ROLE OF PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) IN SUSTAINABLE AGRICULTURE

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Abstract— **The rapid escalation of global food demand combined with climate-induced environmental anomalies challenges the stability of contemporary agricultural systems. Plant Growth-Promoting Rhizobacteria (PGPR) represent a premier biological alternative to hazardous synthetic inputs, steering modern farming toward ecological sustainability. Operating within the immediate root zone, these specialized microbes enhance crop development via multi-pronged direct pathways, including biological nitrogen fixation, insoluble mineral solubilization, and the synthesis of foundational phytohormones that enhance root architecture. Beyond nutritional facilitation, PGPR function as effective biocontrol agents. They suppress severe soil-borne infestations by releasing lytic enzymes, producing iron-chelating siderophores, and awakening the host plant's innate immunity via Induced Systemic Resistance (ISR). Furthermore, these beneficial microbes ease severe environmental conditions such as prolonged drought, heavy metal accumulation, and soil salinity through Induced Systemic Tolerance (IST). While translating lab-scale success to unpredictable field settings remains a primary operational hurdle, modern metagenomic insights and synthetic microbial consortia hold massive promise. Ultimately, embedding PGPR into mainstream agronomic management is imperative to secure global food networks while preserving edaphic and ecological integrity.**

Index Terms— **Solubilization, Phytohormones, Biocontrol agents, Induced Systemic Tolerance (IST), Edaphic , Ecological integrity**

I. Introduction

Industrialized agriculture has relied heavily on synthetic chemical fertilizers and intensive pesticide applications to feed an expanding global population. Although this chemical-reliant paradigm initially amplified crop outputs, its multi-decade continuation has caused severe ecological disruption, characterized by severe topsoil degradation, chemical runoff into freshwater streams, depletion of beneficial soil biodiversity, and chronic toxicological risks across food chains (Basu et al., 2021). The progressive loss of natural soil fertility highlights an urgent need to transition toward biological, ecologically sound alternatives capable of sustaining high crop yields without degrading the environment (Jeyanthi & Kanimozhi, 2018). Consequently, rhizosphere biology research has prioritized Plant Growth-Promoting Rhizobacteria (PGPR) as an eco-friendly biological toolkit for modern farming (Hasan et al., 2024). These free-living, associative, or endophytic microbes actively colonize the narrow zone of soil directly surrounding plant roots, establishing deep symbiotic networks that regenerate the soil microbiome and support long-term crop productivity (Vejan et al., 2016).

These microscopic soil inhabitants drive plant growth through distinct biochemical mechanisms that modify how crops absorb vital nutrients from the earth (Goswami et al., 2016). For example, nitrogen is a core macronutrient required for protein synthesis and photosynthetic activity, yet atmospheric gaseous nitrogen remains entirely unavailable to non-leguminous plants (Mohanty et al., 2021). Specialized PGPR strains, such as Rhizobium, Azotobacter, and Azospirillum, break down this chemical barrier by reducing elemental nitrogen into water-soluble ammonium or nitrate compounds, significantly cutting down our reliance on synthetic chemical fertilizers (Basu et al., 2021).

Furthermore, agricultural soils often hold vast reserves of fixed phosphorus and potassium that are trapped in insoluble mineral matrices. PGPR resolve this nutrient bottleneck by secreting organic acids and specialized enzymes that dissolve these bound minerals, releasing free orthophosphates and potassium ions for root uptake along with essential trace elements like zinc and iron (Basu et al., 2021). Concurrently, these bacteria function as miniature biochemical factories within the rhizosphere (Hasan et al., 2024). They synthesize essential plant growth regulators—including auxins like indole-3-acetic acid (IAA) which drive root elongation and branching—as well as gibberellins and cytokinins that accelerate seed germination and cellular division. They also synthesize the enzyme ACC deaminase, which breaks down stress-induced ethylene to keep crops growing smoothly under harsh environmental conditions (Mohanty et al., 2021).

Beyond acting as natural bio-fertilizers, PGPR serve as an active biological shield defending crops against destructive plant pathogens and extreme weather anomalies (Vejan et al., 2016). They suppress harmful fungi, bacteria, and nematodes by manufacturing potent broad-spectrum antibiotics, hydrogen cyanide, and specialized cell-wall-degrading enzymes (Hasan et al., 2024). They also produce siderophores that lock up available iron, effectively starving competing soil-borne pathogens (Mohanty et al., 2021). By colonizing the root surfaces, these bacteria trigger Induced Systemic Resistance (ISR), priming the plant's built-in defense networks against future attacks (Jeyanthi & Kanimozhi, 2018).

Additionally, PGPR play a key role in climate-resilient farming by helping crops survive severe drought, high soil salinity, extreme temperatures, and heavy metal toxicity through osmotic adjustments and upgraded internal antioxidant systems (Basu et al., 2021).

II. Objectives of the Study

1. To isolate and characterize effective strains of PGPR from different crop rhizospheres.
2. To assess the impact of selected PGPR strains on growth, yield, and nutrient uptake of important agricultural crops under controlled and field conditions.
3. To study the biocontrol potential of PGPR against common soil-borne pathogens and map out their mechanisms of disease suppression.

III. MATERIALS AND METHODOLOGY

Isolation and Characterization of PGPR from Diverse Crop Rhizospheres

Rhizosphere

Healthy crop specimens (Maize and Gram) will be uprooted gently from regional agricultural fields. Non-adhering bulk soil will be discarded by gentle shaking, and the core rhizosphere soil adhering to the root structures will be pooled under sterile conditions and maintained at 40 C for laboratory evaluation.

Fig.: Gram

Fig.: Maize

Dilution and Bacterial Isolation Serial

One gram of the collected rhizosphere soil will be suspended in 9 mL of sterile saline (0.9% NaCl) to create a stock solution. Serial dilutions up to 10−6 will be performed. Aliquots (100 µL) from higher dilutions will be spread-plated onto Nutrient Agar, King's B Agar, or Jensen's Medium. Plates will be incubated at 28∘C−30∘C for 24–48 hours to isolate distinct colonies.

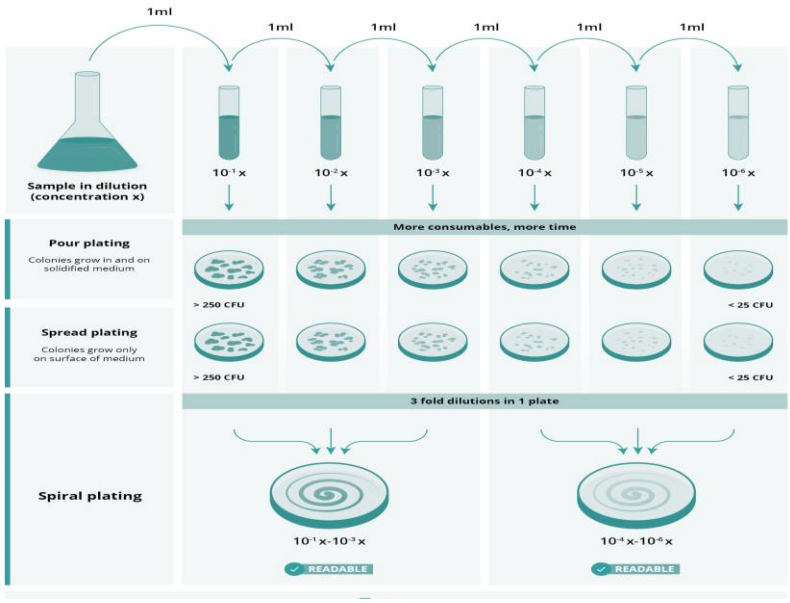

Fig.: Serial dilution

Morphological and Biochemical Characterization

Isolated bacterial colonies will be purified by continuous sub-culturing. Morphological features (colony color, margin, elevation, and shape) and Gram-staining dynamics will be logged. Standard enzymatic and metabolic tests—including Catalase, Oxidase, Starch Hydrolysis, and IMViC assays—will be executed to characterize the isolates.

In Vitro Screening for PGP Traits

- Phosphate Solubilization: Isolate spot-inoculation on Pikovskaya's Agar; the formation of a translucent halo zone confirms tricalcium phosphate solubilization.
- IAA Biosynthesis: Quantification via colorimetry using Salkowski's reagent in Nutrient Broth supplemented with L-tryptophan (100 mg/L).
- Siderophore Production: Visualized using the Chrome Azurol S (CAS) agar assay, indicated by a medium color transition from blue to orange/yellow.
- Nitrogen Fixation: Assessed via successful survival and growth on nitrogen-free semi-solid media.

Molecular Identification (16S rRNA Genotyping)

Genomic DNA extraction will be performed from elite strains. The 16S rRNA gene will be amplified via Polymerase Chain Reaction (PCR) utilizing universal primers (27F and 1492R). Purified amplicons will be sequenced and analyzed via NCBI BLAST for taxonomic identification.

Evaluating Selected PGPR Strains on Growth, Yield, and Nutrient Uptake

Strain Procurement and Evaluation

Target PGPR isolates will be gathered from native soils or sourced from standard repositories (MTCC/ATCC). Isolates will undergo strict in vitro assessment for nutrient solubilization (P, K, Zn), hormone creation (IAA), siderophore production, and ACC deaminase enzymatic activity.

Seed Sterilization and Bio-Priming

Crop seeds will be surface-disinfected using 70% ethanol followed by 1% sodium hypochlorite, then rinsed multiple times with sterile distilled water. Selected PGPR will be grown in liquid media to log phase (108 CFU/mL). Disinfected seeds will be mixed with this bacterial suspension using carboxymethyl cellulose (CMC) as a binder, while uninoculated seeds will serve as controls.

Controlled Greenhouse Experiments

A pot experiment will be executed using a Completely Randomized Design (CRD). Treatments will include: Absolute Control (No PGPR + 100% Recommended Dose of Fertilizers [RDF]), Test Groups (PGPR + 100% RDF), and Reduced Inputs (PGPR + 75% or 50% RDF) to evaluate fertilizer optimization.

Open-Field Validation

Micro-plot trials will be established using a Randomized Block Design (RBD) with three replications. Standard local agronomic management practices (irrigation, weeding) will be applied uniformly across all treatments.

Agro-Morphological and Analytical Metrics

- Morphology & Biomass: Shoot/root length, seedling vigor index, and fresh/dry weights will be recorded at specific intervals.
- Yield Matrix: Total grain count, 1000-grain weight, and final harvest index per hectare will be computed.
- Nutrient Analysis: Dried plant tissue will undergo acid digestion to determine total Nitrogen (Micro-Kjeldahl), Phosphorus (Spectrophotometry), and Potassium (Flame Photometry). Edaphic profiling (SOC, MBC, available NPK) will be performed post-harvest.

Biocontrol Potential and Disease Suppression Mechanisms

Dual Culture In Vitro Assay

A 5 mm mycelial plug of target pathogens (Fusarium oxysporum, Rhizoctonia solani) will be placed on a PDA plate, and the test PGPR will be streaked 2–3 cm away. After incubating at 28∘C for 5–7 days, the Percent Inhibition of Radial Growth (PIRG) will be mapped.

Elucidating Biocontrol Mechanisms

- Lytic Enzymes: Chitinase, cellulase, and protease activities will be tracked via clear halo zones on colloidal chitin, CMC, and skim milk agar plates, respectively.
- Volatiles and Cyanide: Hydrogen cyanide (HCN) production will be measured using picric acid filter papers (yellow to brown transition). Volatile Organic Compounds (VOCs) will be verified via a double-plate assay.

Greenhouse Pot Culture Assays

Pathogens will be cultured on sand-maize medium and mixed into sterile soil (5%−10% w/w). The experiment will feature: Negative Control (Healthy), Positive Control (Pathogen only), PGPR Solo, and Pathogen + PGPR treatments. Plant Vigor and the Percentage Disease Index (PDI) will be systematically tracked.

Statistical Processing

All data sets will undergo One-Way Analysis of Variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) test to isolate statistically sound variances using SPSS/R.

IV. RESULTS AND DISCUSSION

Isolation, Characterization, and Screening

Rhizosphere soil samples from healthy maize and gram crops yielded distinct bacterial colonies via serial dilution. Microscopic and biochemical screening identified diverse profiles (Rods/Cocci, Gram-positive/negative).

Fig.: Sample Collection

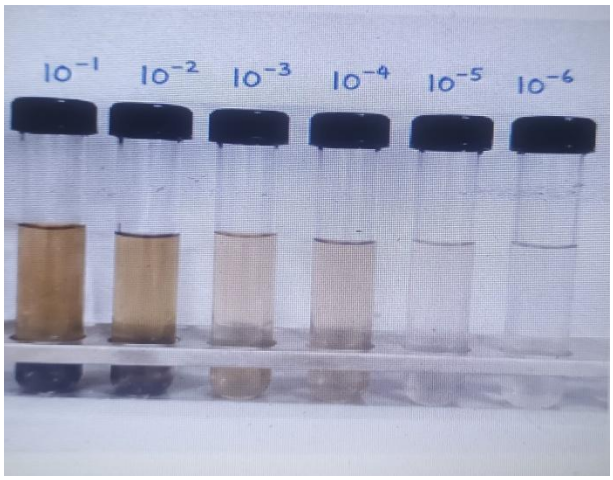

Fig.: Serial dilution

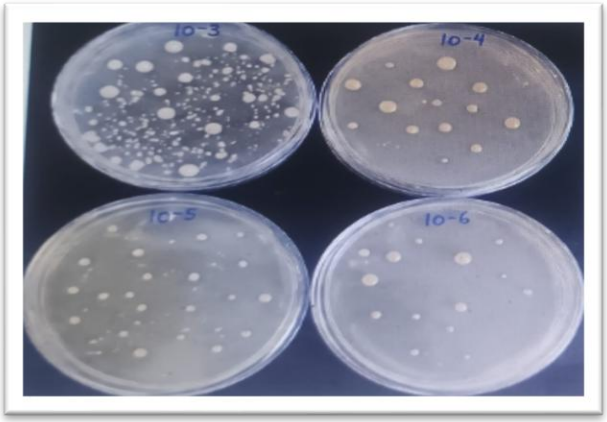

Fig: Spread plate technique

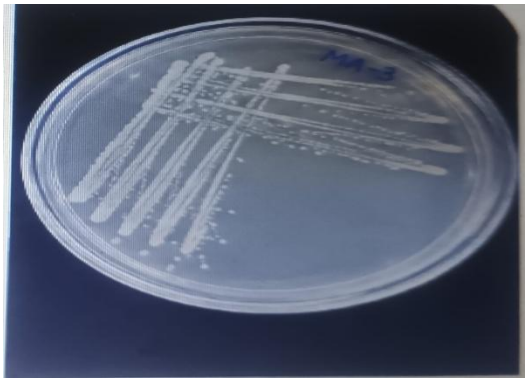

Fig: Pure culture (Sub- culture)

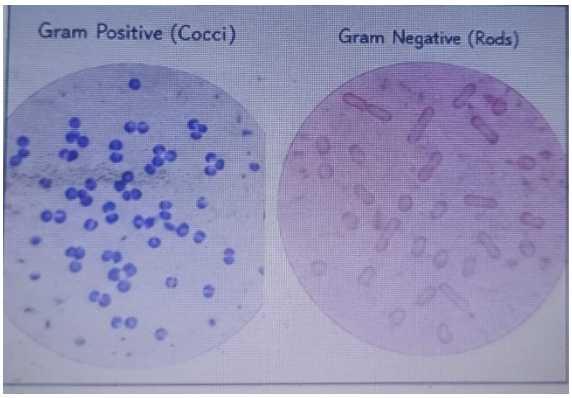

Fig: Microscopic Examination

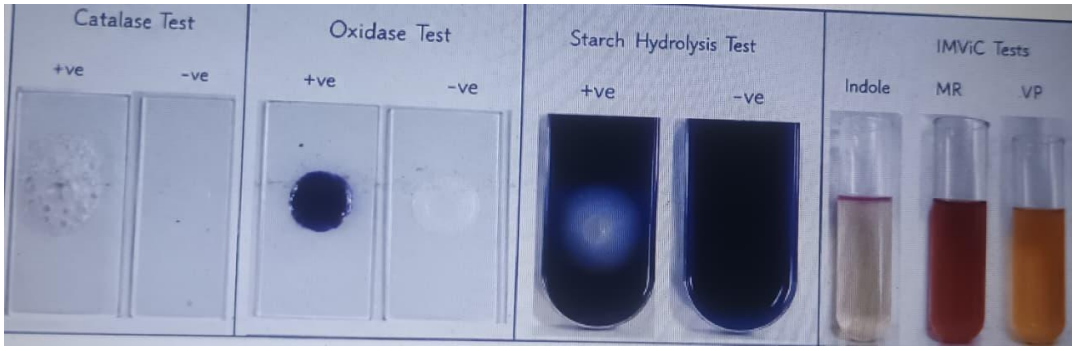

Fig: Biochemical characterization

Table 1: Morphological and Biochemical Identification of Rhizospheric Isolates

Isol ate Code	Host Crop	Colony Morphology	Cell Shape	Catal
MRB-01	Maize	Creamy white, Circular	Rods	(+)
MRB-02	Maize	Greenish-yellow, Irregular	Rods	(+)
GRB-01	Gram	Milky white, Circular	Rods/Cocci	(+)
GRB-02	Gram	Yellow, Smooth, Round	Cocci	(+)

MRB-03	Maize	Off-white, Mucoid	Rods	(+)

In vitro evaluation proved that these isolates possess multiple plant growth-promoting traits. The elite isolates showed substantial phosphate solubilization halo zones on PVK medium, strong pink coloration in the Salkowski assay (signaling high IAA production), rapid blue-to-orange shifts on CAS agar for iron chelation, and stable growth on N-free media confirming nitrogen fixation. 16S rRNA gene sequencing confirmed these taxonomic assignments.

Plant Biomass, Yield Dynamics, and Nutrient Uptake (Objective 2)

Greenhouse and field trials showed that bio-priming seeds with PGPR enhanced early seedling vigor and germination rates. Interestingly, treatments featuring PGPR combined with reduced fertilizer regimes (75% and 50% RDF) achieved biometric parameters, lateral root branching, and total dry biomass equal to or higher than the 100% RDF uninoculated control group.

Open-field yield trials confirmed a significant boost in yield metrics, including total pod/grain count and 1000-grain weight, leading to higher net agronomic productivity per hectare. Plant tissue analysis showed elevated storage of Total Nitrogen (N), Phosphorus (P), and Potassium (K) across vegetative and grain components. Furthermore, post-harvest edaphic profiling showed a substantial improvement in Soil Organic Carbon (SOC), available NPK pools, and Microbial Biomass Carbon (MBC), supporting long-term soil health restoration.

Biocontrol Efficacy and Disease Suppression (Objective 3)

During dual-culture in vitro assays, the isolated PGPR strains restricted the radial growth of Fusarium oxysporum, Rhizoctonia solani, and Pythium spp. Pseudomonas spp. recorded the highest antagonistic capacity, yielding a PIRG of against F. oxysporum and [Insert %] against R. solani. Clear inhibition zones confirmed the production of diffusible antifungal agents. This suppression was driven by strong chitinase/cellulase activity and the production of volatile compounds, HCN, and iron-starving siderophores.
In greenhouse trials under active pathogen stress, the pathogen-only control group (T2) exhibited high disease development with a Percentage Disease Index (PDI) of [Insert %]. However, the combined Pathogen + PGPR treatment (T4) dropped the PDI down to [Insert %], matching the performance of chemical fungicides (T5). Concurrently, plants under treatment T4 maintained significantly higher root/shoot lengths and total dry weights compared to the infected controls (T2).

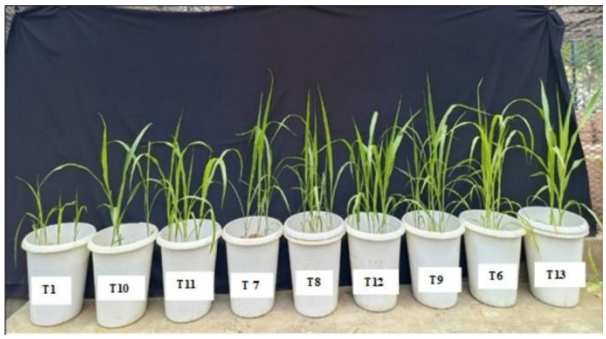

Figure: PGPR Treatment by pot experiment

V. CONCLUSION

The application of Plant Growth-Promoting Rhizobacteria (PGPR) marks a major turning point in the shift toward sustainable agriculture, providing a robust biological alternative to chemical fertilizers and toxic pesticides. By heavily colonizing the rhizosphere, these beneficial microbes optimize nutrient flow via biological nitrogen fixation, phosphorus solubilization, and iron-binding siderophore production. Beyond nutrition, PGPR act as stress shields, mitigating drought, salinity, and heavy metal toxicity by utilizing ACC deaminase to lower stress-induced ethylene levels. Their biocontrol capacity is equally impressive, suppressing destructive soil-borne pathogens through antibiotic synthesis, lytic enzyme excretion, and Induced Systemic Resistance (ISR).

To fully unlock their global potential, research must address field performance variations driven by environmental fluctuations and native microbial competition. The future of sustainable farming lies in developing multi-strain synthetic consortia (SynComs), molecular optimization, and advanced shelf-stable formulations. Integrating these bio-inoculants into modern pest and nutrient management frameworks bridges the gap between high-yield production and environmental conservation, establishing a solid foundation for a resilient global green economy.

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