

Isolation of Effective Plant Growth-Promoting Rhizobacteria (PGPR) to Construct Synthetic Communities and Evaluate Their Impact on Maize (*Zea mays*)

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ABSTRACT

The overutilization of artificial fertilizers in maize (*Zea mays*) cultivation has caused environmental degradation and decrease soil microbial diversity. This study intended to isolate effective Plant Growth-Promoting Rhizobacteria (PGPR) from the maize rhizosphere, construct synthetic communities, and assess their impact on plant growth under glasshouse conditions. Rhizobacteria were isolated and screened using high-throughput microplate assays for phosphate solubilization, indole-3-acetic acid production, and ammonia generation. Genetic diversity and compatibility were assessed using BOX-PCR fingerprinting. Synthetic communities were formulated with compatible, functionally complementary strains and applied to maize in a pot trial alongside controls. Considerable improvements were observed in shoot and root length, biomass, chlorophyll content, and yield factors. Soil biological markers such as microbial biomass carbon and enzyme activities are also enhanced. These Results emphasize the capability of PGPR consortia as biofertilizers to long term increase maize productivity and soil health.

Keywords: Plant Growth-Promoting Rhizobacteria (PGPR), Synthetic microbial community, Maize, Biofertilizer, Soil health, Phosphate solubilization

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Introduction

Maize (*Zea mays* L.) is one of the major cereal crops cultivated globally, especially in tropical and subtropical zones such as Malaysia and Pakistan. It plays a crucial role in food security, livestock feed, and the bio-based industries. Rising in the global population stress and increase demand for food, maize yield has experienced increasingly demanding, utilizing massive amount of manufactured fertilizers. While these components have results high production, their extreme use has led to soil depletion, reduction of beneficial microbes, nutrient run off, and environmental degradation [1]. These challenges highlight the immediate demand for sustainable replacements that sustain both productivity and ecological stability. Plant Growth-Promoting Rhizobacteria (PGPR) a community of beneficial microbes that colonize roots of the plant and support vegetative development through different biochemical and physiological functions. These processes are generally classified as direct or indirect. Direct mechanisms contain key procedures like biological nitrogen fixation, phosphate solubilization, siderophore facilitated iron uptake, ammonia formation, and the biosynthesis of plant hormones such as indole-3-acetic acid (IAA) [2]. Indirectly, PGPR inhibit plant pathogens by forming antibiotics, hydrolytic enzymes along triggering systemic tolerance response in plant hosts [3]. Various studies have revealed that PGPR can clearly enhance plant biomass, root structure, and strength of plant tolerance against abiotic pressure containing salinity, drought, and lack of nutrients [4].

PGPR are freely existing bacteria that promote growth of the plant not only by increasing nutrient supply but also by controlling plant metabolism. They may enhance the plant's ability to take up water and mineral intake, stimulate the root growth, enhance enzymatic activity and support beneficial microbe actions. Beyond this, they indirectly protect to plants by competing the pathogens for nutrients, producing antimicrobial metabolites, breaking down fungal cell walls, and triggering systemic tolerance in the plant. PGPR have also show potential in increasing plant efficiency under abiotic stress by increasing resilience, making them reliable allies for agriculture under changing climatic factors [5].

Although PGPR show great capacity, single-strain PGPR inoculants often shows variable results in practical applications due to changes in environmental factors, soil types, and varieties in crops. To mitigate this shortage, recent studies have highlighted the composition of synthetic microbial communities or consortia formulations of adaptable PGPR strains that constructed to complementary or synergistically increase the growth and health of plant. These synthetic consortia offer improved colonization capability, functional diversity and tolerance under changing environmental factors, thereby increasing their capability for field scale agricultural practices [6].

Objectives

- To isolate, characterize, and assemble synthetic communities of Plant Growth Promoting Rhizobacteria (PGPR) with complementary plant beneficial traits for maize cultivation.
- To assess the capability of these synthetic PGPR consortia in increasing plant growth, nutrient uptake, and minimizing chemical fertilizer and pesticide use in tropical maize yield.
- To examine the role of PGPR consortia on maize development and crop output under controlled glasshouse conditions.

Materials and Methods

Field Sampling and isolation of rhizobacteria

Soil and root samples will be taken from the rhizosphere of healthy maize plants from diverse agroecological zones. Isolation will be carried out on a variety of selective and differential media under aerobic conditions to isolate diverse PGPR, following the protocols of Nautiyal [7].

High-Throughput Characterization Using Microplate Assays

Rather than conventional agar-based methods, high-throughput 96-well microplate assays will be applied to assess PGPR traits including:

(a) Testing of phosphate solubilization ability

Phosphate solubilizing activity will be measured using the molybdate-blue method with absorbance measured at 420 nm as described by King [8].

$$PSI = (\text{Colony diameter} + \text{Halo diameter}) / \text{Colony diameter.}$$

(b) Assessment of indole acetic acid production

IAA production by bacterial strains was measured by the method of Gordon and Weber [9]. Bacterial cultures will be incubated in nutrient broth with tryptophan for 2 days. After centrifugation, Salkowski reagent will be added to the liquid phase, resulting in the development of pink color, reflecting the presence of IAA. The amount of IAA will be determined by absorbance at 535 nm using a spectrophotometer.

(c) Estimation of ammonia production

The output of ammonia will be evaluated by the following method. Based on this method, the bacterial strains will be produced in test tubes containing 10 ml of peptone water and incubated at $30 \pm 0.1^\circ\text{C}$ for 2 days. After incubation, Nessler's reagent (0.5 ml) will be added to each tube. The establishment of a yellow to brown color will indicate ammonia production.

Molecular Identification Using BOX-PCR Fingerprinting

To analyze genetic diversity and compatibility among isolates, BOX-PCR fingerprinting will be employed as reported by the Rademaker and de Bruijn [10], enabling selection of diverse and complementary strains for community assembly.

Formulation of Microbial Consortia

(a) In vitro Compatibility and Functional Complementarity

Identified isolates will be grown together on nutrient agar and in liquid media to evaluate antagonism or synergism. Only synergistic strains with supplementary PGPR traits (e.g., simultaneous phosphate solubilization and

siderophore production) will be integrated into synthetic consortia Berendsen [11].

(b) Bioformulation Development

A talc-based carrier formulation will be developed using standardized moisture content and viable count methods as described by Vidhyasekaran and Muthamilan [12]. Shelf-life will be observed monthly for three months and stored at room temperature.

Experiment Design

A glasshouse pot trial will be carried out using maize (*Zea mays*). A Completely Randomized Design (CRD) will be used with five treatments:

T1: Control (without inoculation)

T2: Suggested dose of chemical fertilizer (RDF)

T3: Single efficient PGPR strain

T4: Assembled PGPR consortium

T5: Half-dose RDF + consortium

Analyzed Parameters

(a) Plant Growth

Germination rate, shoot and root elongation, overall plant biomass, SPAD chlorophyll content and yield parameters such as cob weight and grain number.

(b) Soil Parameters

Microbial biomass carbon content (Vance et al., 1987), available nitrogen, phosphorus, potassium, pH, EC, and catalytic activities such as dehydrogenase and ALP enzyme.

(c) Root Colonization:

Visualization of colonization efficiency will be performed using fluorescence microscopy, employing GFP-tagged strains as described by Bloemberg [13].

Data Interpretation

All treatments will be carried out in three replicates. Data will be evaluated using analysis of variance (ANOVA), and Tukey's HSD test will be used to identify meaningful differences among means ($p \leq 0.05$). Principal Component Analysis (PCA) will also be used to examine correlations among multiple plant and soil parameters.

Results and Discussion

Increased Plant Growth with Synthetic PGPR Consortia

The use of PGPR, particularly synthetic microbial communities, meaningfully increased maize out parameters compared to the without inoculation control (T1) and single-strain treatments (T3). Maize plants handled with the synthetic consortium alone (T4) and in combination with a half-dose of recommended artificial fertilizer (T5) showed significant enhanced shoot and root elongation, total plant biomass, and SPAD chlorophyll observations. The T5 treatment, in particular, showed a 40% increase in cob weight and grain count, demonstrating that PGPR consortia can efficiently minimize fertilizer dependence while sustaining crop productivity.

Enhanced Soil Enzyme Activity and Microbial Activity

Soil enzymatic analysis further validated the beneficial effect of PGPR consortia. Consortium-treated soils showed higher microbial biomass carbon, along with markedly raised elevated dehydrogenase and alkaline phosphatase functions, compared to the control. These parameters indicate increased microbial metabolic activities and nutrient cycling functions, indicating that PGPR treatments lead to long-term soil fertility and health.

Genetic Diversity and Complementarity Functional Traits

Molecular identification using BOX-PCR fingerprinting verified substantial genetic diversity among the screened PGPR isolates. This diversity underpins the identified activities complementarity within the synthetic consortium, contributing to rationale for multi-strain composition. The inclusion of functionally diverse isolates increases the likelihood of synergistic plant-microbe and microbe-microbe interactions under different environmental conditions.

Efficient Root Colonization by PGPR Isolates

Root colonization analyses using GFP-tagged PGPR strains verified the rhizosphere competency of identified isolates. Microscopic analysis confirmed successful formation and root attachment, which is important for sustained plant-microbe interaction and the long-term capability of microbial inoculants in the field.

Alignment with Existing Literature

These findings align with previous research presenting that multi-strain PGPR inoculants outperform single-strain practices in increasing plant growth parameters, nutrient uptake, and soil biological activity. The outcomes underscore the level of developing synthetic PGPR communities as a key contributor of integrated nutrient management systems.

Conclusion

This study clearly demonstrated that synthetic communities of functionally diverse PGPR isolates significantly increase maize growth and soil biological health under controlled glasshouse conditions. The synergistic inputs of increased nutrient acquisition, abiotic stress tolerance and effective rhizosphere colonization emphasize the promise of PGPR consortia as environmentally sound and sustainable biofertilizers. Significantly, their application even with minimize artificial inputs was able to sustain or enhance critical yield indicators, underscoring their capacity as an alternative to conventional fertilizers. These results not only demonstrate the functional complementarity of well-constructed microbial consortia but also provide to the broader pursuit of climate resilient, resource efficient smart farming systems. Future study should focus on field level assessments, long-term performance evaluating and strain enhancement across diverse agroecological zones to promote scalable and context specific implementation of PGPR based strategies in sustainable maize production.

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