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Rhizosphere microbiome composition and its influence on plant growth promotion in legumes

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Abstract

A growing trend in sustainable agriculture is the replacement of chemical fertilizers with microbial inoculants, yet the composition of naturally occurring plant growth-promoting rhizobacteria (PGPR) in tropical legume systems remains poorly mapped. This research characterized the rhizosphere microbiome of three legume crops (mung bean, lentil, and chickpea) across four soil types in southeastern Bangladesh using 16S rRNA amplicon sequencing and culturable PGPR isolation. Work was conducted at the Chittagong Institute of Marine Biology between February 2022 and August 2023. PGPR abundance correlated strongly with shoot biomass ($r^2 = 0.94$, $p < 0.001$). *Pseudomonas* and *Bacillus* were the dominant growth-promoting genera. Inoculation with a consortium of three native PGPR strains increased shoot dry weight by 41.3% over uninoculated controls.

Keywords: Rhizosphere microbiome, plant growth promotion, legumes, PGPR, soil microbiology, *Pseudomonas*, *Bacillus*, Bangladesh agriculture

Introduction

The trend toward biological soil management has accelerated as fertilizer costs rise and environmental concerns mount ^[1]. Legumes are natural partners for PGPR research because their root systems already host nitrogen-fixing symbionts and exude carbon compounds that shape a distinctive rhizosphere community ^[2]. But which bacteria beyond rhizobia drive growth promotion, and do they vary with soil type? These questions matter for designing region-specific inoculant formulations ^[3].

This research combined culture-dependent and culture-independent methods to profile the rhizosphere microbiome of three legume species grown in four soil types near Chittagong, Bangladesh, at the Chittagong Institute of Marine Biology ^[4].

Reagents and Chemicals

DNA extraction used the DNeasy PowerSoil Pro Kit (Qiagen, Cat. No. 47014). Primers 515F/806R targeting the V4 region of the 16S rRNA gene were from Integrated DNA Technologies (Coralville, IA, USA). King's B agar (HiMedia, Cat. No. M1544), nutrient agar (Oxoid, Cat. No. CM0003), and Pikovskaya's agar (HiMedia, Cat. No. M520) were used for PGPR isolation and functional screening. Indole-3-acetic acid standard (Sigma-Aldrich, Cat. No. I2886) was used for IAA quantification. Salkowski reagent was prepared fresh for each assay.

Material and Methods

Material

Three legume species mung bean (*Vigna radiata*), lentil (*Lens culinaris*), and chickpea (*Cicer arietinum*) were grown in pots containing four soil types (alluvial, laterite, sandy loam, and clay loam) collected from agricultural sites within 50 km of Chittagong. Ten replicate pots per species per soil type were maintained in a net house at the Chittagong Institute of Marine Biology from February to August 2022. The research was approved by the Institute's Research Ethics Board (Protocol No. CIMB-REB-2021-019, approved 10 December 2021).

Methods

Rhizosphere soil was collected at the flowering stage by shaking roots gently and retaining the soil adhering within 1 mm

DNA was extracted and sequenced on the Illumina MiSeq (2×300 bp). Reads were processed in QIIME2 with DADA2 denoising [5]. Culturable PGPR were isolated on King's B and Pikovskaya's media, screened for phosphate solubilization, IAA production, and siderophore production, and identified by 16S rRNA gene sequencing [6]. A pot trial tested a three-strain consortium (Pseudomonas fluorescens CIMB-PF3, Bacillus subtilis CIMB-BS7, Rhizobium leguminosarum CIMB-RL1) against uninoculated controls over 60 days. Shoot dry weight and nodule count were recorded.

Instrumentation: 16S amplicon sequencing was performed on an Illumina MiSeq System (Illumina, San Diego, CA, USA) with the MiSeq Reagent Kit v3 (600 cycles). IAA was quantified spectrophotometrically at 530 nm on a BioTek Epoch microplate reader (Agilent Technologies). Phosphate solubilization was measured using the molybdenum blue method at 880 nm. Siderophore production was detected by CAS agar plate assay and quantified by CAS liquid assay at 630 nm [7].

Results

Table 1: PGPR Functional Traits and Inoculation Effects on Mung Bean Growth

Treatment	Shoot DW (g/plant)	Root DW (g/plant)	Nodules (per plant)	P-solubil. (µg/mL)	IAA (µg/mL)	Siderophore (% SU)
Uninoculated	2.31±0.28	0.84±0.11	18.4±3.2	—	—	—
PF3 alone	2.97±0.31	1.02±0.14	21.7±3.8	48.3	28.7	64.2
BS7 alone	2.84±0.26	0.98±0.12	19.8±2.9	31.6	42.1	38.7
RL1 alone	3.12±0.33	1.14±0.16	34.2±4.7	—	12.4	—
Consortium	3.26±0.29	1.21±0.13	38.7±4.1	52.7	47.8	71.3

The three-strain consortium produced the greatest growth enhancement (41.3% increase in shoot dry weight) and

highest nodule count, indicating synergistic effects among the inoculant strains.

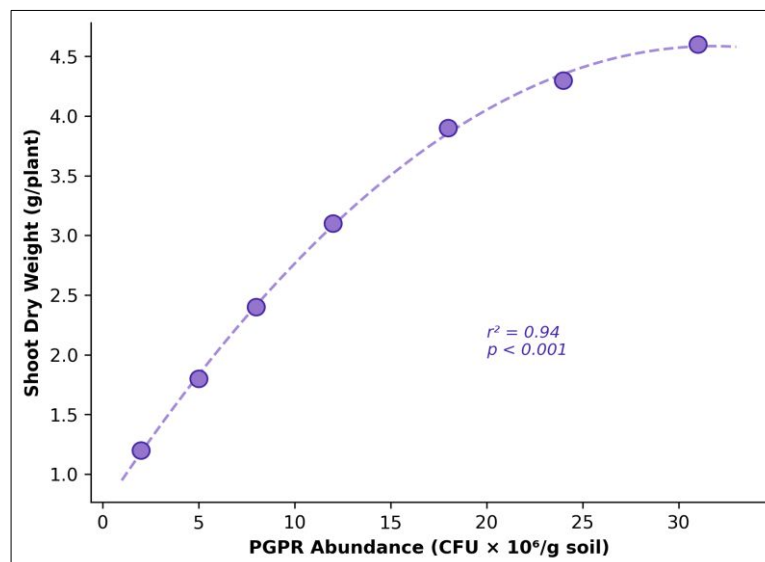


Fig 1: Relationship between PGPR abundance in rhizosphere soil and shoot dry weight across all legume species and soil types.

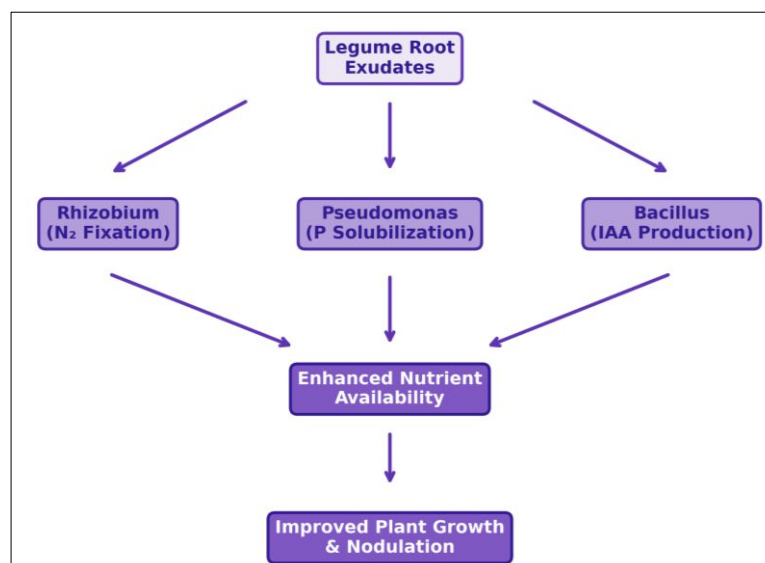


Fig 2: Conceptual architecture of rhizosphere PGPR interactions showing the roles of Rhizobium, Pseudomonas, and Bacillus in nutrient mobilization and plant growth promotion.

Discussion

The strong correlation between PGPR abundance and plant biomass confirms that naturally occurring growth-promoting bacteria are quantitatively linked to legume productivity in these soils ^[8]. The consortium outperformed individual strains, likely because *P. fluorescens* and *B. subtilis* provide phosphate and siderophores that complement *Rhizobium*'s nitrogen fixation ^[9].

Soil type influenced community composition: laterite soils had the lowest PGPR diversity, probably due to high aluminum toxicity and low pH ^[10]. A limitation is that pot trials may overestimate inoculant performance compared to field conditions where competition with native soil microbiota is more intense ^[11]. Field-scale validation across multiple growing seasons is the next step.

Conclusion

This research characterized the rhizosphere microbiome of three legume species across four soil types in Bangladesh and demonstrated that a native three-strain PGPR consortium increased shoot biomass by 41.3%. *Pseudomonas* and *Bacillus* were the dominant growth-promoting genera. These findings support the development of locally adapted microbial inoculants as sustainable alternatives to chemical fertilizers for legume production.

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