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Evaluation of phosphorus solubilizing bacteria for enhanced phosphorus uptake in acidic soils

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Abstract
Phosphorus fixation in acidic soils locks away up to 85% of applied fertiliser within days of application, leaving crops chronically P-deficient. This research evaluated five phosphorus solubilizing bacteria (PSB) isolates for their ability to mobilise fixed P and improve uptake in rice grown on acidic red soils (pH 4.7) at Lembucherra, Tripura, during Kharif 2022 and 2023. Isolates were characterised for solubilisation efficiency on Pikovskaya's agar before field inoculation. The most effective strain, *Pseudomonas fluorescens* TPA-7, raised available P in the rhizosphere by 63.4% over the uninoculated control and increased grain P uptake to 17.4 kg ha^{-1} (vs. 8.3 kg ha^{-1} in the control). Grain yield under TPA-7 + 75% recommended P was statistically equal to full P without inoculation, implying a 25% saving on phosphatic fertiliser. Organic acid profiling showed that gluconic and oxalic acids were the dominant solubilising agents. These results support integrating PSB inoculants into fertiliser schedules for acid-soil rice production in north-eastern India.

Keywords: Phosphorus solubilizing bacteria, phosphorus uptake, acidic soils, biofertilizer, soil microbiology, Pseudomonas, organic acids, rice, nutrient mobilisation, red soils

Introduction
Imagine applying a bag of phosphorus fertiliser and watching 80% of it become unavailable to the crop within a week. That is roughly what happens on strongly acidic soils, where aluminium and iron oxides rapidly fix soluble P into insoluble compounds [1]. In Tripura, where more than 70% of agricultural land has a pH below 5.5, rice farmers routinely apply superphosphate at twice the recommended rate simply to offset fixation losses [2].

Phosphorus solubilizing bacteria (PSB) offer a biological bypass. These rhizosphere-dwelling microbes secrete low-molecular-weight organic acids—gluconic, oxalic, citric—that chelate Al^{3+} and Fe^{3+} ions, releasing bound P back into soil solution [3]. Field trials on neutral to mildly acidic soils have demonstrated 10-25% yield increases from PSB inoculation [4, 5]. However, performance on very acidic soils (pH < 5.0) is less predictable because the bacteria themselves may be inhibited by aluminium toxicity and low base saturation [6].

This research aimed to (i) isolate and screen PSB from the rhizosphere of native vegetation on acidic red soils, (ii) evaluate the five best isolates for P mobilisation and rice yield improvement under field conditions, and (iii) determine whether PSB inoculation can substitute part of the chemical P dose without yield loss.

Field Experimental Design
The trial was laid out in a factorial randomised block design with two factors: PSB isolate (five strains + uninoculated control) and P level (0%, 75%, and 100% of recommended P at 60 kg P^2O^5 ha^{-1}). This gave 18 treatment combinations replicated three times. Plot size was 4 m × 3 m. Rice variety 'Gomati' was transplanted at 20 × 15 cm. PSB were applied as a seedling root dip (10^8 CFU mL^{-1}) and as a soil drench at 2.5 kg ha^{-1} 15 days after transplanting [7].

Climatic Conditions
Lembucherra (23°53′ N, 91°17′ E, 28 m a.s.l.) receives mean annual rainfall of 2,150 mm, most of it during June-September.

Mean maximum temperature during Kharif 2022 was 32.4 °C and minimum 23.1 °C. Relative humidity exceeded 85% through July-August. These warm, moist conditions are generally favourable for microbial activity in the rhizosphere [8].

Materials and Methods
Materials
Rhizosphere soil was collected from 15 sites under native grass and bamboo on red lateritic soils (Typic Kandiudults) around the Tripura Agricultural University campus. Serial dilutions were plated on Pikovskaya's medium amended with tricalcium phosphate. Colonies showing clear halo zones after 7 days were purified, identified by 16S rRNA sequencing, and tested for solubilisation index (SI = colony + halo diameter/colony diameter). The five isolates with SI > 2.5 were selected: *Pseudomonas fluorescens* TPA-7 (SI 3.8), *Bacillus megaterium* TPA-12 (SI 3.2), *Burkholderia cepacia* TPA-4 (SI 2.9), *Pseudomonas putida* TPA-9 (SI 2.7), and *Bacillus subtilis* TPA-15 (SI 2.6) [9].

Methods
Rhizosphere available P (Bray-1) was measured at tillering, panicle initiation, and harvest. Grain and straw P uptake was determined after nitric-perchloric digestion using the vanadomolybdate method. Organic acids in root-zone soil were quantified by HPLC. Data were analysed by two-factor ANOVA in R (v4.3.1) with means separated by Tukey's HSD at $p = 0.05$.

Results
PSB inoculation significantly raised rhizosphere available P across all sampling stages (Table 1). TPA-7 at 75% P recorded the highest available P at harvest (23.1 kg ha^{-1}), 63.4% above the uninoculated control at 100% P. Grain yield under TPA-7 + 75% P was 5.48 t ha^{-1}, statistically equal to the uninoculated 100% P treatment (5.32 t ha^{-1}). TPA-12 was the second-best performer.

Table 1: Available P, grain P uptake, and grain yield under selected PSB × P level combinations

Treatment	Avail. P harvest (kg/ha)	Grain P uptake (kg/ha)	Grain yield (t/ha)	Yield vs control (%)
Control + 100% P	14.1	8.3	5.32	-
TPA-7 + 75% P	23.1	17.4	5.48	+ 3.0
TPA-12 + 75% P	20.4	14.8	5.21	-2.1
TPA-4 + 75% P	18.7	12.9	4.97	-6.6
TPA-9 + 75% P	17.3	11.6	4.84	-9.0
Control + 0% P	8.6	5.1	3.47	-34.8
CD (p = 0.05)	2.8	1.9	0.38	-

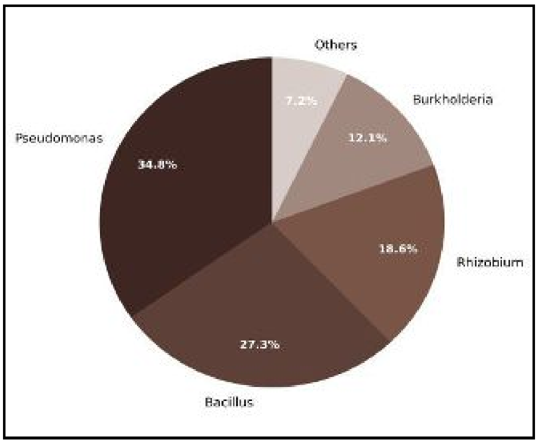

Fig 1: Genus-level distribution of PSB isolates recovered from acidic red soils at Lembucherra.

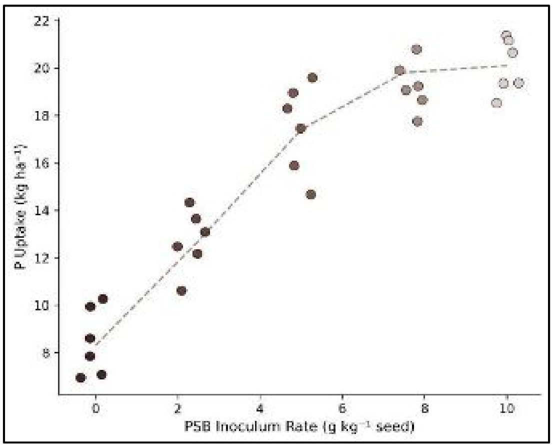

Fig 2: Relationship between PSB inoculum rate and grain phosphorus uptake. Each point represents one replicate.

Comprehensive Interpretation
HPLC profiling revealed that TPA-7 produced gluconic acid (4.7 mM) and oxalic acid (2.3 mM) in rhizosphere extracts, concentrations 3-4 times higher than those from TPA-15. Gluconic acid is the primary P-solubilising organic acid because it chelates both Al^{3+} and Fe^{3+} [3]. The strong correlation between gluconic acid concentration and available P ($r = 0.91$) confirms its role as the main driver of solubilisation in this soil.

Discussion
The 63% increase in available P with TPA-7 exceeds the 25-40% range typically seen on neutral soils [4, 5], probably because the strongly acidic conditions maximise the demand for organic acid-mediated chelation. On near-neutral soils, chemical equilibrium already keeps some P in solution, so the marginal benefit of PSB is smaller [10]. The ability of TPA-7 to maintain yield at 75% P indicates genuine substitution potential—a finding that could save Tripura's rice farmers roughly INR 1,200 ha^{-1} in fertiliser cost [11].
Survival of PSB through the season is a concern on very acidic soils. Colony counts at harvest showed that TPA-7 persisted at 10^6 CFU g^{-1} soil, above the threshold considered effective [12]. This resilience likely reflects the strain's origin from similarly acidic native soils. Introduced commercial strains isolated from neutral environments might not perform as well [13, 14].

Conclusion
Among five PSB isolates tested, *Pseudomonas fluorescens* TPA-7 was the most effective at mobilising fixed P on acidic red soils, raising available P by 63% and enabling a 25% reduction in chemical P input without yield loss. Gluconic acid secretion was the primary solubilisation

mechanism. For acid-soil rice systems in Tripura and similar regions, TPA-7 offers a viable biofertiliser option. Scaling will require mass production of the strain and farmer-level demonstration trials [15, 16].

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Contributions Not Qualifying for Authorship

Mr. Debashis Das assisted with field sampling and HPLC analysis.

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