total of 36 isolates, all exhibited positive results for production of siderophore, evidenced by presence of purple or yellow halo zones in CAS agar medium. Notably, the isolate, namely, GM2c corresponds to Soybean (*Glycine max*) from Western Maharashtra region (as illustrated in Fig. 7), displayed the largest halo zone, rest of each measuring over 12 mm, indicating their significant siderophore-producing capabilities (Table 4.12). These results showed that siderophilic bacteria were abundant in the rhizosphere soil.

Plant growth promoting rhizobacteria (PGPR) produce siderophores to help plants get iron from the soil. Siderophores are low molecular weight compounds that bind to iron and transport it into plant cells. Siderophore production is considered a valuable plant growth promotion trait in several PGPR. Pseudomonas species are known for their remarkable siderophore-producing ability.

Iron (Fe) serves as a crucial nutrient for nearly all living organisms (Neilands, 2014; Pahari and Mishra, 2017). In this study, all isolates shared common characteristics of siderophore production on solid CAS blue agar medium. Several researchers have reported siderophore producing ability in different PGPR isolates as *Ralstoniamannitolilytica* (Paul and Datta, 2016), *Pantoeadispersa* (Panwar *et al*., 2016) and *Acinetobacter pittii* (Kumari *et al*., 2018).

Habibi *et al*. (2019) reported that *Pseudomonas* species were more active in terms of siderophore production than species of other genera. It is interesting to note that no Pseudomonas isolate was identified in our study. The different results may have been due to the bacterial species varying with the rhizospheres of different plants and different locations.

Table 4.12 Ability of PGPR isolates to produce Siderophores.

	Sr. No.	PGPR Isolates	[a]Diameter of Colony (mm)	[a]Diameter of Halo Zone (mm)	[a]SPI
Marathwada Region	1	ZM1a	12.00 ±0.33	26.00 ±0.14	3.16 ±0.33
	2	ZM1b	11.00 ±0.45	25.00 ±0.22	3.27 ±0.21
	3	ZM1c	11.00 ±0.78	24.00±0.20	3.18 ±0.18
	4	GM1a	9.00 ±0.67	25.00 ±0.10	3.77 ±0.67
	5	GM1b	10.00 ±0.89	24.00 ±0.47	3.40 ±0.58
	6	GM1c	9.00 ±0.42	26.10 ±0.36	3.90 ±0.89
	7	CA1a	8.00 ±0.62	28.00 ±0.12	4.50 ±0.72
	8	CA1b	8.00 ±0.33	29.00 ±0.23	4.62 ±0.66
	9	CA1c	7.00 ±0.22	24.00 ±0.11	4.42 ±0.54
Western Maharashtra Region	10	ZM2a	12.00 ±0.18	13.00 ±0.22	2.08 ±0.42
	11	ZM2b	13.00 ±0.21	15.00 ±0.42	2.15 ±0.67
	12	ZM2c	14.00 ±0.52	13.00 ±0.56	1.92 ±0.11
	13	GM2a	9.00 ±0.88	34.00 ±0.78	4.77 ±0.18
	14	GM2b	9.00 ±0.82	30.00 ±0.24	4.33 ±0.25
	15	GM2c	8.00 ±0.56	31.00 ±0.18	4.87 ±0.33
	16	CA2a	6.00 ±0.67	15.00 ±0.22	3.50 ±0.35
	17	CA2b	7.00 ±0.22	13.00 ±0.56	2.85 ±0.67
	18	CA2c	6.00 ±0.18	14.00 ±0.66	3.33 ±0.78
Vidarbha Region	19	ZM3a	6.00 ±0.10	11.00 ±0.32	2.83 ±0.89
	20	ZM3b	7.00 ±0.13	12.00 ±0.25	2.71 ±0.64
	21	ZM3c	7.00 ±0.53	14.00 ±0.33	3.00 ±0.35
	22	GM3a	8.10 ±0.16	16.10 ±0.89	2.98 ±0.32
	23	GM3b	7.00 ±0.24	14.00 ±0.18	3.00 ±0.24
	24	GM3c	10.00 ±0.32	18.00 ±0.20	2.80 ±0.22
	25	CA3a	11.00 ±0.61	18.10 ±0.12	2.64 ±0.52
	26	CA3b	12.00 ±0.45	19.00 ±0.32	2.58 ±0.25
	27	CA3c	11.00 ±0.26	18.00 ±0.67	2.63 ±0.67
Konkan Region	28	ZM4a	6.00 ±0.15	12.00 ±0.81	3.00 ±0.33
	29	ZM4b	7.00 ±0.62	15.00 ±0.32	3.14 ±0.35
	30	ZM4c	6.00 ±0.34	12.00 ±0.78	3.00 ±0.67
	31	GM4a	6.10 ±0.67	14.10 ±0.47	3.31 ±0.78
	32	GM4b	6.00 ±0.44	15.00 ±0.33	3.50 ±0.12
	33	GM4c	7.00 ±0.51	18.00 ±0.21	3.57 ±0.11
	34	CA4a	11.00 ±0.81	19.10 ±0.66	2.73 ±0.18
	35	CA4b	12.00 ±0.11	20.00 ±0.45	2.66 ±0.25
	36	CA4c	11.00 ±0.20	19.00 ±0.23	2.72 ±0.33

[a]Datais the mean values of three replicates. Means±SE (p≤0.05).

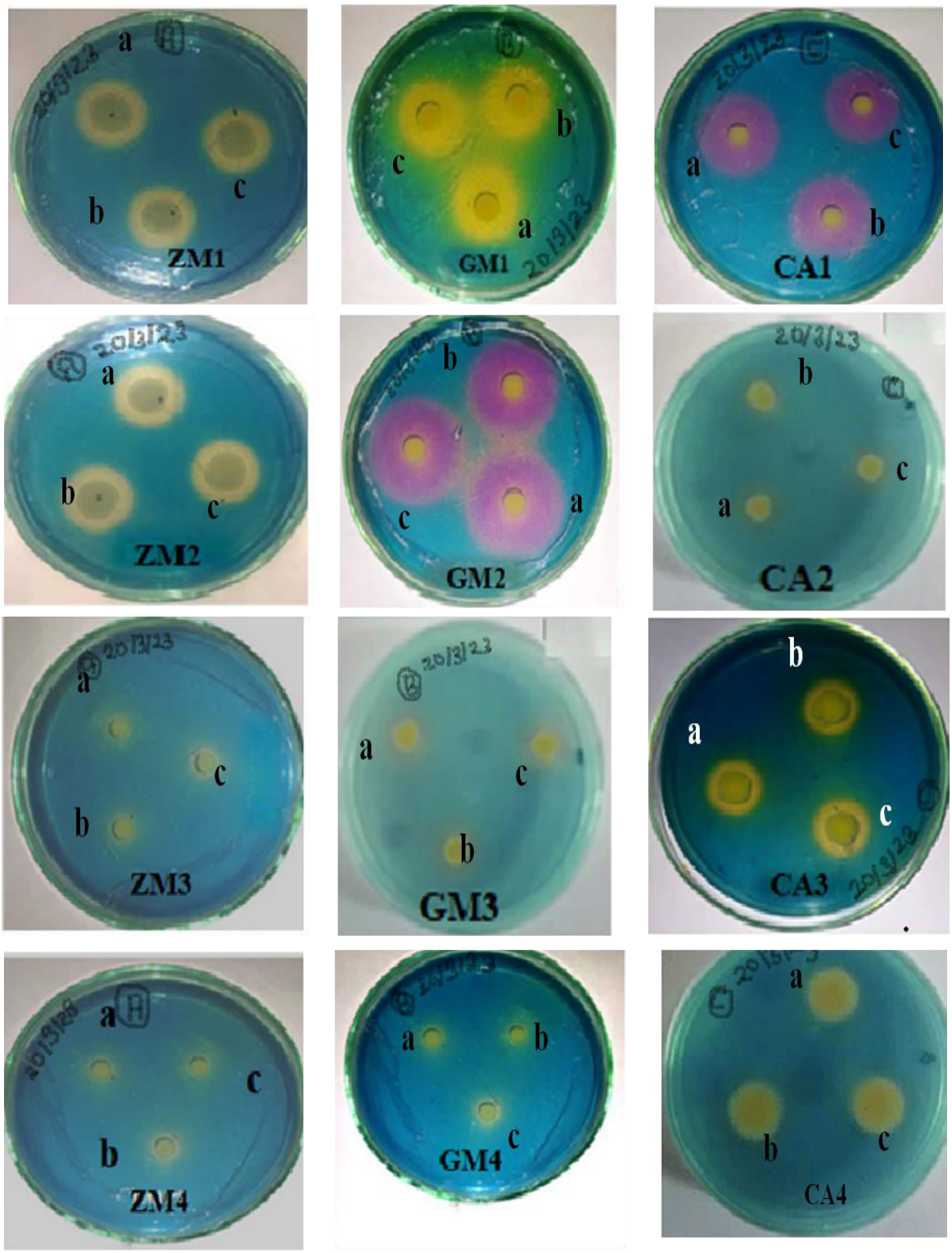


Fig. 7 Releasing of Siderophore on CAS agar medium by selected PGPR isolates after 7 days of incubation at 28±2 °C.

Amylase production

Bacteria can produce a large number of extracellular enzymes to improve their adaptability to the environment. Currently, the research of amylase mainly focuses on industrial production and promoting the absorption and utilization of starch in animals. Satoh *et al*. 1997, found that the bacterium *Streptococcus bovis* in the rumen of animals has very strong raw starch adsorption and degradation abilities due to the expression of the gene that produces extracellular amylase, which can improve the digestion ability of animals

In thus study the bacterial isolates were formed clear zone which means in Starch Agar Medium they promisingly found to degrade starch (Fig. 8). The isolate CA2c (13mm) from the Western Maharashtra region's Chilli (*Capsicum annuum*) exhibited the greatest solubilization index in comparison to all the isolates tested for amylase production in Starch Agar Medium. (Table 4.13).

Amylase-producing *Pseudomonas fluorescence* strains had been previously isolated from different plant rhizospheres & evaluated on the basis of a variety of parameters (Karnwal, 2011). Similar findings are noticed (Kathiresan and Manivannan, 1996) with *Penicillium fellutanum* isolated from mangrove rhizosphere soil.

Yao *et al*. (2021) also found that *B. subtilis* WS9 can efficiently produce α-amylase by enhancing the signal peptide SPRpmG, which is 2.9-fold greater than the original strain, also considered that the accumulation of *B. subtilis* extracellular amylase can be enhanced through signal peptide optimization .

According to Zheng *et al*. (2021), the presence of α-amylase strongly influences the adaptation strategies of bacterial metabolism and stimulates the production of metabolic signals such as acetic acid. This acid induces numerous changes in the physicochemical properties of the bacterial surface, such as hydrophobicity and surface charge, which directly affects the interactions that these bacteria are able to establish. This makes the presence of positive results even more surprising, which can be explained by the production of secondary metabolites by the actinobacteria itself .

Table 4.13 Estimation of PGPR ability of amylase production.

	Sr. No.	PGPR Isolates	[a]Diameter of Colony (mm)	[a]Diameter of Halo Zone (mm)	[a]API
Marathwada Region	1	ZM1a	5.00±0.15	13.00 ±0.11	3.60 ±0.66
	2	ZM1b	5.00 ±0.26	13.00 ±0.25	3.60 ±0.22
	3	ZM1c	3.00 ±0.67	12.00 ±0.45	5.00 ±0.42
	4	GM1a	6.00 ±0.89	11.00 ±0.67	2.83 ±0.18
	5	GM1b	7.00 ±0.90	12.00 ±0.78	2.71 ±0.33
	6	GM1c	6.00 ±0.22	12.00 ±0.89	3.00 ±0.35
	7	CA1a	4.00 ±0.67	00	---
	8	CA1b	5.00 ±0.66	00	---
	9	CA1c	3.00 ±0.52	4.00 ±0.11	2.33 ±0.22
Western Maharashtra Region	10	ZM2a	4.00 ±0.18	17.00 ±0.18	5.25±0.78
	11	ZM2b	5.00 ±0.25	19.00 ±0.20	4.80 ±0.42
	12	ZM2c	4.10 ±0.10	20.10 ±0.21	5.90 ±0.67
	13	GM2a	8.00 ±0.11	24.00 ±0.33	4.00 ±0.45
	14	GM2b	6.00 ±0.33	27.00 ±0.56	5.50 ±0.56
	15	GM2c	6.00 ±0.32	28.10 ±0.67	5.68 ±0.22
	16	CA2a	6.00 ±0.56	15.10 ±0.22	3.51 ±0.67
	17	CA2b	4.10 ±0.22	14.00 ±0.18	4.41 ±0.33
	18	CA2c	2.00 ±0.14	12.00 ±0.20	7.00 ±0.25
Vidarbha Region	19	ZM3a	3.00 ±0.72	13.00 ±0.25	5.33 ±0.18
	20	ZM3b	2.00 ±0.35	8.00 ±0.22	5.00 ±0.22
	21	ZM3c	3.00 ±0.11	15.00 ±0.66	6.00 ±0.32
	22	GM3a	4.00 ±0.33	14.00 ±0.21	4.50 ±0.78
	23	GM3b	3.00 ±0.45	13.00 ±0.56	5.33 ±0.45
	24	GM3c	4.00 ±0.66	13.10 ±0.60	4.27 ±0.21
	25	CA3a	5.10 ±0.18	14.00 ±0.25	3.74 ±0.78
	26	CA3b	5.00 ±0.25	15.00 ±0.22	4.00 ±0.89
	27	CA3c	4.00 ±0.12	16.00 ±0.10	5.00 ±0.12
Konkan Region	28	ZM4a	5.00 ±0.41	15.00 ±0.18	4.00 ±0.25
	29	ZM4b	3.00 ±0.80	8.00 ±0.12	3.66 ±0.33
	30	ZM4c	4.00 ±0.25	13.00 ±0.15	4.25±0.56
	31	GM4a	3.00 ±0.11	7.00 ±0.56	3.33 ±0.78
	32	GM4b	4.00 ±0.76	11.00 ±0.78	3.75±0.12
	33	GM4c	4.00 ±0.22	10.10 ±0.11	3.52 ±0.18
	34	CA4a	4.00 ±0.16	11.00 ±0.67	3.75±0.20
	35	CA4b	6.00 ±0.11	13.00 ±0.21	3.16 ±0.18
	36	CA4c	4.10 ±0.10	12.00 ±0.33	3.92 ±0.11

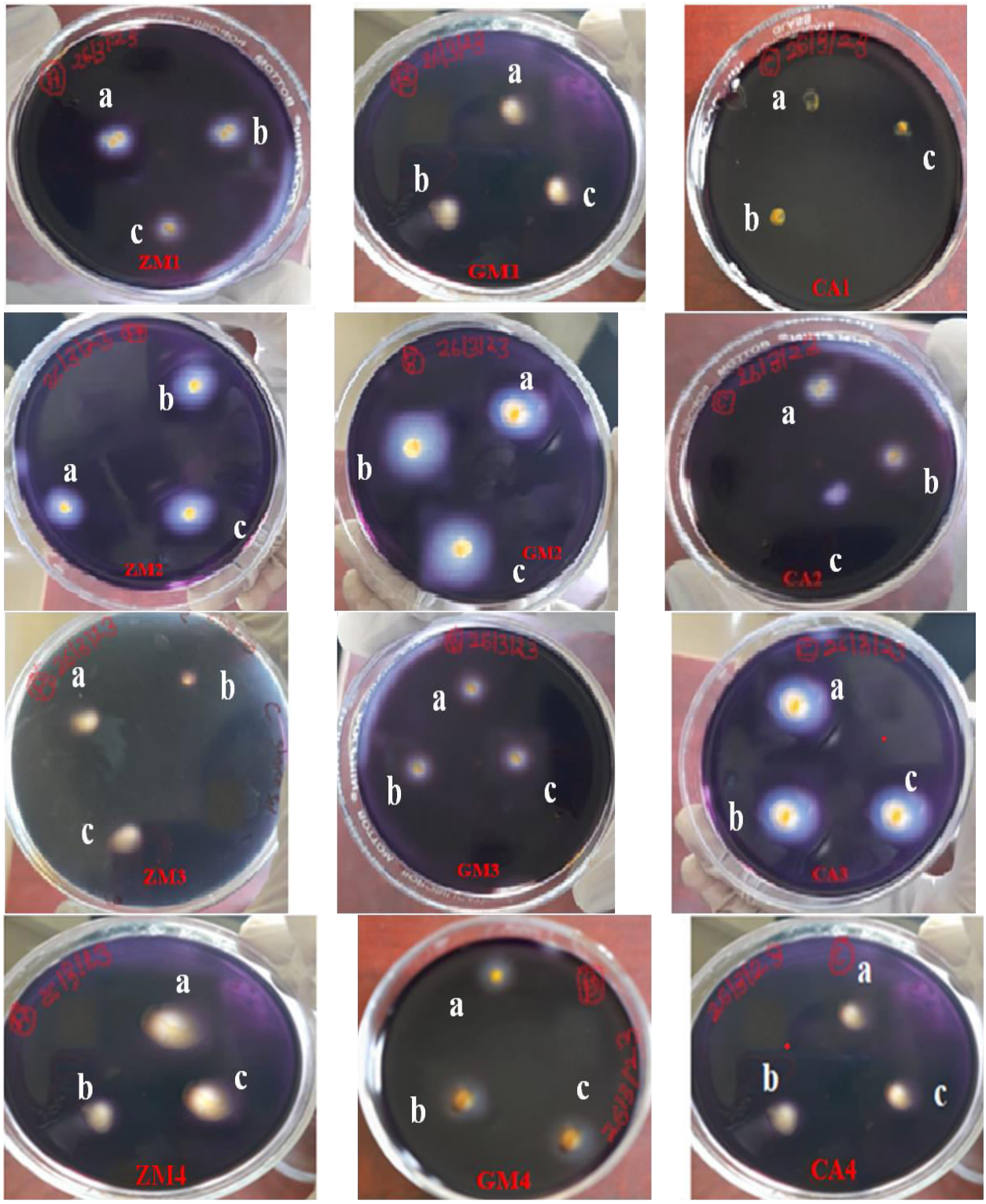


Fig. 8 Production of Amylase by selected PGPR isolates on Starch Agar Medium after 7 days of incubation at 28±2 °C.

Production of Indole-3-acetic acid (IAA)

The PGPR isolates demonstrating various PGP traits were chosen to evaluate Indole-3-acetic acid (IAA) production in L-tryptophan absence. The conversion of red pink color confirmed positive test result for IAA production. The selected strains (one from each crop) indicated positive result except for GM1 and CA2. Based on the quantitative estimation of IAA; the isolates were identified as promising candidates, a finding that is supported by Karnwal (2009). The isolate ZM3 produced maximum (15.91 µg/mL) of IAA as recorded in Table 4.14 (Fig. 9).

Table 4.14 Estimation of bacterial isolates for the IAA production.

Sr. No.	PGPR Isolates	Production of IAA by isolates		
		OD at 530 nm	Concentration (µg/mL)	Result
1	ZM1	1.034	11.49	Positive
2	GM1	0.081	0.9	Negative
3	CA1	1.325	14.72	Positive
4	ZM2	0.332	3.69	Positive
5	GM2	1.384	15.37	Positive
6	CA2	0.035	0.39	Negative
7	ZM3	1.432	15.91	Positive
8	GM3	1.381	15.34	Positive
9	CA3	1.335	14.83	Positive
10	ZM4	1.421	15.79	Positive
11	GM4	1.021	11.35	Positive
12	CA4	0.379	4.22	Positive

Among diverse array of phytohormones, Indole-3-acetic acid (IAA) stands out as abundant; crucial plant growth regulator produced by bacteria (Venturi and Keel, 2016). Their pivotal function in overall development of plants has been well-established in scientific research (Gowtham *et al*., 2017). As per earlier reports production of IAA can vary amongst different bacterial species which is also controlled by the conditions of culture, growth stage as well as substrates availability (Spaepen *et al*., 2007). Mussa *et al*. (2018) found high IAA production by Enterobacter strain in presence of 0.1% L- tryptophan.

Similarly, Kumari *et al*. (2018a), observed higher production of IAA by *Pseudomonas aeruginosa* strain BHU B13–398 & *Acinetobacter pittii* strain BHU B13-397 with 0.1% DL-tryptophan after two days of incubation period. The lessening in indole acetic acid synthesis during the incubation period may be attributed to the release of degrading enzymes, including indole acetic acid oxidase & peroxidase (Arora *et al*., 2015; Ozdal *et al*., 2017).

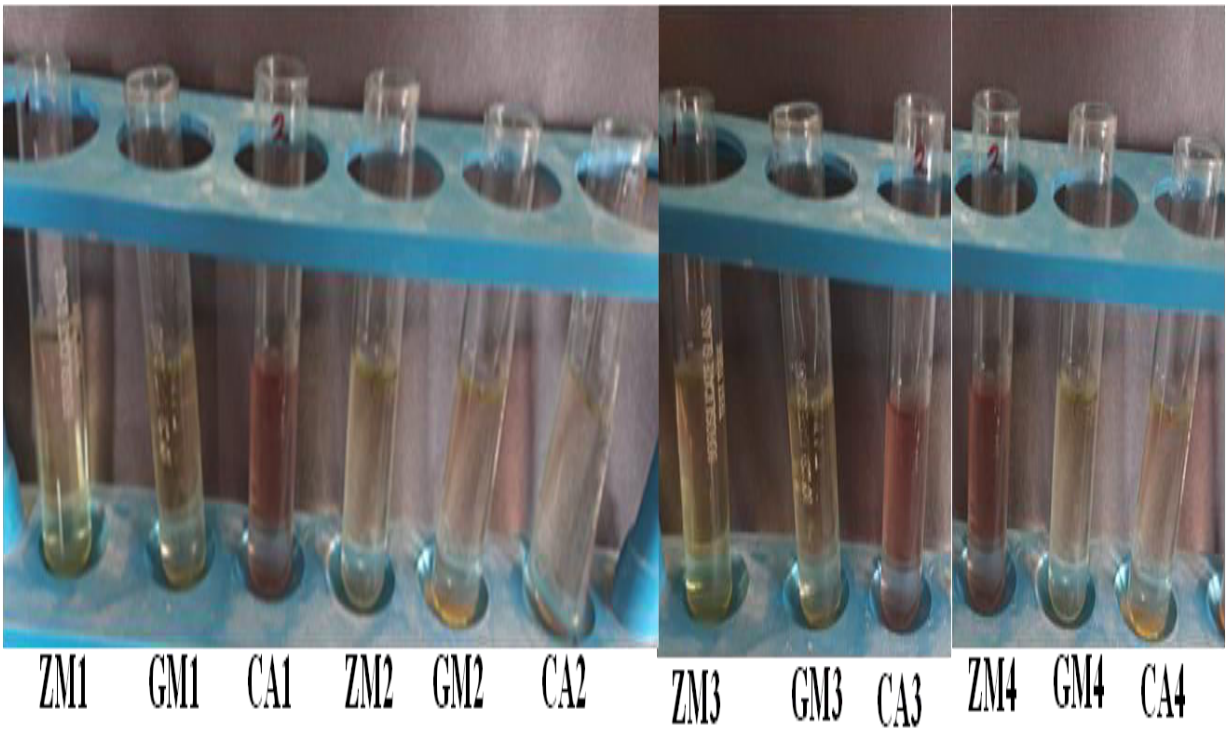
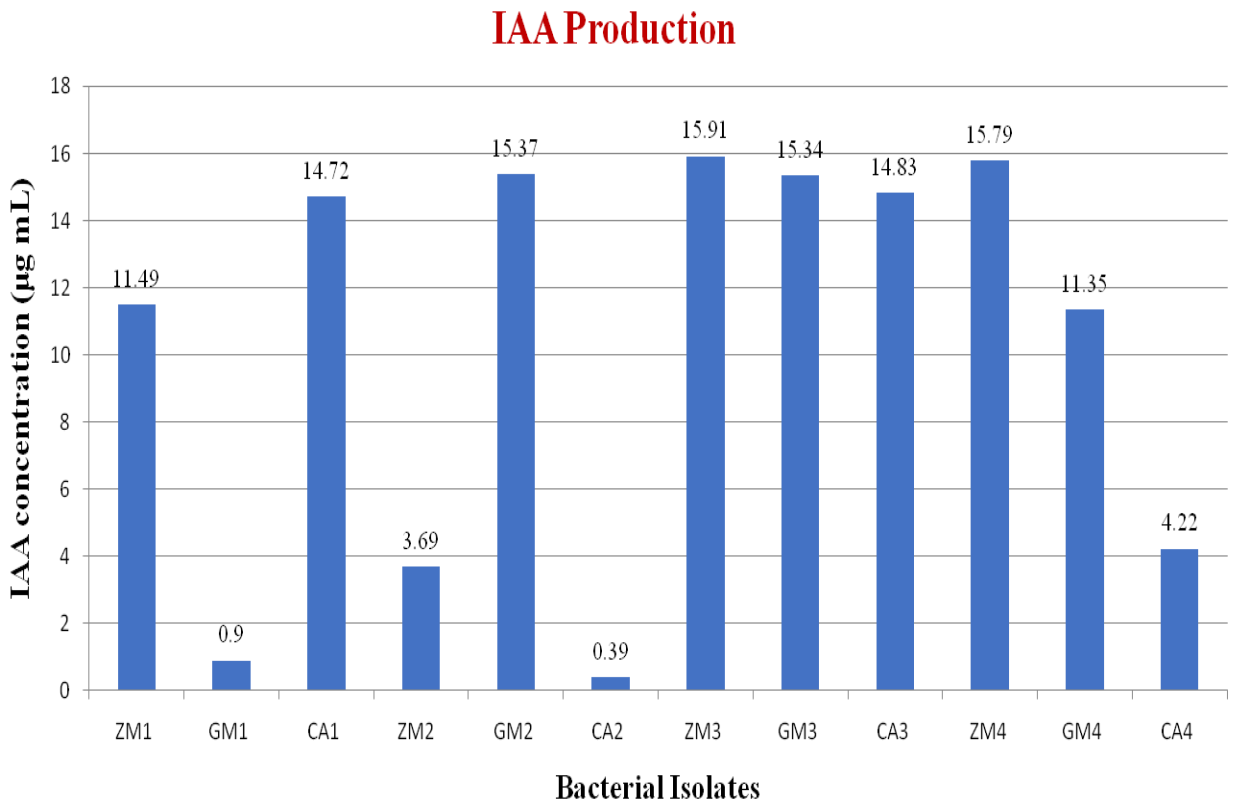


Fig. 9 Screening of bacterial isolates for qualitative estimation of IAA.



Graph 2. Quantitative assesment of Indole-3-acetic acid (IAA) by selected bacterial isolates**.**

Catalase, Urease and Oxidase test

Air bubbles formed in all the isolates tested which indicated the presence of positive catalase activity (Hydrogen Peroxide Test) except CA2 (Fig. 10). Among all the bacterial isolates, GM1, CA2 and CA3 were found positive for Oxidase test (Fig. 11). Isolates GM2, CA2 and CA3 were found positive for Urease test (Fig. 12).

It is predicted that strains demonstrating catalase activity possess remarkable resistance to chemical, mechanical, & environmental stressors (Joseph *et al*., 2007; Kumar *et al*., 2012) Air bubble formation indicated positive catalase test (Mollah *et al*., 2020). Urease, an enzyme that performs a vital part in the decomposition of urea into ammonia, is among the most commonly evaluated soil enzymes (Rodriguez-Caballero *et al*., 2017). A positive oxidase test result in

PGPR isolates is indicated by a color change to bright purple within 5-10 seconds (Kesaulya *et al*., 2021).

Table 4.15 Analysis of PGPR isolates for Catalase test.

Sr. No.	PGPR Isolates	Result
1	ZM1	Positive
2	GM1	Positive
3	CA1	Positive
4	ZM2	Positive
5	GM2	Positive
6	CA2	Negative
7	ZM3	Positive
8	GM3	Positive
9	CA3	Positive
10	ZM4	Positive
11	GM4	Positive
12	CA4	Positive

Table 4.16 Analysis of PGPR isolates for Urease test.

Sr. No.	PGPR Isolates	Results
1	ZM1	Negative
2	GM1	Negative
3	CA1	Negative
4	ZM2	Negative
5	GM2	Positive
6	CA2	Positive
7	ZM3	Negative
8	GM3	Negative
9	CA3	Positive
10	ZM4	Negative
11	GM4	Negative
12	CA4	Negative

Table 4.17 Analysis of PGPR isolates for Oxidase test.

Sr. No.	PGPR Isolates	Results
1	ZM1	Negative
2	GM1	Positive
3	CA1	Negative
4	ZM2	Negative
5	GM2	Negative
6	CA2	Positive
7	ZM3	Negative
8	GM3	Negative
9	CA3	Positive
10	ZM4	Negative
11	GM4	Negative
12	CA4	Negative

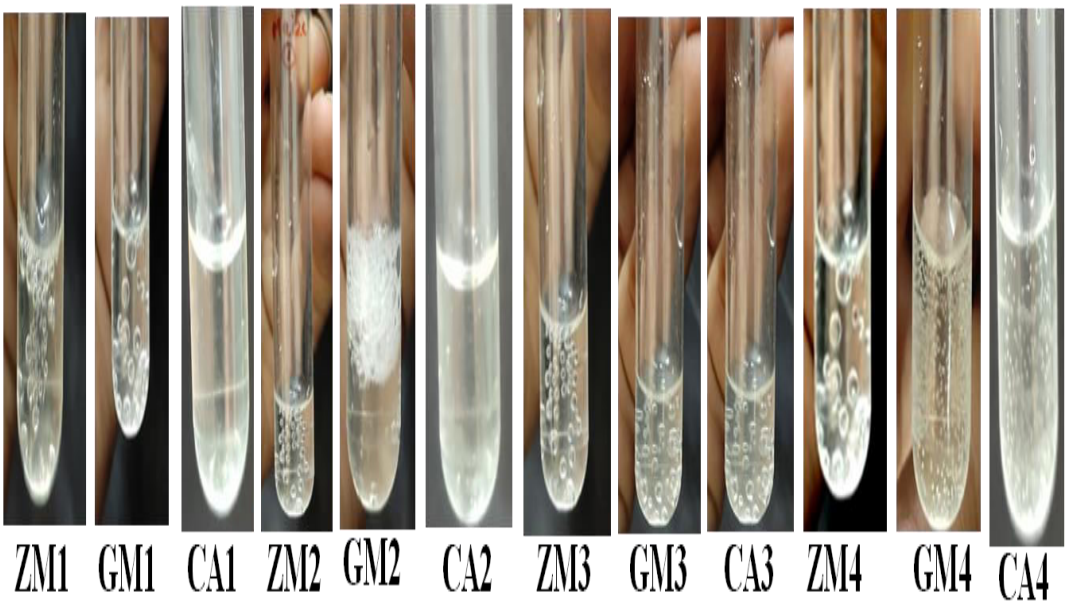


Fig. 10 Screening of bacterial isolates for Catalase test.

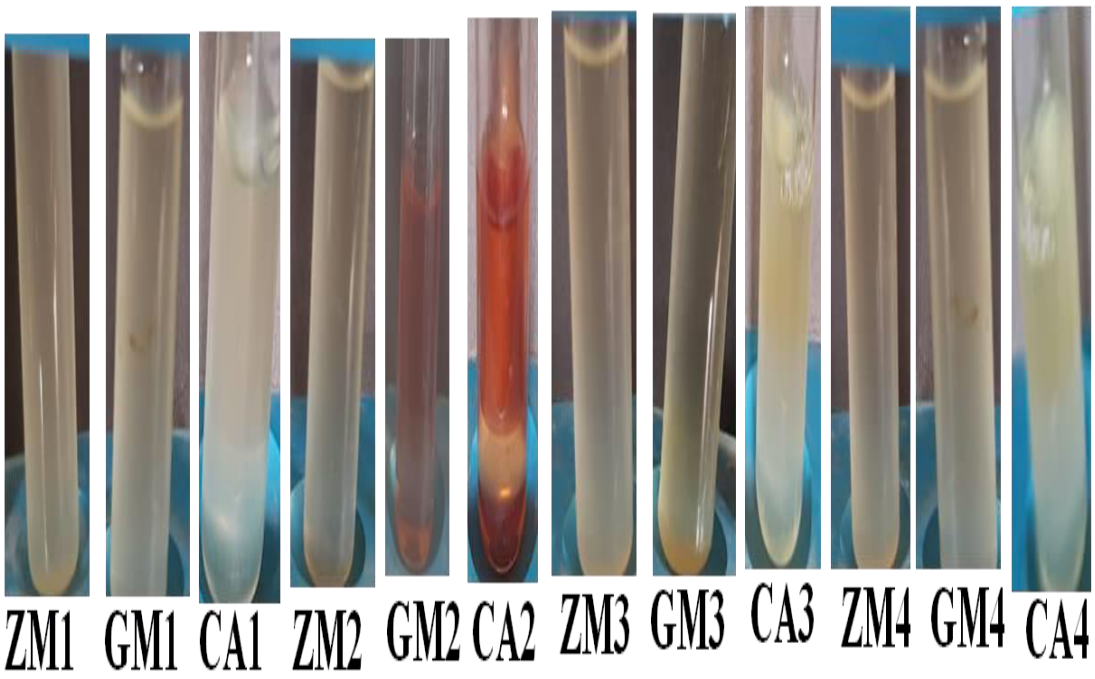


Fig. 11 Screening of bacterial isolates for Urease test.

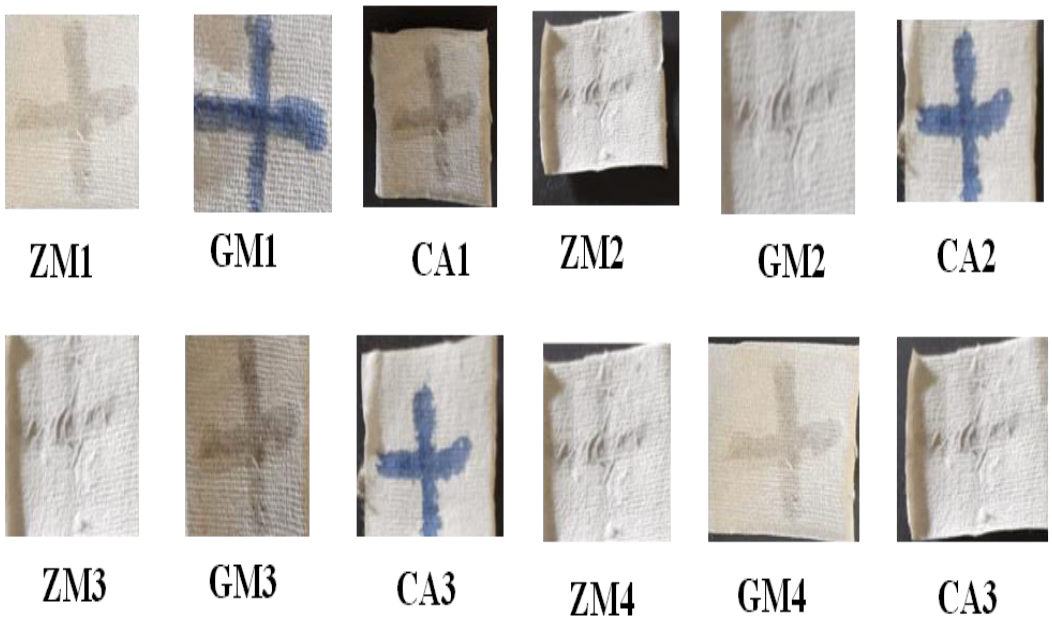


Fig. 12 Screening of bacterial isolates for Oxidase test.

Molecular characterization of selected PGPR isolates using 16S rRNA technique

1. DNA Amplification via PCR

Rhizobacterial DNA of 12 selected PGPR isolates namely; ZM1, GM1, CA1, ZM2, GM2, CA2, ZM3, GM3, CA3, ZM4, GM4, CA4 respectively from different regions of Maharashtra was amplified with primer set 27F and 1492R.

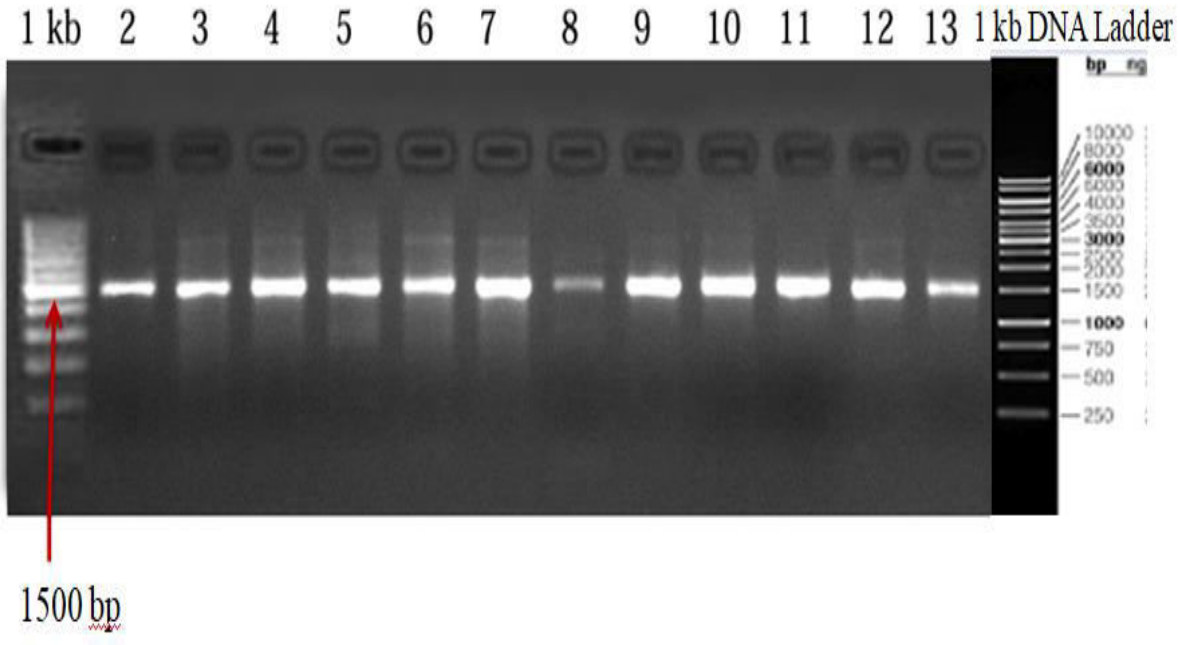


Fig. 13 PCR amplification of PGPR 16 S rRNA gene profiles. Lane 1: Molecular weight marker (1kb ladder), Lane 2-13 represent partial 16 S rRNA of selected PGPR isolates *i.e.* ZM1, GM1, CA1, ZM2, GM2, CA2, ZM3, GM3, CA3, ZM4, GM4, CA4 respectively.

2. Sequencing of amplified 16S rRNA

Amplified 16S rRNA genes from all selected rhizobacterial isolates were eluted, purified. Sanger sequencing was employed to sequence purified PCR products at Eurofins Genomics India Pvt. Ltd. in Bengaluru, India. The 16S rDNA sequences obtained after sequencing had been

assembled in SeqMan software and to identify individual strains complete sequences had been subjected to BLAST, NCBI. The initial sequence of (≅30-50bp) each forward and reverse reaction was trimmed and made a contig sequence that was used for further analysis (Appendix V).

3. Analysis of Sequences

The 16S rRNA gene sequences obtained had beenexaminedutilizing the BLAST and compared with the GenBank database. Sequences of PGPR isolates from four different sites showed the closest matches with two distinct bacterial classes. Rhizobacterial isolates were identified based on sequence similarities with the GenBank database (Table 4.18).

Phylogenetic tree of four isolates using MEGA version 11 software showed *Pseudomonas* species (*Pseudomonas guariconensis, Pseudomonas plecoglossicida, Pseudomonas mosselii* and *Pseudomonas aeruginosa*), *Aeromonas* sp (*Aeromonas caviae- 2, Aeromonas hydrophila*), *Acinetobacter* sp, *Delftiatsuruhatensis* and *Stenotrophomonas maltophilia.*

Pseudomonas is a genus of bacteria that can be beneficial to plants- Producing phosphate solubilizing compounds, production of siderophores, antagonistic effects against phytopathogenic fungi, improving plant tolerance to droughts, high salinity stresses and synthesis of phytohormones as *Pseudomonas plecoglossicida* produces auxins and cytokinins.

Aeromonas species produce a variety of enzymes (Chitinases, Nucleases, Amylases) that are associated with pathogenicity and environmental adaptability Eg. enhancing plant resistance to dehydration.

Delftia tsuruhatensis functions in plants- Plant growth promotion, Organic pollutant degradation, Antagonistic activity against plant pathogens, Rhizosphere colonization, IAA production, Phosphate solubilization *etc*. *Acinetobacter* sp. contributes to the mineralization, can increase the shoot height, root length.

Stenotrophomonas maltophilia- Sulfur & nitrogen cycles, beneficial plant-microbe interaction, Degradation of complex compounds and pollutants, osmoprotectantstransport, biocontrol activity, Production of phytohormones. *S. maltophilia* thus involved in bioremediation and phytoremediation strategies

Table 4.18 Molecular characterization through 16S rRNA gene sequencing technique and identification of selected PGPRs.

Regions	Sr. No	PGPR Isolates	Closest NCBI Database match	Similarity (%)	E Value	Accession No.
Marathwada	1	ZM1	Pseudomonas guariconensis	99.67%	0.0	PP754219
	2	GM1	Pseudomonas putida	100.00%	0.0	PP754220
	3	CA1	Pseudomonas aeruginosa	100.00%	0.0	PP754221
Western Maharashtra	4	ZM2	Pseudomonas sp.	99.24%	0.0	PP754222
	5	GM2	Pseudomonas sp.	97.00%	0.0	PP754223
	6	CA2	Delftia tsuruhatensis	100.00%	0.0	PP754224
Vidarbha	7	ZM3	Acinetobacter sp.	94.66%	0.0	PP754225
	8	GM3	Stenotrophomonas maltophilia	100.00%	0.0	PP754226
	9	CA3	Aeromonas caviae	100.00%	0.0	PP754227
Konkan	10	ZM4	Aeromonas caviae	99.25%	0.0	PP754228
	11	GM4	Aeromonas caviae	100.00%	0.0	PP754229
	12	CA4	Aeromonas hydrophila	99.15%	0.0	PP758187

Several approaches are available for bacterial identification, including morphological and biochemical characterization. However, these methods are often challenging and time-consuming, and they may not reliably identify microorganisms at various levels, let alone at strain level (Franco-Duarte *et al*., 2019). Many closely related species isolated in PGP studies are indistinguishable using conventional techniques. Misidentification is common in traditional methods due to various unknown phenotypic traits that frequently arise under different cultural conditions (Cherkaoui *et al*., 2010).

In contrast, the 16S rRNA technique provides an efficient and reliable method for identifying bacterial communities at the species level. The 16S rRNA genes are highly conserved and are universally present across all bacterial genomes (Klappenbach *et al*., 2000). The molecular identification process for all bacteria is standardized, requiring only DNA extraction, 16S rRNA amplification, and sequencing (Reller *et al*., 2007). Additionally, using 16S rRNA sequences from various bacteria offers a more accurate approach to bacterial identification than traditional methods, significantly reducing the likelihood of misidentification (Boivin-Jahns *et al*., 1995).

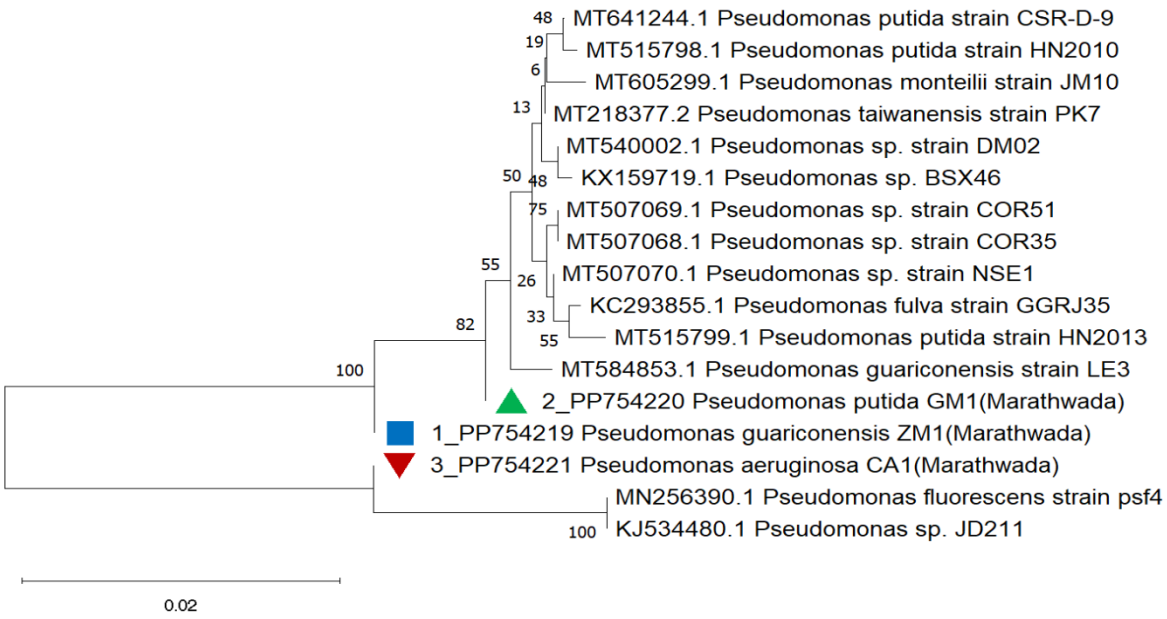


Figure 14 Phylogenetic tree on the basis of 16S rRNA gene sequences of isolates ZM1, GM1 & CA1 from **Marathwada region** showing the relationship between some related taxa, using MEGA XI by Neighbor-Joining method at bootstrap value of n=1000. At branch points, bootstrap values are displayed as percentages. Bar displays 2nt (nucleotide) substitution per 100nt.

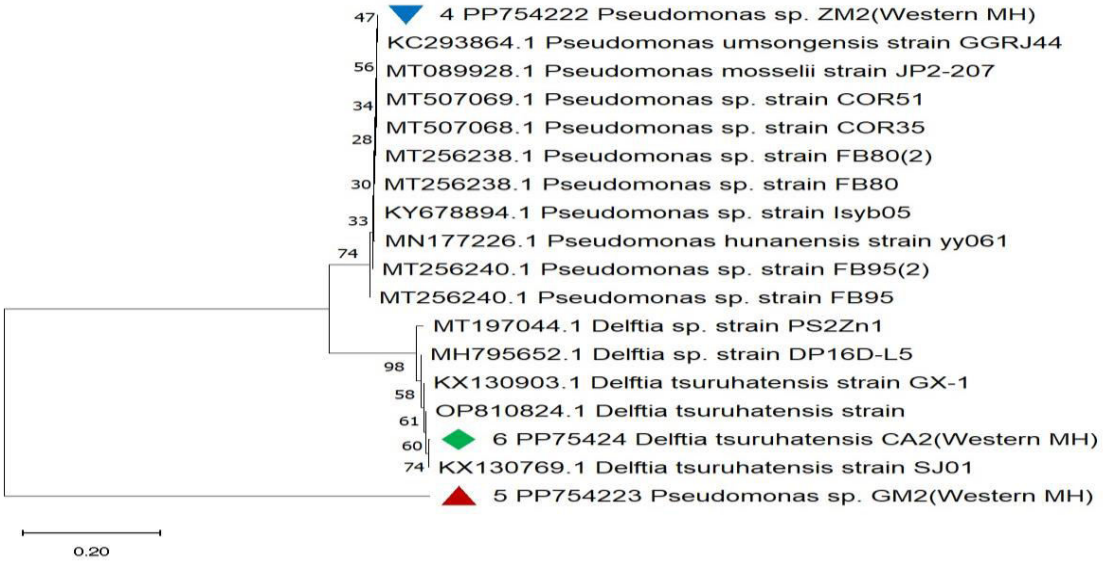


Figure 15 Phylogenetic tree on thebasis of 16S rRNA gene sequences of isolates ZM2, GM2 & CA2 from **Western MH region** showing the relationship between some related taxa, using MEGA XI by Neighbor-Joining method at bootstrap value of n=1000. Bootstrap values are demonstrated as percentages at branch points. Bar displays 2nt (nucleotide) substitution per 100nt.

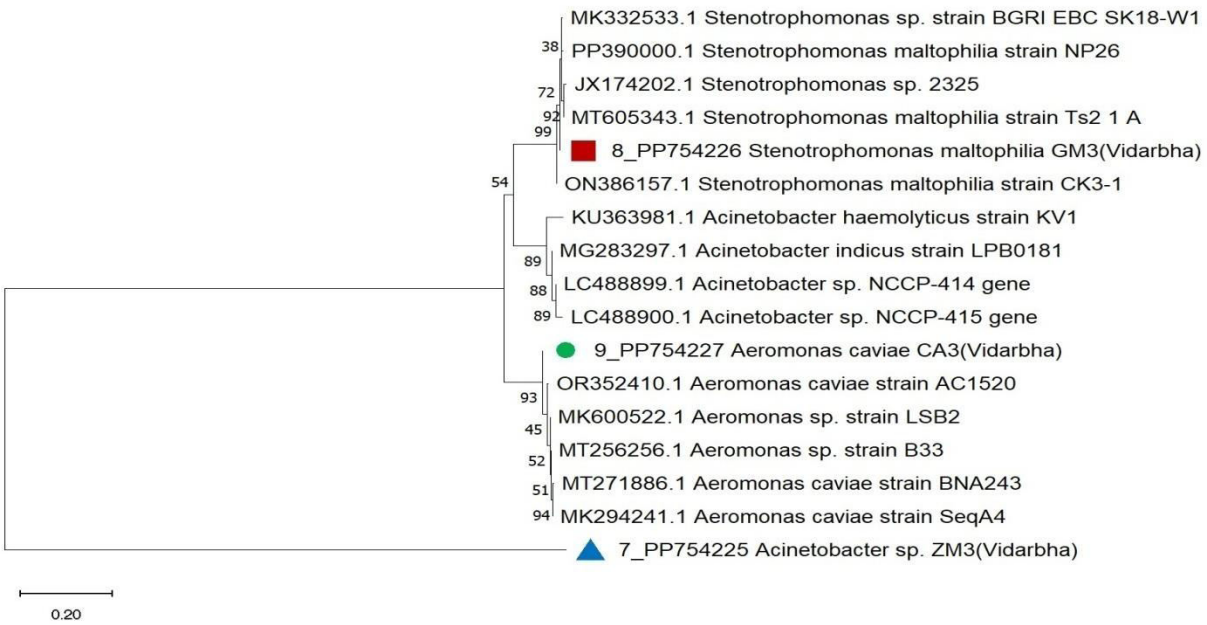


Figure 16 Phylogenetic tree on the basis of 16S rRNA gene sequences of isolates ZM3, GM3 & CA3 from **Vidarbha region** showing the relationship between some related taxa, using MEGA XI by Neighbor-Joining method at bootstrap value of n=1000. Bootstrap values are displayed as percentages at the branch points. Bar displays 2nt (nucleotide) substitution per 100nt.

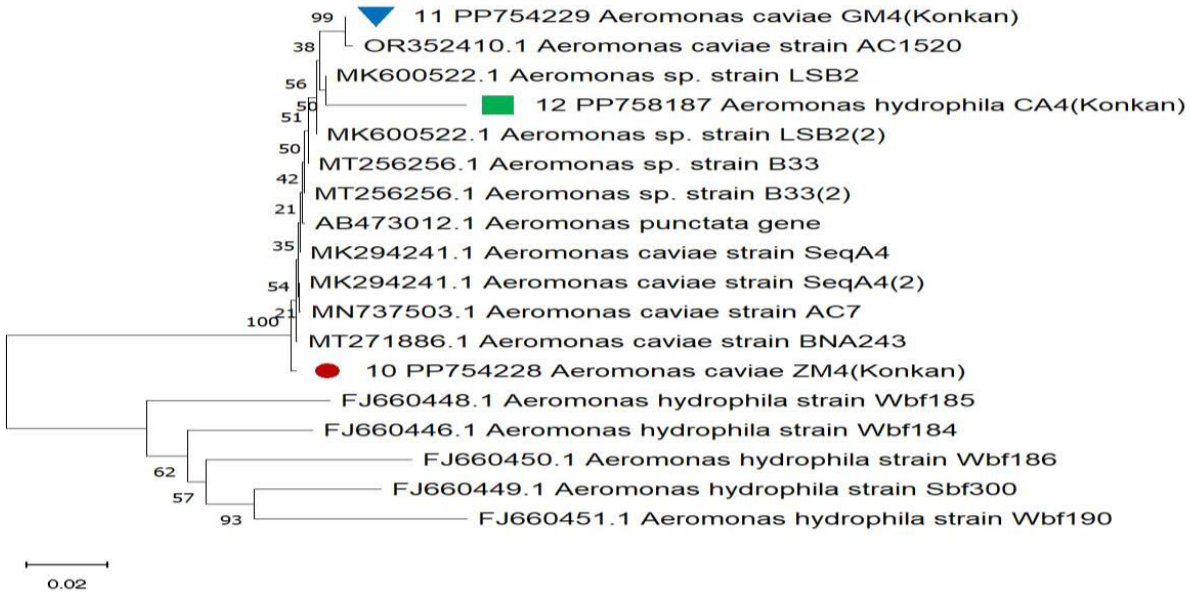


Figure 17 Phylogenetic tree on the basis of 16S rRNA gene sequences of isolates ZM4, GM4 & CA4 from **Konkan region** showing the relationship between some related taxa, using MEGA XI by Neighbor-Joining method at bootstrap value of n=1000. Bootstrap values are revealed as percentages at branch points. Bar displays 2nt (nucleotide) substitution per 100nt.

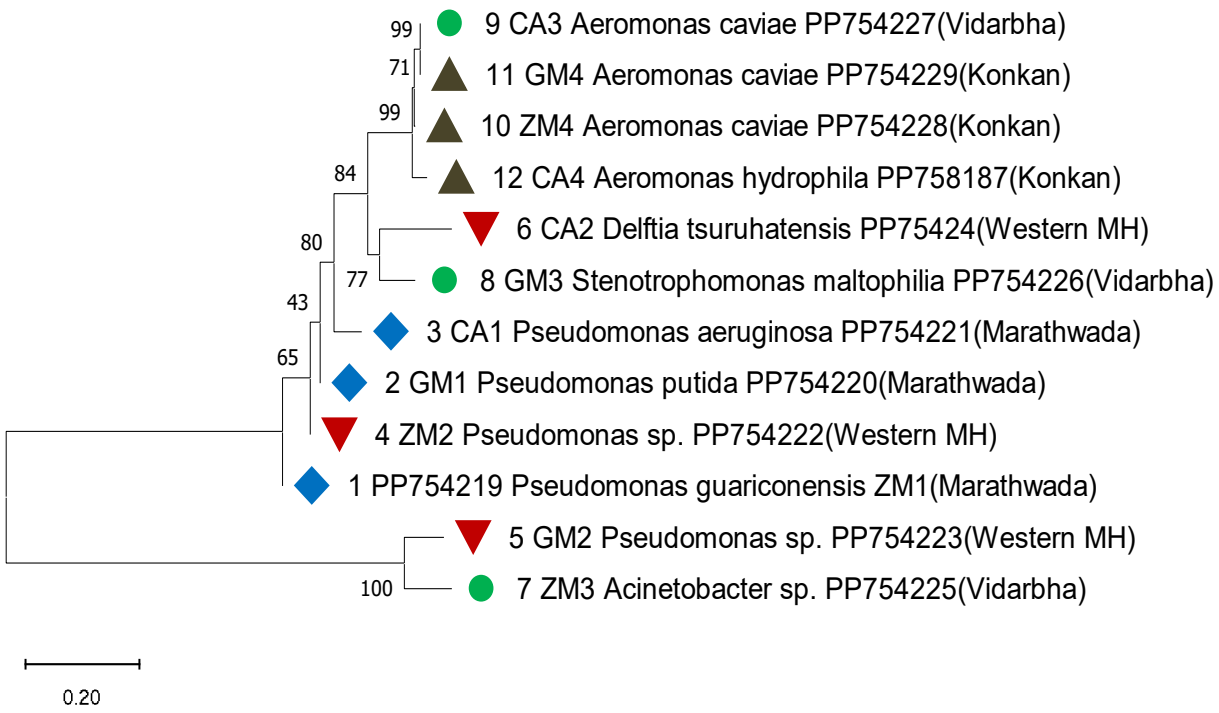


Figure 18 Phylogenetic tree on thebasis of 16S rRNA gene sequences of selected isolates showing relationship between some related taxa, using MEGA 11 by Neighbor-Joining method at bootstrap value of n=1000. Bootstrap values are revealed as percentages at branch points.

Phylogenetic analysis

The phylogenetic tree of PGPR isolates is separated into two main clades- two isolates *i e*. GM2-Pseudomonas sp. (PP754223) from Western MH and ZM3-Acinetobacter sp. (PP754225) from Vidarbha region showed single clade. While all the other isolates of different regions are grouped in first main cluster. Therefore, on the basis of 16S rRNA gene sequence data, all PGPR isolates seem to be paraphyletic except the two isolates. Only isolated PGPRs' 16S rRNA sequences were included in phylogenetic tree.

The BLAST search outcomes of 16S rRNA gene sequences from all isolates, when compared to similar bacterial sequences in the NCBI GenBank database, aligned closely with specific genera or species, indicating that while 16S rRNA gene sequence is frequently employed, it may serve primarily as a genetic marker for bacterial classification.

4. Standardization of media composition and evaluation of growth kinetic parameters of selected rhizobacteria.

Standardization of media composition

In the present study, three PGPR isolates- CA2, ZM3, and GM3;were selected to optimize their physical and nutritional parameters using a synthetic medium. Our results showed that these parameters had a significant impact on bacterial growth. The carbon source plays a crucial role in biosynthesis and energy generation during the cultivation of microorganisms. Rapidly metabolized sugars are often linked to fast bacterial growth, though they tend to result in low secondary metabolite productivity (Crueger and Crueger, 2004; Fuchslin *et al*., 2012). Primary & secondary metabolite production as well as biomass accumulation can be impacted by the rate at which the carbon source is digested (de Villegas *et al*., 2002).

a. Effect of different Carbon sources on the growth

To identify the most effective carbon source for supporting maximum growth of the bacterial isolates, 1% concentrations of glucose, maltose, and sucrose were individually tested in Schlegel's Medium (SM). After 32 hours of incubation, isolates CA2, ZM3, and GM3 exhibited the highest growth on glucose, while the lowest growth was observed with maltose and sucrose. Carbon sources also influenced digression of pH of the medium by growth of bacterial isolates. All three isolates CA2, ZM3, and GM3 showed maximum digression in medium pH in existence of glucose (Table 4.19).

In this investigation, maximum cell growth of all 3 isolates was achieved after 32 hours of incubation when glucose had been employed as carbon source. The greatest decrease in pH was also observed with glucose as the carbon source. This pH reduction can be attributed to the formation of gluconic acid, as these bacteria metabolize glucose via the Entner-Doudoroff (ED) pathway (Lynch *et al*., 2019). Yuan *et al*. (2008) demonstrated that glucose is essential for enhancing bacterial cell growth. Additionally, Slininger and Shea-Wilbur (1995) reported that *Pseudomonas fluorescens* produced high biomass in glucose-supplemented media.

b. Effect of nitrogen source on growth

The impact of reduced & nitrate forms of nitrogen on bacterial isolates growth was investigated using various nitrogen sources, that include potassium nitrate, ammonium sulfate, & ammonium chloride, each tested individually in the medium. The results showed that the rhizobacterial isolates were able to utilize a broad range of nitrogen sources, though their efficiency in using different sources varied. All three bacterial isolates demonstrated a preference for the reduced form of nitrogen over the nitrate form. The isolate CA2 showed higher growth ($\cong 1.56\times10^{10}$ CFU mL^{-1}) in ammonium chloride followed by ammonium sulphate when used as nitrogen sources in SM. ZM3 isolate showed maximum growth ($\cong 2.42\times10^{10}$ CFU mL^{-1}) when ammonium sulphate was employed as a nitrogen source subsequent to ammonium chloride. GM3 isolate showed maximum growth ($\cong 1.87\times10^{10}$ CFU mL^{-1}) when ammonium sulphate had beenemployed as a nitrogen source subsequent to ammonium chloride (Table 4.19).

Nitrogen is essential for synthesizing amino acids, enzyme cofactors, purines, pyrimidines, certain carbohydrates, and lipids, making up about 10% of the dry weight of most organisms. Many industrial microorganisms mayemploy various inorganic and organic nitrogen sources. The choice of nitrogen source is crucial in cultivating microorganisms effectively (Sayyed *et al*., 2005). Ammonium is considered the optimal nitrogen source for bacteria (Merrick and Edwards, 1995). While many bacterial strains can utilize nitrate as a nitrogen source, it must be transported by specific transporters (Gonzalez *et al*., 2006) and undergo a two-step reduction—first by assimilatory nitrate reductase and then by nitrite reductase—before being converted to NH_4^+ for assimilation (McCarty, 1995). Slininger and Shea-Wilbur (1995) observed that *Pseudomonas fluorescens* produced higher biomass when ammonium sulphate was employed as nitrogen source.

c. Effect of pH on cell growth

The effect of different pH levels (5, 7 and 8) on bacterial growth was investigated, revealing that pH significantly influenced the growth of the selected rhizobacteria. The impact of pH varied among the isolates. Isolate CA2 exhibited the highest growth (approximately 1.69×10^{10} CFU mL^{-1}) at pH 7, subsequent to pH 8, after 32 hours of incubation. Isolate ZM3 showed maximum growth (approximately 2.62×10^{10} CFU mL^{-1}) at pH 7, subsequent to pH 8, with minimal growth at pH levels 5. Isolate GM3 also demonstrated maximum growth

(approximately 1.94×10^{10} CFU mL^{-1}) at pH7, with lower growth at pH8, and negligible growth at pH 5. Overall, isolates CA2, ZM3, and GM3 preferred a nearly neutral pH for optimal growth, while both high and low pH levels inhibited cell growth (Table 4.19).

All three selected isolates showed maximum growth at 7 pH & 37 °C in synthetic medium. Similarly Dastager *et al*. (2009) described that a strain of *Pantoea* sp. showed growth in 4-11 pH range, but maximum at 7 pH. Pseudomonas fluorescens showed optimal growth rate and biomass accumulation over broad ranges (7-8) of pH (Slininger and Shea-Wilbur, 1995).

d. Effect of temperature on cell growth

Impact of varying temperatures on the cell growth of selected PGPR isolates was assessed by incubating the rhizobacterial cultures at 28°C, 33°C & 37°C. Isolates CA2, ZM3, and GM3 demonstrated maximum growth at 37°C, with moderate growth at the 28°C. Additionally, the most significant change in pH for all three isolates was observed at 37°C (Table 4.19).

Temperature performed a critical function in cell growth of PGPR, influencing their colonization and activity in the rhizosphere. It affects PGPR cell growth, metabolism, and enzyme activity, which in turn impacts their ability to synthesize substances that promote plant growthfor example cytokinins, gibberellins & auxins. Most PGPR species thrive at temperatures of 25-30°C, with optimal growth typically occurring between 20-28°C. At higher temperatures, PGPR cell growth and metabolic activity may decrease, while lower temperatures can reduce enzyme activity and the production of plant growth-promoting substances.

Every bacterium requires a specific temperature range for optimal growth and metabolism. Zvidzai *et al*. (2015) reported that *Enterobacter asburiae* grows and produces cellulase enzyme most effectively at a pH of 6 and a temperature of 40°C. Meanwhile, Monteiro *et al*. (2016) found that *Bacillus amyloliquefaciens* 629 colonizes plants most effectively at 28°C and produces the lipopeptide surfactin at an optimal temperature of 15°C. In this study, the PGPRs exhibited high growth at temperatures varying from 25–35°C, with substantial growth observed at 40°C & even 50°C. This characteristic is particularly interesting for the potential use of these PGPRs in biofertilizer production and their application in agricultural soils, where temperatures often fluctuate and may increase.

e. Effect of NaCl concentration on cell growth

Effect of various concentrations of sodium chloride (2.5 % and 5 %) on cell growth of the selected rhizobacterial isolates was studied and results are presented in Table 4.19. The three rhizobacterial isolates exhibited varying responses to different salt concentrations, although their growth was better in the control conditions. Isolates CA2 and ZM3 showed maximum growth (approximately 0.26×10^{10} CFU mL^{-1} and 0.69×10^{10} CFU mL^{-1}, respectively) at 2.5% NaCl, but growth diminished significantly as the NaCl concentration increased. The GM3 isolate was sensitive to sodium chloride, showing no growth at any concentration. Additionally, isolate CA2 exhibited a pH reduction of 0.83, from 7.0 to 6.17, while ZM3 showed a pH decrease of 0.93, from 7.0 to 6.07 (Table 4.19).

Numerous studies have demonstrated that salinity negatively impacts microbial growth and activity (Andronov *et al*., 2012; Bakhshandeh *et al*., 2014). Khanghahi *et al*. (2018) described NaCl tolerance in zinc-solubilizing strains at varying salt concentrations and found that *Agrobacterium tumefaciens* and *Rhizobium* sp. could grow on NA medium with NaCl concentrations varying from 0.5%-4%. Son *et al*. (2006) observed, a *Pantoeaag glomerans* strain could cultivate in media that contain up to 5% NaCl, while another strain was tolerant to NaCl concentrations between 0-7% (Dastager *et al*., 2009). The capability to adapt to salt stress is crucial for survival and growth of rhizobacteria in salt-affected fields (Bakhshandeh *et al*., 2014). It previously noted different strains of bacteria exhibit varying levels of tolerance to NaCl, with most rhizobacterial isolates thriving optimally at 0.5% NaCl (Shahab and Ahmed, 2008). Different bacterial strains have specific optimal physical and nutritional requirements for their growth and biomass accumulation. Thus, optimizing these parameters for new strains could enhance the industrial viability of biotechnological processes.

Promising PGPR isolates which execute well under laboratory conditions are likely to yield positive results under more realistic conditions, such as in pot cultures trials. According to a variety of studies, PGPR inoculants can protect plants from disease, increase plant development, and boost biochemical and yield indicators (Vejan *et al*., 2016). While numerous reports highlight the advantageous impact of PGPR on growth & development of plants, further research is needed to discover potential impacts of bacteria in rhizospheric zone, particularly their function in promoting growth of plants & exhibiting antagonistic behaviours against plant

pathogens. The use of PGPR is increasingly recognized for promoting sustainable agricultural practices, improving crop yield, enhancing stress tolerance, and boosting disease resistance. The effects of rhizobacterial isolates on growth of plants can be categorized as neutral, detrimental, or beneficial (Kloepper *et al*., 1989).

Table 4.19 Effect of different physical & nutritional parameters on growth of selected PGPR isolates. OD values at 32 h are reported.

Physical and nutritional parameters	**CA2**		**ZM3**		**GM3**	
	OD_{600}	**CFU ($\times 10^{10}$)**	**OD_{600}**	**CFU ($\times 10^{10}$)**	**OD_{600}**	**CFU ($\times 10^{10}$)**
Glucose	**9.227**	**1.491**	**2.108**	**2.149**	**5.157**	**1.739**
Maltose	0.282	0.046	0.177	0.180	0.237	0.080
Sucrose	7.530	1.217	0.188	0.192	0.124	0.042
Ammonium chloride	**9.652**	**1.560**	**2.324**	2.370	4.827	1.628
Ammonium sulphate	8.574	1.385	2.424	**2.472**	**5.121**	**1.727**
Potassium nitrate	6.222	1.005	2.108	2.149	3.549	1.197
5 pH	1.494	0.241	0.124	0.126	0.221	0.074
7 pH	**10.502**	**1.697**	**2.573**	**2.624**	**5.757**	**1.942**
8 pH	6.776	1.095	0.910	0.928	3.204	1.081
28 °C	9.722	1.571	2.511	2.560	5.686	1.918
33 °C	9.985	1.614	2.849	2.906	5.897	1.989
37 °C	**10.750**	**1.737**	**3.249**	**3.313**	**6.184**	**2.086**
NaCl (0 %)	10.750	1.737	3.249	3.313	6.184	2.086
NaCl (2.5 %)	**1.608**	**0.260**	**0.683**	**0.697**	**0.151**	**0.051**
NaCl (5 %)	0.252	0.041	0.147	0.150	0.158	0.053

*Data are the mean values of three replicates.

5.The effect of Plant Growth Promoting Rhizobacteria (PGPR) in selected regional crops.

Promising PGPR isolates that performed well under laboratory conditions are expected to produce good results under more realistic conditions in pot cultures or field trials. Several researchers reported the ability of PGPR inoculants to enhance plant growth, biochemical & yield parameters and plant protection from diseases (Vejan *et al*., 2016). Although, several

reports are available on beneficial effects of PGPR on plant growth and development, but still investigations are required on the potential effects of bacteria invading the rhizospheric zone to examine their impact on the promotion of plant growth and their antagonistic behavior against plant pathogens. The use of PGPR is increasing for sustainable agricultural practices to improve crop yield, stress tolerance and disease resistance. The effect of rhizobacterial isolates can be described as neutral, detrimental and beneficial on plant growth and development (Kloepper *et al*., 1989).

Selection of PGPR isolates:

On the basis of *in-vitro* screening and evaluation of PGP traits of isolates, total of six best PGPR isolates had been selected to evaluate their efficacy on PGP activity and attributes of chilli, soybean, maize in pot experiment and mung bean in field trial. To evaluate the efficiency of PGPR isolates, pot and field experiment was conducted using isolates that showed high PGP activities under *in vitro* studies. In pot experiment, it has been detected that PGPR inoculation substantially improved crops growth (chilli, soybean and maize). In field experiment on Mung bean crop, it has been observed that PGPR treatments notably improved crops growth and development in all parameters studied.

Table 4.20 List of PGPR isolates with their Plant Growth Promoting traits.

Sr. No.	PGPR Isolates	PGP Traits					
		Phosphate Solubilization	Potassium Solubilization	Zinc Solubilization	IAA Production	Siderophore Production	Ammonia Production
1	CA2	+	+	+	+	+	+
2	CA1	+	+	-	-	+	+
3	GM2	+	+	+	+	-	+
4	ZM3	+	+	+	+	+	+
5	GM3	+	+	+	+	+	+
6	ZM4	+	+	+	-	+	+

Physico-chemical analysis of soil

Soil's physico-chemical analysis had been executed before sowing PGPR treated seeds in pots. Soil was alkaline in nature and EC was found in normal range. Organic carbon was observed in sufficient amounts but available P, K & N were low in soil (Table 4.20).

Table4.21 Physico-chemical analysis of soil for pot experiment.

Sr. No.	Parameters	Field Soil
1	pH	7.96
2	EC (mS/cm)	0.29
3	Organic Carbon (%)	0.77
4	Available Nitrogen (Kg/ha)	91.76
5	Available Phosphorous (Kg/ha)	11.63
6	Available Potassium (Kg/ha)	115.00

A. Pot experiment

A trial had been performed in pots in a simple randomized design to find the best PGPR isolates for the growth enhancement in chilli, soybean and maize crops. The details of results are briefly described below and summarized Tables 4.22-4.24. Promising PGPR isolates that performed well under laboratory conditions are expected to produce good results under more realistic conditions in pot experiments.

Seed Sterilization:

After being surface sterilized for two minutes with 0.1% HgCl2, the seeds were rinsed six times with sterile distilled water. The seeds spent roughly twelve hours submerged after being dipped in PGPR suspension.

Figure 19 Seed treatment with 0.1 % HgCl2 for 2 min.

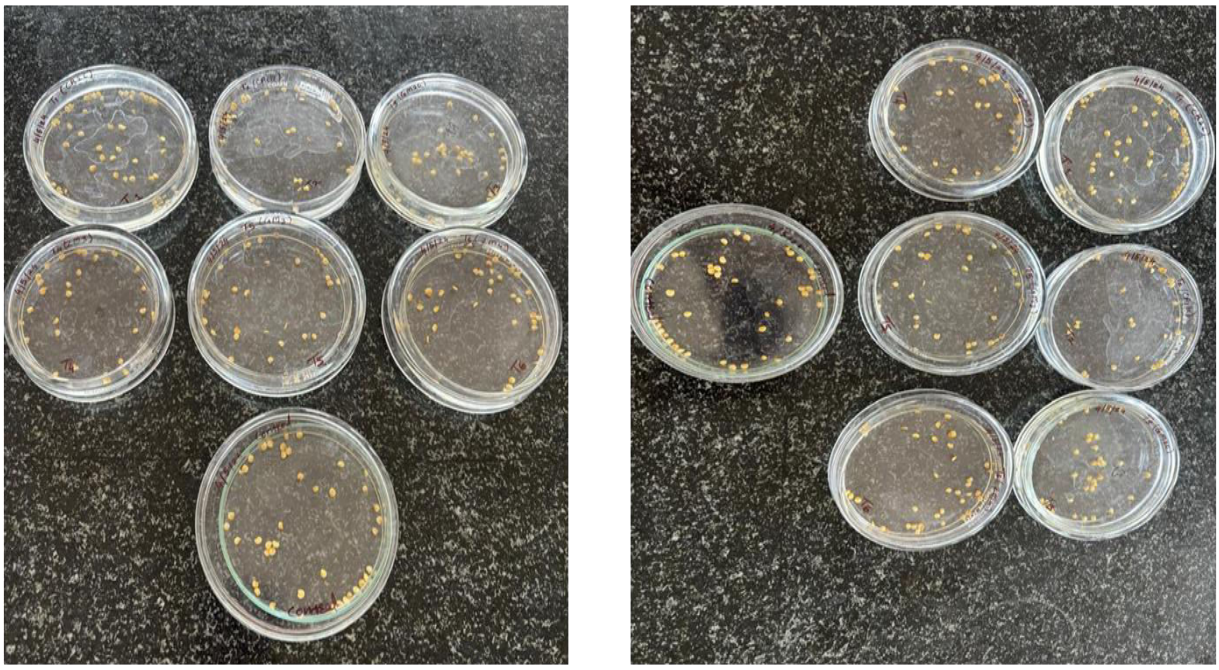


Figure 20 Seed treatments with selected rhizobacterial suspensions.

Figure 21 Preparation of Pots to study different PGPR treatments with selected rhizobacterial suspensions.

1. **Effect of selected PGPR on Chilli Crop**

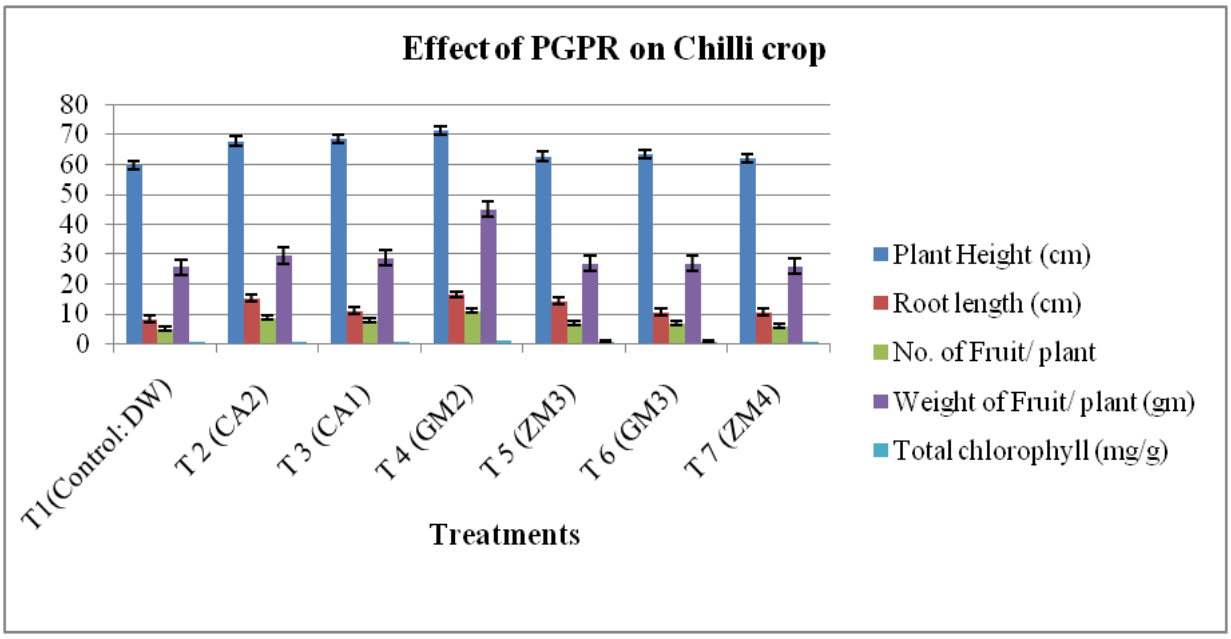
The treatment T4 GM2 (*Pseudomonas* sp.) treated seed, exhibited substantial greatest outcome in plant height (71.20cm), fruits/plant number (11), weight of fruits/plant (g) (45.00 gm), root length (16.50cm) and total chlorophyll content (1.20 mg) followed by T2 CA2 (*Delftia tsuruhatensis*) treated seed when compared with other treatments. This indicated the significant growth over the control plants which were without PGPR treatments *i. e*. in Control plants plant height (59.60), root length (8.33), fruits/plant number (5), fruits/plant weight (g) (25.70) and total chlorophyll content (0.82). *Pseudomonas* sp. had beendisplayed to be a PGP agent for *C. annuum*.

The activities of PGPB align with findings by Dhanalakshmi *et al*. (2014), who reported a maximum leaf count in okra & chili PGPR treatment. Similarly, Pathak *et al*. (2013) observed an enhanced number of leaves in guava when PSB. In line with these results, Densilin *et al*. (2010) also demonstrated comparable effects. Consistent with the present study, previous research by Ramakrishnan and Selva Kumar (2012), Mirzakhani *et al*. (2009), and Berova *et al*. (2010) has demonstrated that both biofertilizers & organic fertilizers increase fruit weight in crops such as tomato, safflower, and *Capsicum annuum* (chilli), respectively. Applying nitrogen-fixing bacteria enhances fruit yield because nitrogen, an essential protein component required for protoplasm development, encourages cell growth and division. Improved development of generative organs has been linked with faster growth of vegetative organs. Studies by Atiyeh *et al*. (2000), Hashemimajid *et al*. (2004), and Berova *et al*. (2010) further highlight vermicompost's positive effect on yield, showing a marked growth in pericarp thickness & average fruit mass.

Table 4.22 Effect of PGPR treatments on growth parameters of Chilli (*Capsicum annuum* L) crop.

Treatments	**[a]Plant Height (cm)**	**[a]Root length (cm)**	**[a]No. of Fruit/plant**	**[a]Weight of Fruit/plant (gm)**	**[a]Total chlorophyll (mg/g)**
T1(Control: DW)	59.60±0. 28	8.33±0.62	5±0.97	25.70±0.38	0.82±0.65
T 2 (CA2)	67.60±0.66	15.20±0.37	9±0.65	29.50±0.12	1.11±0.78
T 3 (CA1)	68.50±0.03	11.20±0.90	8±0.23	28.70±0.74	1.04 ±0.09
T 4 (GM2)	**71.20±0.84**	**16.50±0.03**	**11±0.85**	**45.00±0.66**	**1.20±0. 53**
T 5 (ZM3)	62.50±0.12	14.30±0.23	7±0.79	27.00±0.08	0.98±0. 97
T 6 (GM3)	63.40±0.25	10.50±0.45	7±0.18	27.00±0.66	0.95±0.33
T 7 (ZM4)	62.00±0.33	10.5±0.21	6±0.11	26.00±0.42	1.07±0.20

[a]Datais the mean values of three replicates. Means±SE (p≤0.05).



Graph 3. Effect of PGPR treatments on growth parameters of Chilli (*Capsicum annuum* L) crop.

Figure 22 Chilli plants treated with selected rhizobacterial suspensions in pots.

2. Effect of selected PGPR on Soybean Crop

One of the largest crops in the world, soybeans (Glycine max L.) are valued mainly for their high protein & oil content, which enables their wide application in a variety of agro-industrial sectors. (Hart, 2017; Nguyen, 2018). The amount of diverse growth metrics had a substantial beneficial effect by co-inoculating soybean with PGPR.

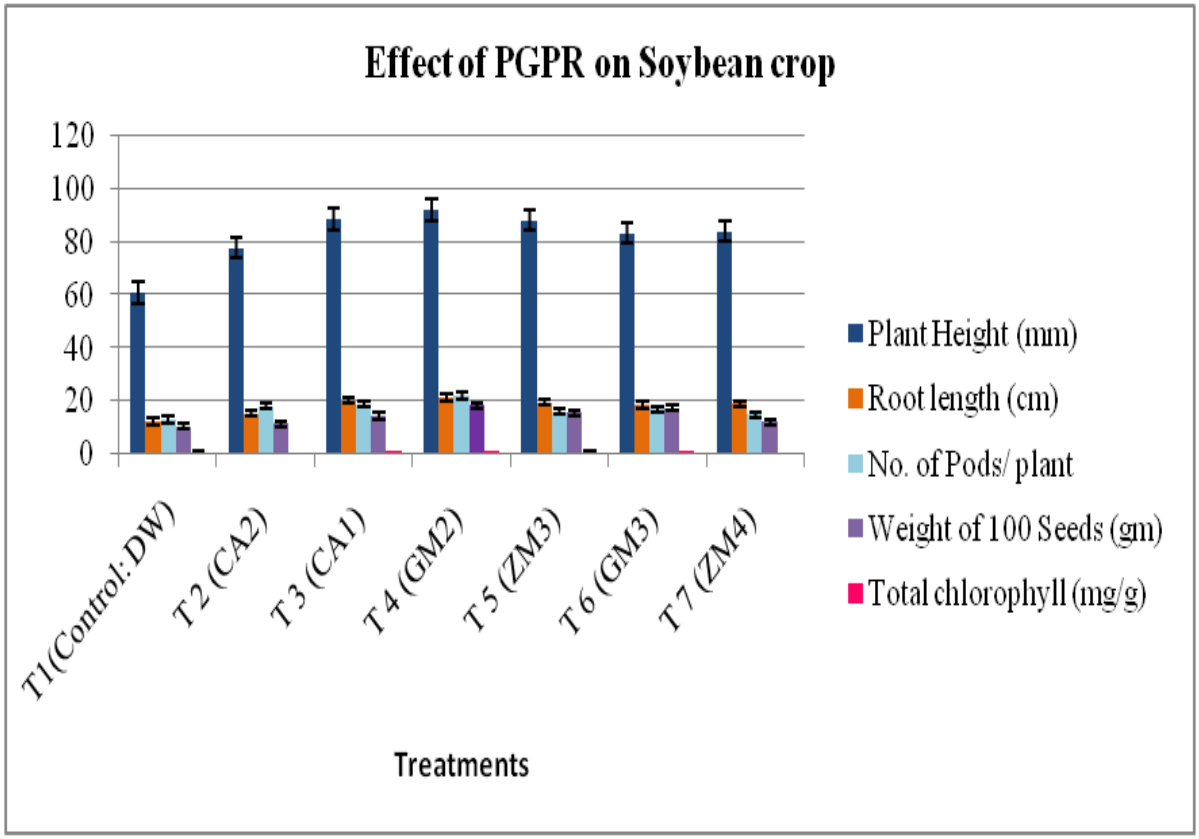
The treatment T4 GM2 (*Pseudomonas* sp.) treated seed, exhibited notably optimum outcome in plant height (92.00cm), number of pods/plant (20.35), weight of 100 seeds (g), (18.33gm), root length (21.20cm) and total chlorophyll content (1.20mg) followed by T3 CA1 (*Pseudomonas aeruginosa*). It indicated the significant growth in all parameters over the control plants which were without PGPR treatments *i. e*. in Control plants plant height (60.70cm), root length (12.20 cm), number of pods/ plant (12.20), weight of 100 Seeds (gm) (10.67) and total chlorophyll content (0.90).

Similar findings were reported by Rubin, Van Groenigen, and Hungate (2017), who observed that PGPR application increased shoot & root biomass by 28% & 35%, correspondingly, across various plant species. Zeffa *et al*. (2018) also demonstrated the benefits of inoculating maize with *Azospirillum* spp., with inoculated plants yielding 651 kg ha^{-1} more than the control group. It is widely accepted that PGPR's production of phytohormones is a key mechanism supporting host plant development, particularly enhancing root system growth (Olanrewaju, Glick & Babalola, 2017; Puente *et al*., 2018). Additionally, bacterial phytohormones have an impact on the symbiotic connection among rhizobia & legumes (Stacey *et al*., 1995; Imada *et al*., 2017). Auxins generated by PGPR are believed to promote rhizobia in this situation through boosting the quantity of root hairs, sites of interaction for soybeans (Schmidt, Messmer & Wilbois, 2015). Tewari & Arora stated a 50% rise in germination when seeds were inoculated with EPS-producing *Pseudomonas aeruginosa* PF23 under stress conditions. It has been demonstrated that EPS, which is produced by a variety of PGPR, helps crop plants through enhanced root colonization, seed germination, as well as stress tolerance.

Table 4.23 Effect of PGPR treatments on growth parameters of Soybean (*Glycine max* L) crop.

Treatments	[a]Plant Height (cm)	[a]Root length(cm)	[a]No. of Pods/plant	[a]Weight of 100 Seeds (gm)	[a]Total chlorophyll (mg/g)
T1(Control: DW)	60.70±0.23	12.20±0.67	13±0.23	10.67±0.70	0.90±0.55
T 2 (CA2)	77.60±0.90	15.30±0.07	18±0.18	11.53±0.64	1.04±0.08
T 3 (CA1)	88.50±0.24	20.40±0.18	19±0.47	17.40±0.05	1.20±0.73
T 4 (GM2)	**92.00±0.84**	**21.20±0.23**	**22±0.26**	**18.33±0.75**	**1.10±0.08**
T 5 (ZM3)	88.30±0.07	19.50±0.19	16±0.70	15.64±0.10	0.94±0.66
T 6 (GM3)	83.20±0.03	18.50±0.06	17±0.06	14.50±0.70	1.10±0.52
T 7 (ZM4)	84.00±0.12	19.10±0.26	15±0.82	12.20±0.25	1.03±0.31

[a]*Datais the mean value of three replicates. Means±SE ($p \leq 0.05$).*



Graph 4 Effect of PGPR treatments growth parameters of Soybean (*Glycine max* L) crop.

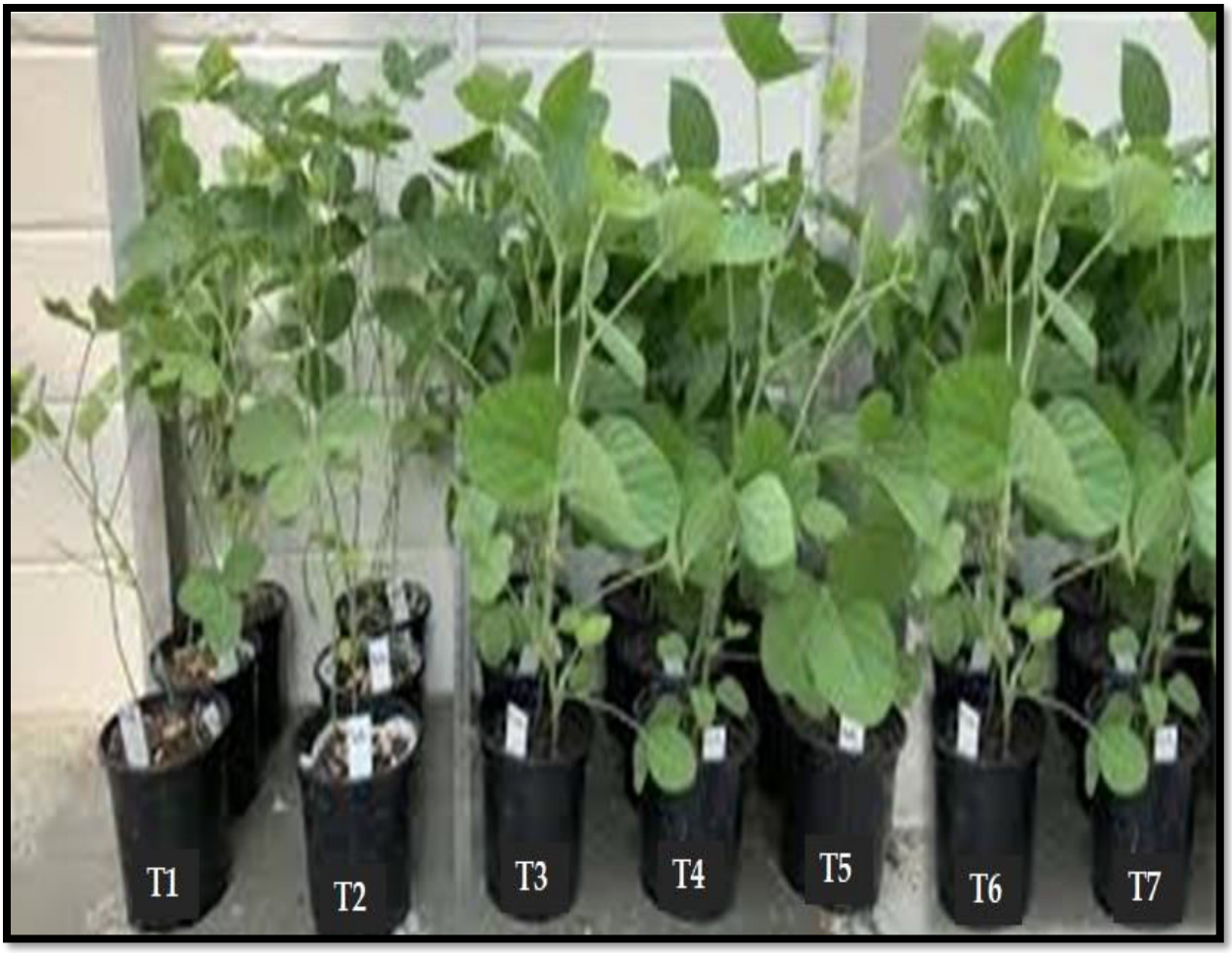


Figure 23 Effect of PGPR treatments growth parameters of Soybean (*Glycine max* L) crop.

3. Effect of selected PGPR on Maize Crop

Recent research in India has focused on PGPR's effects on maize crop growth. Studies indicate that PGPR can greatly boost maize growth by enhancing nutrient absorption, inducing systemic resistance, and fostering beneficial soil microbial communities.

The treatment T7 ZM4 (*Aeromonas caviae*) treated seed, demonstrated notably the best outcome in plant height (146.70cm), root length (29.90cm), Fresh Weight (19.67g), Dry weight (3.87g) and total chlorophyll content (1.04mg) followed by T5 ZM3 (*Acinetobacter* sp). PGPR treatments indicated the significant growth deference in all growth parameters over the control plants which were without PGPR treatments *i. e*. in Control plants, plant height (93.30 cm), root

length (22.40cm), Fresh Weight (10.03g), Dry weight (2.97g) and total chlorophyll content (0.84mg).

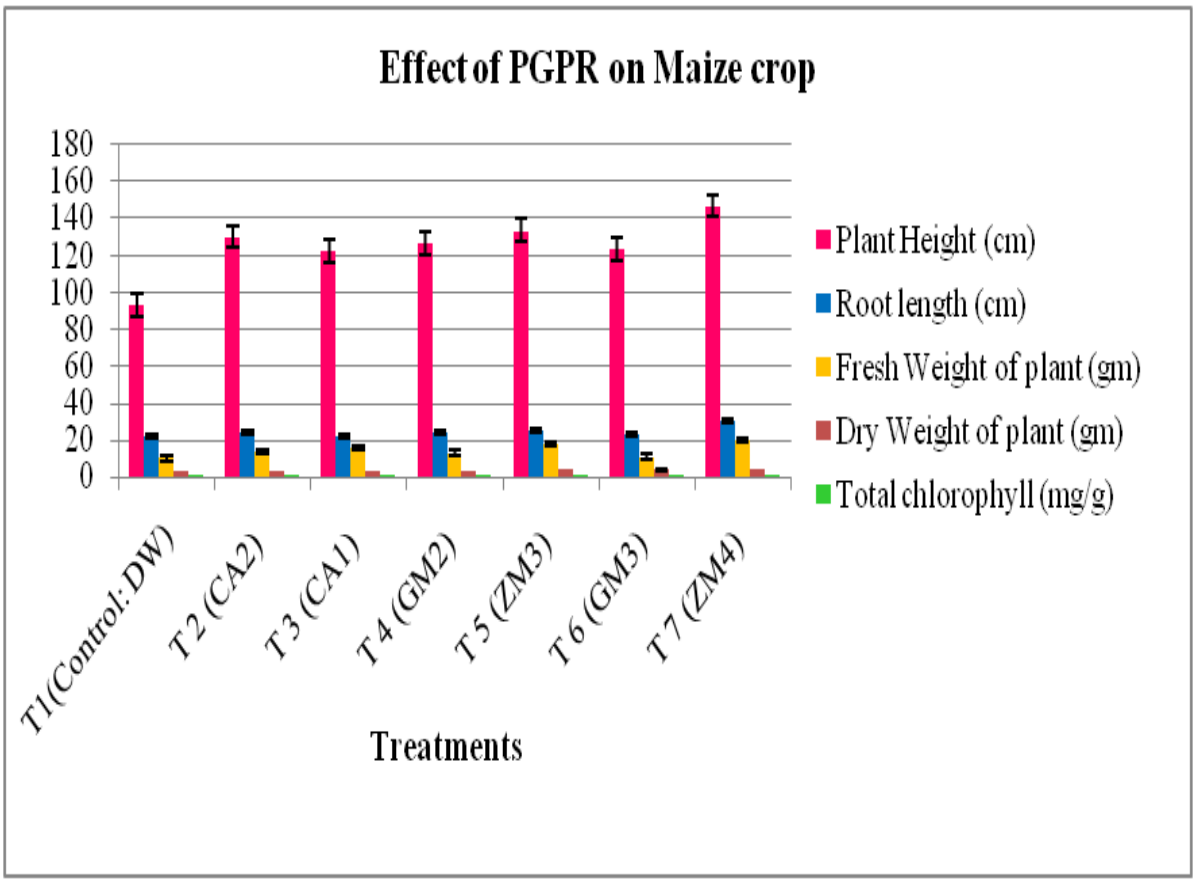
Comparable rises in plant height and leaf area had been noted in various crops inoculated with *Azospirillum*, *Azotobacter*, & *Pseudomonas* strains (Martinez-Toledo *et al*. 1988; Siddiqui and Shaukat, 2002; Burd *et al*. 2000). This increase may be associated with the hormone production by *Azospirillum* isolates (Tien *et al*., 1979), including auxins, gibberellins, and cytokinins. Furthermore, the *Pseudomonas* (PSD6) PGPR functions as a biological control agent against phytopathogens as well as enhances plant growth by suppressing these diseases through the production of diverse chemicals (Hill *et al*. 1994).

Similarly, other researchers have reported enhancements in dry weight, plant height, & grain yield of several crop plants because of PGPR inoculation (Khalid *et al*. 2004; Biswas *et al*. 2000a, b). Maize treated with PGPR exhibited maximumrise in dry weight in comparison to the uninoculated control. Plants infected with a mixture of *P. fluorescens* & *P. putida* exhibited a 53.72% rise in shoot biomass and a 108.71% rise in root biomass (Gholami *et al*., 2009).

Table 4.24 Effect of PGPR treatments on growth parameters of Maize crop.

Treatments	**[a]Plant Height (cm)**	**[a]Root length (cm)**	**[a]Fresh Weight of plant (gm)**	**[a]Dry Weight of plant (gm)**	**[a]Total chlorophyll (mg/g)**
T1(Control: DW)	93.30±0.52	22.40±0.03	10.03±0.23	2.97±0.80	0.84±0.04
T 2 (CA2)	130.00±0.10	23.80±0.18	13.42±0.08	3.24±0.24	1.04±0.21
T 3 (CA1)	122.00±0.03	22.20±0.20	15.87±0.12	3.16±0.50	0.94±0.33
T 4 (GM2)	126.70±0.22	23.70±0.25	13.06 ±0.67	3.21±0.34	1.03±0.78
T 5 (ZM3)	133.30±0.32	24.90±0.05	17.67±0.88	3.9±0.56	1.10±0.40
T 6 (GM3)	123.30±0.66	23.30±0.11	10.96±0.91	3.69±0.21	0.98±0.21
T 7 (ZM4)	**146.70±0.71**	**29.90±0.25**	**19.67±0.20**	**3.87±0.06**	**1.04±0.18**

[a]Datais the mean value of three replicates. Means±SE (p≤0.05).



Graph 5 Effect of PGPR treatments growth parameters of Maize crop.

The comparable outcomes have been documented in pot experiments of different crops for examplemaize, pea, chickpea, & barley with Acinetobacter rhizosphaerae isolate (Gulati *et al*., 2009), maize with *Burkholderia cepacia* (Zhao *et al*., 2014) and wheat with *Stenotrophomonas* sp., *Acetobacter pasteurianus* and *Stenotrophomonas* sp. (Majeed *et al*., 2015). Goswami *et al*. (2014b) stated that bacterial isolates increased plant length (32 %), fresh biomass (44 %) and dry biomass (43 %) compared to the control of Arachis hypogea plant. Likewise, plant growth-promoting effects of *Pseudomonas* BHU B13-398 & *Bacillus* BHU M strain on mung bean growth were reported by Kumari *et al*. (2018b). Additionally, inoculations of mung bean with rhizospheric isolates have been shown to significantly enhance plant growth in pot experiments (Shaharoona *et al*., 2006; Ali *et al*., 2010).

Figure 24 Effect of PGPR treatments growth parameters of Maize crop

B. Field trial of the selected PGPR on Mungbean crop

Effect of PGPR isolates on growth, biochemical and yield parameters of *V. radiata* (Mungbean) was evaluated in the field in a completely randomized block design (Figure 25 & 26). The details of results are briefly described below and summarized in Graph 6 and Table 4.25.

Plant Growth parameters

The growth parameters namely, shoot and root length, pods per plant, number of seeds per pod and weight of 100 seed were found to be significantly influenced by all treatment of selected PGPR isolates over the control.

PGPR treatments significantly influenced shoot and root length per plant over the control. Treatment T 4 was best amongst the all PGPRs treatment and increased shoot and root length per plant by over the control.

The treatment T4 GM2 (*Pseudomonas* sp.) treated seed, exhibited notably optimum outcome in plant height (66.70cm), number of pods/plant (50), weight of 100 seeds (g), (4.90gm), root length (24.70cm) and total chlorophyll content (1.20mg) followed by T3 CA1 (*Pseudomonas aeruginosa*). It indicated the significant growth in all parameters over the control plants which were without PGPR treatments *i. e*. in Control plants plant height (43.60 cm), root length (18.40 cm), number of pods/ plant (39), weight of 100 Seeds (gm) (3.97) and total chlorophyll content (0.82).

Similar findings have been reported in pot experiments of different crops such as pea, chickpea, maize, and barley with *Acinetobacter* rhizosphaerae isolate (Gulati *et al*., 2009), maize with Burkholderia cepacia (Zhao *et al*., 2014) and wheat with *Stenotrophomonas* sp., Acetobacter pasteurianus and Stenotrophomonas sp. (Majeed *et al*., 2015). Goswami *et al*. (2014b) reported that the bacterial isolates increased plant length (32 %), fresh biomass (44 %) and dry biomass (43 %) compared to the control of *Arachis hypogea* plant.

Soil microbes may also make accessible nutrient to the plant by producing chelating substances and organic acids leading to mineral solubilization (Rodriguez *et al*., 2004; Chen *et al*., 2006; Osman *et al*., 2010). Phosphate solubilizing Bacillus strain was reported to enhance growth parameter, yield, N, P and K contents in the shoots and seeds of mung bean and maize (Ahmad *et al*., 2019). The plant growth-promoting potential of PGPR isolates are highly influenced by environmental factors. Previous studies showed that soil pH, temperature and carbon availability determine the actions of these PGPR isolates (Garcia-Pausas and Paterson, 2011).

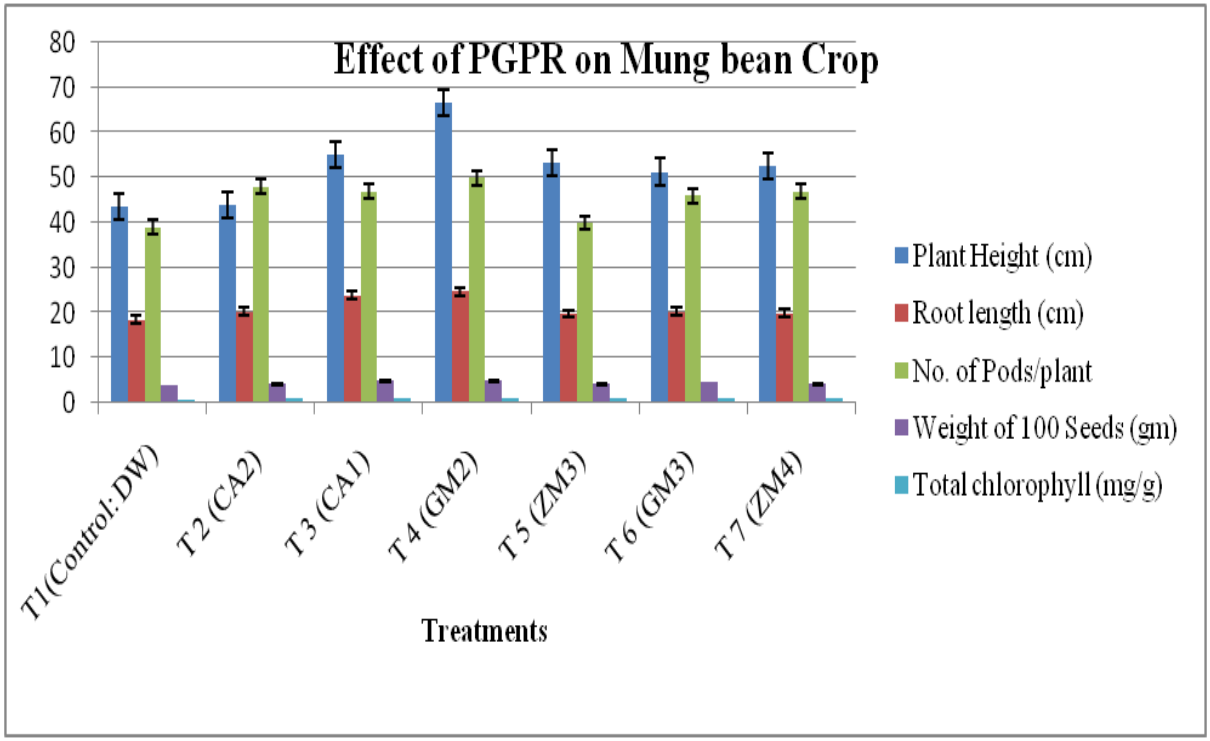
Aamir *et al*. (2013) reported increment in plant length (14 %), number of pods (18 %),1000 grain weight (13 %) and grain yield (23 %) and NPK content in seed (26 %, 4 % and 17 %, respectively) by Rhizobium isolate over the control in mung bean plant. Several other earlier studies on mung bean also endorse the findings obtained in the present research (Kumar *et al*., 2012; Ali *et al*., 2010; Kumar *et al*., 2014). Dey *et al*. (2004) evaluated efficiency of nine *Pseudomonas* sp. on peanut continuously for three years and observed significant enhancement in root length, plant biomass, pod yield and plant height of peanut than the control of peanut by two isolates, PGPR1 and PGPR2.

The results of field experiments, in the present study confirmed the beneficial effects of PGPR isolates on the growth and yield of mung bean is possibly due to better nutrient availability for plant. Yet, there were differences among the bacterial strains in their ability to sustain plant growth.

Table 4.25 Effect of PGPR treatments on growth parameters of *V. radiata* (Mungbean).

Treatments	**[a]Plant Height (cm)**	**[a]Root length (cm)**	**[a]No. of Pods/plant**	**[a]Weight of 100 Seeds (gm)**	**[a]Total chlorophyll (mg/g)**
T1(Control: DW)	43.60±1.21	18.40±0.05	39±0.20	3.97±0.70	0.82±0.02
T 2 (CA2)	44.00±0.10	20.20±0.19	48±0.14	4.16±0.51	1.04±0.03
T 3 (CA1)	55.00±0.05	23.90±0.07	47±0.82	4.87±0.52	1.10±0.04
T 4 (GM2)	**66.70±0.21**	**24.70±0.22**	**50±0.63**	**4.90±0.32**	**1.20±0.08**
T 5 (ZM3)	53.30±0.30	19.80±0.20	40±0.09	4.24±0.34	1.07±0.10
T 6 (GM3)	51.30±0.62	20.30±0.12	46±0.81	4.69±0.20	1.03±0.10
T 7 (ZM4)	52.70±0.70	19.90±0.24	47±0.21	4.21±0.08	1.11±0.18

[a]*Datais the mean value of three replicates. Means±SE ($p \leq 0.05$).*



Graph 6 Effect of PGPR treatments growth parameters of Mung bean crop.

Figure 25 Field preparations for experiment on *V. radiata* (Mungbean) according to RCBD.

Figure 26 Field view of experiment on *V. radiata* (Mungbean) at 60 DAS. T1 (Control), T2 (CA2), T3 (CA1), T4 (GM2), T5 (ZM3), T6 (GM3) and T7 (ZM4).

Chapter 5

Summary and Conclusions

The current investigation had been aimed at isolating PGPR from soil samples, studying and quantifying their PGP traits, characterizing them on morphological and biochemical basis, identifying them using molecular tools and evaluating their efficacy in promoting plant growth under field conditions.

PGPR are soil-dwelling bacteria that have a beneficial and environmentally favorable influence on plant health. A range of root-colonizing bacteria is included in PGPR, enhancing crop development and yield by promoting plant growth and strengthening resilience to biotic as well as abiotic stress (Cakmakci *et al*., 2007; Maksimov *et al*., 2011). To enhance plant growth and production, PGPR are essentially helpful, free-living soil bacteria that display a variety of growth-promoting characteristics.

Farmers commonly use chemical fertilizers to boost crop yields, but a significant portion of the soluble inorganic NPK content in these fertilizers quickly becomes immobilized in the soil, making it inaccessible to crops. Additionally, leaching of these chemicals increases the residual load of synthetic fertilizers in soil and nearby water sources, posing risks to local flora and fauna (Adesemoye and Kloepper, 2009). Excessive reliance on chemical fertilizers not only impacts food quality but also poses risks to human health. The negative effects of these fertilizers on soil fertility have led to a growing interest in understanding soil dynamics in a more nuanced way.

To address soil fertility issues, it is essential to foster a well-functioning ecosystem beneath the soil, supported by hundreds of naturally occurring microorganisms. PGPR offer a promising substitute to reduce chemical inputs by enhancing various soil processes, such as organic matter decomposition, soil formation, nutrient recycling, mineral solubilization, growth regulatorsproduction, degradation of organic pollutants, as well as resilience to biotic & abiotic stresses. Recognizing importance of PGPR and their potential to reduce chemical fertilizer use, this study has been undertaken with the following objectives:

1. To collect soil samples from different regions of Maharashtra and isolation of bacteria.
2. To identify and multiply bacterial isolates based on morphological, biochemical and molecular characteristics.
3. To standardize media and evaluation of growth parameters of rhizobacteria.
4. To evaluate different bacterial isolates *in vitro* for their ability to promote plant development.
5. To study impact of PGPR in selected regional crops.

PGPR presents numerous benefits for enhancing plant growth and development. Various microbes employ diverse mechanisms to promote plant health. Ortiz-Castro *et al*. (2013) classified these mechanisms into two main categories: direct and indirect. Among the direct mechanisms, phytohormone production by PGPR performs a vitalfunction in promoting growthof plants, impacting both symbiotic & non-symbiotic roots (Patten and Glick, 1996; Glick, 2014).

Soil microbiota also plays a vital role in phosphorus dynamics, aiding in its solubilization and mineralization to ensure plants can access otherwise unavailable forms (Fankem *et al*., 2006). Potassium-solubilizing bacteria are particularly beneficial to agriculture (Basak and Biswas, 2009). Additionally, certain soil microorganisms facilitate zinc uptake in plants by making it accessible through acidification, organic acidsproduction, exchange, & chelation reactions (Chang *et al*., 2005). PGPR-produced siderophores enhance the bacteria's competitive edge by supporting balanced nutrition, contributing to both antibiotic activity and iron uptake for plants sharing the same environment (Glick, 1995).

The current investigation has been intended at isolating PGPR from soil samples, studying and quantifying their PGP traits, characterizing them on morphological and biochemical basis, identifying them using molecular tools and evaluating their efficacy in promoting plant growth in pot experiments.

From this perspective 12 soil samples were collected from economically important crops (Maize, Soybean, Chilli) of different regions of Maharashtra Marathwada, Western Maharashtra,

Vidarbha & Konkan). Utilizing serial dilution method on NA medium overall 1174 bacteria had been isolated and screened for PGP traits.

Overall36 isolates had been found positive for different PGP traits like phosphate, potassium and zinc solubilization, siderophore & Amylase production.

The largest phosphate solubilization zone along with PSI among the 36 bacterial isolates that were examined for phosphate solubilization had been displayed by CA4a in the current investigation (25mm & 13.50mm, correspondingly). Isolates numbers were further narrowed down on the basis of single PGP traits and evaluated qualitative and quantitative.Some examples as Burkholderiacepacia (Pande *et al*., 2017, 2019), B. tropica, B. unamae and B. cepacia (Ghosh *et al*., 2016) were reported as good phosphate solubilizers.

GM4b demonstrated the highest (12) solubilized potassium concentration of the supplemented insoluble potassium with high value of KSI on 7th DAI.Numerous bacterial strains have been identified from crop rhizosphere that could solubilize potassium from minerals (Zeng *et al*., 2012). All selected PGPR isolates lowered pH of medium by probably releasing acids and / or enzymes as reported earlier (Bennett *et al*., 1998).

Amongst all selected isolates, CA2c showed maximum zone of clearance - 51 mm and Zinc Solubilizing Index (11.20).Fasim *et al*. (2002), observed a clear halo zone around spotted *Pseudomonas aeruginosa* bacteria on a medium having ZnO as an insoluble Zn source. 4 Pawar *et al*. (2015) isolated *Burkholderia cenocepacia, Pseudomonas aeruginosa and Pseudomonas striata* that could solubilize zinc.

CA1b (4.62) and GM2c (4.87) isolates were found most efficient siderophore producers, GM2c exhibited maximum halo zone diameter that is, 31mm. *Burkholderia* genus is well recognized for producing an extensive diversity of secondary metabolites that include siderophore (Mamta *et al*., 2010; Asghar *et al*., 2011).Among all tested isolates for Amylase Production, CA2c showed highest zone diameter (12 mm).

Total of 12 isolates had been finally selected on thebasis of morphological and some biochemical tests and was further screened for IAA production, Catalase activity, Urease and Oxidase test.Four bacterial isolates (GM2, GM3, ZM3 and ZM4) produced more than 15 µg mL

IAA under in vitro conditions. In the present investigation, the bacteria isolated produced IAA in the absence of tryptophan, a finding that is supported by Karnwal (2009) who tested *P. aeruginosa* and *P. fluorescens*. Similar observations were recorded by Zahir *et al*. (2010) in *Rhizobium phaseoli* strain.

All isolates were found positive for Catalase Activity (Hydrogen Peroxide Test) except CA2 isolates. Isolates GM2, CA2 and CA3 were found positive for Urease test.Among all the bacterial isolates, GM1, CA2 and CA3 were found positive for Oxidase test.

Based on the *in vitro* study of PGP traits, four best PGPRs from each site (Total of 12 isolates) were selected for morphologically and biochemical characterization. These bacteria had beenrecognizedby employing 16S rRNA gene sequencing technique.The 16S rDNA sequences obtained after sequencing were assembled in SeqMan software and to identify individual strains complete sequences were subjected to BLAST, NCBI. Phylogenetic tree had been built of four isolates using MEGA version 11 software.

The isolates' gene sequences have been entered into GenBank, & accession numbers have been assigned. 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).

PGPR isolates were showing maximum similarity with- *Pseudomonas* species (*Pseudomonas guariconensis, Pseudomonas plecoglossicida, Pseudomonas mosselii* and *Pseudomonas aeruginosa*), *Aeromonas*sp (*Aeromonas caviae- 2, Aeromonas hydrophila*), *Acinetobacter*sp, *Delftiatsuruhatensis* and *Stenotrophomonas maltophilia*from database of Genbank.

The effect of physical (pH and temperature) and nutritional parameters (various carbon and nitrogen sources) on the growth of three selected rhizobacteria, *Delftia tsuruhatensis* CA2, *Acinetobacter* sp. ZM3, and *Stenotrophomonas maltophilia* GM3; in shake flask level was investigated in Schlegel medium. The culture conditions for these three isolates were optimized for production of maximum biomass during growth phase. Growth kinetic parameters were studied on the best PGPR CA2.

The efficacies of the best six PGPR isolates were tested on chilli, soybean and maize plants in pot conditions resulting in significant increase in growth parameters. The treatment T4 GM2 (*Pseudomonas* sp.) treated seed, demonstrated notably greatest outcome in height of plant (71.20cm), fruits/plant number (11), weight of fruits/plant (g) (45.00 gm), root length (16.50cm) and total chlorophyll content (1.20 mg) followed by T2 CA2 (*Delftia tsuruhatensis*) treated seeds, in contrast to alternative treatments. It has been demonstrated that *Pseudomonas* sp. promotes growth of *Capsicum annuum*.

On Soybean crop, the treatment T4 GM2 (*Pseudomonas* sp.) treated seed, exhibited notably greatest outcome in plant height (92.00cm), number of pods/plant (20.35), weight of 100 seeds (g), (18.33gm), root length (21.20cm) and total chlorophyll content (1.20mg) followed by T3 CA1 (*Pseudomonas aeruginosa*). Similar findings were reported by Rubin, Van Groenigen, and Hungate (2017).

Likewise in Maize crop the treatment T4 GM2 (Pseudomonas sp.) treated seed, exhibited notably best outcome in plant height (146.70cm), root length (29.90cm), Fresh Weight (19.67g), Dry weight (3.87g) and total chlorophyll content (1.04mg) followed by T5 ZM3 (*Acinetobacter* sp). Several crops injected with strains of *Azospirillum*, *Pseudomonas*, as well as *Azotobacter* exhibited comparable enhancements in plant height & leaf area (Martinez-Toledo *et al*. 1988; Siddiqui and Shaukat, 2002; Burd *et al*. 2000).

The results specific to treatments showed consistency in their performance when the conditions were scaled up from pot to field conditions. In the field trial on Mung bean crop, treatment T4 GM2 (*Pseudomonas* sp.) treated seed, exhibited notably best outcome in plant height (66.70cm), number of pods/plant (50), weight of 100 seeds (g), (4.90gm), root length (24.70cm) and total chlorophyll content (1.20mg) followed by T3 CA1 (*Pseudomonas aeruginosa*). On the basis of growth enhancement of mung bean in field trial, *Pseudomonas* sp GM2 isolate and *Pseudomonas aeruginosa* CA1 isolates could be used as bio inoculants for nourishing the soil under field conditions for mung bean cultivation.

These results emphasize the pivotal role of PGPR in solubilizing essential nutrients like phosphate, potassium, and zinc, as well as producing siderophores and Indole-3-Acetic Acid, all vital for enhancing plant growth. The significance of PGPR in contemporary agriculture cannot

be overstated; their ability to produce crucial chemicals is indispensable for crop cultivation and sustainable agricultural practices.

Thus, to retain long-term soil fertility and maintain crop productivity, identification of region-specific and crop specific PGPR strains is strongly advised. The study's promising findings open avenues for future research. Additionally, efforts should focus on commercialization, farmer training, and developing climate-resilient crop varieties. Collaborative research involving various disciplines can provide holistic insights, fostering sustainable and eco-friendly agricultural practices.

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Appendix I

CULTURE MEDIUM

A. Nutrient medium (pH 7.2±0.2)

S. No.	Components	Quantity (g L^{-1})
1	Peptic digest of animal tissue	5.0
2	Sodium chloride	5.0
3	Beef extract	1.5
4	Yeast extract	1.5

B. Pikovskaya's media (pH 7.2±0.2)

S. No.	Components	Quantity (g L^{-1})
1	Dextrose	10
2	Tri calcium phosphate	5.0
3	Ammonium sulphate	0.5
4	Potassium chloride	0.2
5	Magnesium sulphate	0.1
6	Manganese sulphate	0.0001
7	Ferrous sulphate	0.0001 8
8	Yeast extract	0.5
9	Agar	20

C. Aleksandrow Agar (pH 7.2±0.2)

S. No.	Components	Quantity (g L^{-1})
1	Glucose	5.0
2	Magnesium sulphate	0.5
3	Calcium carbonate	0.1
4	Potassium alumino silicate	2.0
5	Ferric chloride	0.005
6	Calcium phosphate	2.0
7	Agar	20

D. Modified Pikovskaya agar medium (pH 7.2±0.2)

S. No.	Components	Quantity (g L^{-1})
1	Dextrose	10.0
2	Ammonium sulphate	1.0
3	Potassium chloride	0.2
4	Dipotassium hydrogen phosphate	0.1
5	Magnesium sulphate heptahydrate	0.2
6	Zinc oxide	1.0
7	Agar	20

E. Siderophore production on Chrome Azurol S (CAS) agar plate

Glassware

All glassware for siderophore production were treated with 6 N HCl for 24 h to remove residual iron and rinsed thrice with water and finally with distilled water to avoid iron contamination.

All glassware used for siderophore detection and estimation were kept in 6 M HCl for 24 h and rinsed 3 times with distilled water to remove all traces of iron.

The CAS plates were prepared by following steps

i. CAS indicator solution

60.5 mg of Chrome Azurol S (CAS) was dissolved in 50 mL of Milli Q water. 10 mL of Fe (III) solution (27 mg FeCl3 6H2O and 83.3 µL concentrated HCl in 100 mL mili Q water), along with 72.9 mg hexadecyl trimethyl ammonium bromide (HDTMA) dissolved in 40 mL distilled water. The HDTMA solution was added slowly while stirring, resulting in a dark blue solution (100 mL total volume), which was then autoclaved.

ii. Buffer solution

30.2 g of PIPES buffer was dissolved in 750 mL of salts solution containing KH2PO4, 0.3 g; NaCl, 0.5 g and NH4Cl, 1.0 g. The pH of the solution was adjusted to 6.8 using 6N NaOH. The total volume was brought to 800 mL using distilled water, and 20 g agar added to the solution while stirring and heat and melt. The solution was then autoclaved.

iii. Basal Medium

Glucose, 2 g; mannitol 2 g; MgSO4.7H2O .493 g; CaCl2 0.011 g; MnSO4.H2O 0.00117 g; H3BO30.0014 g; CuSO4.5H2O 0.00004 g; ZnSO4.7H2O 0.0012 g and NaMoO4. 2H2O 0.001g were added in flask and made the volume to 70 mL using distilled water. This solution was then autoclaved separately.

iv. Casamino acid Solution

This solution was prepared by dissolving 3 g casamino acid in 27 mL distilled water and dissolved thoroughly on magnetic stirrer. The solution was filter sterilized using 0.45 µm membrane filter.

Preparation of CAS Agar Plates

Sterilized PIPES buffer solution cooled to 50 ºC then CAS indicator solution, basal medium and CAS amino acid solution were added along the glass wall with sufficient stirring to mix all components without bubble formation. Medium was poured into sterilized petri plates and left to solidify.

F. Siderophore Inducing Medium (SIM)

Siderophore inducing medium was prepared by adding buffer solution, basal medium and CAS amino acid solution in same ratio as in CAS agar medium except agar and CAS indicator solution.

G. Peptone broth (pH 7.2±0.2)

Peptone broth was prepared by adding 4 g peptone to a flask and made up final volume to 1 litre with distilled water.

H. Sulphide Indole Motility (SIM) (pH 7.2±0.2)

S. No.	Components	Quantity (g L^{-1})
1	Peptone	30.0
2	Beef extract	3.0
3	Ferrous ammonium sulfate	0.2
4	Sodium thiosulfate	0.025
5	Agar	3.0

I. Glucose Nutrient Broth (pH 6.9±0.2)

S. No.	Components	Quantity (g L^{-1})
1	Peptone	07
2	Potassium dehydrogenate phosphate	05
3	Dextrose	05

J. Skim Milk Agar medium (pH 7.2±0.2)

S. No.	Components	Quantity (g L^{-1})
1	Skim milk powder	100.0
2	Peptone	5.0
3	Agar	15.0

K. Starch Agar medium (pH 7.2±0.2)

S. No.	Components	Quantity (g L^{-1})
1	Peptone	5.0
2	Beef extract	3.0
3	Starch	2.0
4	Agar	15.0

L. Simmon Citrate Agar medium (pH 6.9±0.2)

S. No.	Components	Quantity (g L^{-1})
1	Ammonium dihydrogen phosphate	1.0
2	Dipotassium hydrogen phosphate	1.0
3	Sodium chloride	5.0
4	Sodium citrate	2.0
5	Magnesium sulfate	0.2
6	bromothymol blue	0.08
7	Agar	15

M. Luria-Bertani (LB) medium (pH 7.2±0.2)

S. No.	Components	Quantity (g L^{-1})
1	Tryptone	10
2	Yeast extract	5
3	Sodium chloride	10

N. Modified Schlegel's Medium (SM) Solution I

S. No.	Components	Quantity (g L^{-1})
1	Sodium hydrogen phosphate	9.0
2	Potassium dihydrogen phosphate	1.3
3	Ammonium chloride	1.0
4	Potassium chloride	1.0
5	Magnesium sulfate	0.2

O. Trace element solution (10 mL/L)

S. No.	Components	Quantity (g L^{-1}) (mg/100 mL)
1	Zinc sulphate heptahydrate	10
2	Manganese chloride tetrahydrate	03
3	Boric acid	30
4	Sodium molybdate dihydrate	03
5	Cobalt chloride hexahydrate	20
6	Copper chloride dihydrate	01
7	Nickel chloride hexahydrate	02

All components of **Solution I** were dissolved one by one to avoid precipitation in 400 mL distilled water, added 10 mL of trace element solution and made up the volume to 900 mL with distilled water. The pH was adjusted to 6.8 before sterilization.

Solution II: Dissolve 50 mg ferric ammonium citrate and 100 mg calcium chloride dehydrate in 50 mL distilled water and made volume up to 250 mL with distilled water.

Solution III: Dissolve 10 g glucose in 50 mL distilled water and made volume up to 90 mL with distilled water.

In order to avoid precipitate formation, all four solutions were prepared and sterilized separately by autoclaving. After cooling mixed 900 mL of **Solution I**, 10 mL of **Solution II** and 90 mL of **Solution III** to prepare 1 litre of the basal mineral medium preparation.

Appendix II

General buffers and stock solutions.

S. No.	Solutions Names	Components
1	0.8 % Agarose	0.8 g of low melting point agarose was dissolved in 100 mL 1 X TAE buffer solution
2	5M NaCl	29.25 g of NaCl was taken and dissolved in 100 mL distilled water, autoclaved and stored at room temperature.
3	Chloroform: Iso-amyl alcohol (24:1)	672 mL of chloroform was added to 28 mL of isoamyl alcohol. It was readily mixed and stored at -4ºC
4	CTAB buffer	2 g of CTAB was dissolved in 98 mL TE buffer (pH 8), autoclaved and preheated before use.
5	DNA loading buffer (10 X)	0.25% (w/v) bromophenol blue and 0.25% (w/v) xylene cyanol were mixed in 30% (v/v) glycerol in Milli Q water.
6	Ethidium Bromide 10 mg mL^{-1}	Ethidium Bromide solution was dissolved in distilled water (stock solution).
7	Lambda DNA	To prepare working solution for electrophoresis, 5 µL of 1 kb λ DNA ladder was mixed with 5 µL of distilled water. 2 µL of this λ DNA solution was loaded with 3 µL of loading dye.
8	Phenol : Chloroform : Iso-amyl alcohol (25:24:1)	700 mL of phenol was added to 672 mL of chloroform followed by 28 mL of isoamyl alcohol to each sample.
9	TAE (50 X) TrisAcetate EDTA buffer	121 g of Tris-base was dissolved in 50 mL of 0.5 M EDTA (pH 8.0), and 28.6 mL glacial acetic acid. Adjusted volume to 500 mL with Milli Q water.
10	10 TE (1 X)	10 mM Tris salt solution (pH 8) mixed with 1 mM EDTA solution (pH 8) in 1:1 ratio, autoclaved and stored at 4°C.

Appendix III

SOLUTIONS AND REAGENTS

A. Barton's reagent

For preparation of Barton's reagent following two solutions were prepared:

Solution A: 25 g ammonium molybdate was dissolved in 400 mL distilled water.

Solution B: 1.25 g ammonium metavanadate was dissolved in 300 mL of boiling water, cooled and then 250 mL concentrated HNO3 was added to it. Then Solution A and B were mixed and volume made up to one litre.

B. Salkowvysky's Reagent

Add 1 mL of 0.5M FeCl3 in 49 mL of 35 % HClO4 with continuous stirring.

C. Chrome azurol S (CAS) Assay Solution

This solution was prepared in following steps:

- **2 mM CAS stock solution:** 0.121 g CAS in 100 mL water
- **1 mM Fe stock solution:** 1 mM FeCI3.6H2O in 10 mM HCI
- **Piperazine buffer:** Dissolve 4.307 g piperazine in 30 mL water. Add 6.75 mL concentrated HC1 (12M) to bring the pH to 5.6
- **HDTMA:** Dissolve 0.0219 g HDTMA in 50 mL distilled water in a 100 mL Volumetric Flask.

  Mix 1.5 mL of 1 mM Fe stock solution with 7.5 mL CAS solution and add to the HDTMA in the Volumetric Flask then piperazine solution added to the Volumetric Flask and bring volume up to 100 mL with water.
- **Shuttle solution:** 0.2 M 5-Sulfosalicylic acid.

D. Picric acid solution (0.5%)

This solution was prepared by adding 0.5 g picric acid and 2 g sodium carbonate in 50 mL distilled water and final volume was made up to 100 mL with distilled water.

E. Oxidase reagent

0.1 g tetra methyl-p-phenylene diamine dihydrochloride was added in 10 mL of distilled water.

F. Kovac's reagent

5 g of p-dimethyl aminobenzaldehyde was added to 75 mL of isoamyl alcohol and then 25 mL of concentrated hydrochloric acid was slowly added.

G. Sodium hydroxide solution (2.5 N)

10 g NaOH was dissolved in sufficient distilled water and final volume made up to 100 mL with distilled water.

H. Sodium silicate solution (10 %)

10 g sodium silicate was dissolved in sufficient distilled water and final volume was made up to 100 mL with distilled water.

I. 1-amio-2-naphthole-4-sulphonic acid

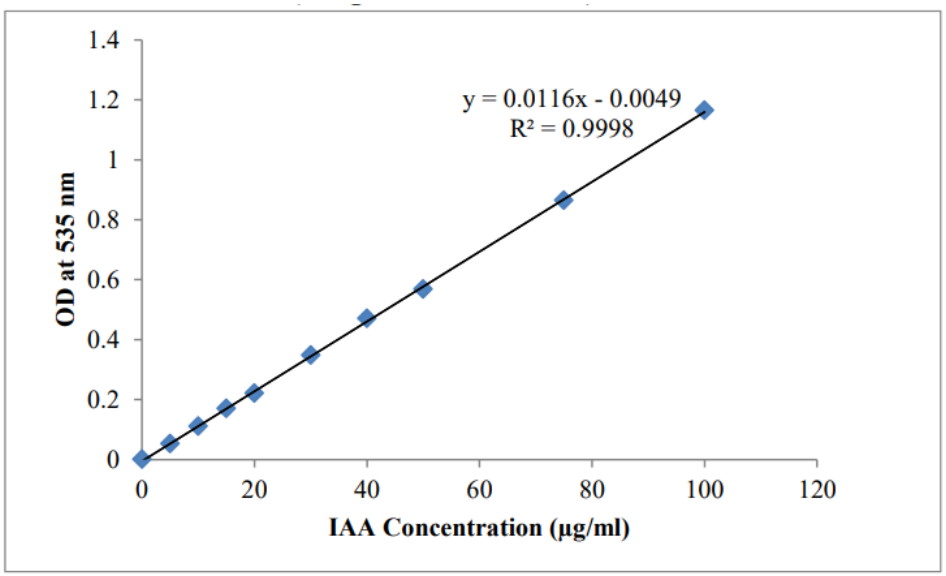
0.5 g 1-amio-2-naphthole-4-sulfonic acid was dissolved in 195 mL of 15 % sodium bisulphide solution to which 5 mL of 20 % sodium sulphate solution was added. The solution was kept in amber coloured bottle.

J. Molybdic acid reagent (2.5%)

6.25 g of ammonium molybdate was dissolved in 175 mL distilled water to which 75 mL of 10 N sulphuric acid was added.

Appendix IV

Standard curve of IAA (Tang and Bonner, 1948)



Appendix V

SUBMITTED SEQUENCES WITH ACCESSION NUMBERS IN PARANTHESIS

1. **>1_ZM1_Pseudomonas guariconensis(Marathwada)**

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   GGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCGAGC
   TAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAG
   ATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGAC
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2. **>2_GM1_Pseudomonas putida_PP754220(Marathwada)**

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3. **>3_CA1_Pseudomonas aeruginosa_PP754221(Marathwada)**

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4. **>4_ZM2_Pseudomonas sp._PP754222(Western MH)**

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5. **>5_GM2_Pseudomonas sp._PP754223(Western MH)**

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6. **>6_CA2_Delftia tsuruhatensis_PP75424(Western MH)**

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7. **>7_ZM3_Acinetobacter sp._PP754225(Vidarbha)**

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8. **>8_GM3_Stenotrophomonas maltophilia_PP754226(Vidarbha)**

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9. **>9_CA3_Aeromonas caviae_PP754227(Vidarbha)**

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10. >10_ZM4_Aeromonas caviae_PP754228(Konkan)

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11. >11_GM4_Aeromonas caviae_PP754229(Konkan)

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12. >12_CA4_Aeromonas hydrophila_PP758187(Konkan)

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TAGATAGCTTAACCTTCGGGAGGGCGTTACCACGGTGGATCGGGGTATACGA
AATGAAACTGACAATGTG

List of Publications

Published Papers

1. Gavande, S.S., Maurya, A. & Sharma, S. Isolation and characterization of plant growth promoting rhizobacteria (PGPR) from rhizosphere of major crops grown in Marathwada region of Maharashtra, India. *Vegetos* 37, 637–648 (2024). https://doi.org/10.1007/s42535-023-00779-y

2. Gavande, S.S. Genomics in Crop Plants: an Overview. *AgriBiotech e-Newsletter* 01:04 (2021).

3. Gavande, S.S. Molecular mechanisms for cellular osmoregulation in plants. *AgriBiotech e-Newsletter* 01:06 (2021).

4. Gavande, S.S., Sharma, S., Ekatpure, S., Hembade, V. & Maurya, A. Evaluating Growth Promoting Potential of Crop Specific PGPR in Konkan Region of Maharashtra for Sustainable Agriculture. *Biopesticides International* 20(2):275-284 (2024). https://doi.org/10.59467/BI.2024.20.275

List of Conference and Seminar attended

1. National Conference on Agriculture, Applied and Life Sciences: Current Research. Effect of promising liquid microbial cultures on growth attributes of maize. 5PSRM2022/059:52pp.

2. National Conference on Present and future prospective of life sciences research for sustainable development and biodiversity conservation.

   03-04 March 2023.

3. ICMBSDG-2023 International Conference. Growth promotion activity of plant growth promoting rhizobacteria (PGPR) isolated from Konkan region of Maharashtra, India.

   24-25 November 2023.

4. International Seminar on Career Opportunities of Research and Higher Education in Foreign Universities. GRQRF4-CE000034.

   08-Apr-23.

5. National Seminar on Recent Advanced in Chemical Science and Technology.

   04-Mar-23.