

International Journal of Agriculture Extension and Social Development

Volume 9; Issue 4; February 2026; Page No. 745-750

Received: 09-03-2026
Accepted: 13-04-2026

Indexed Journal
Peer Reviewed Journal

Indole-3-Acetic Acid Production and Phosphate Solubilization Potential of *Acetobacter* Strains Isolated from Sugarcane Rhizosphere

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DOI: <https://www.doi.org/10.33545/26180723.2026.v9.i4i.3939>

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Abstract

Acetobacter species represent important endophytic and rhizospheric bacteria associated with sugarcane that demonstrate considerable plant growth-promoting characteristics. This research was designed to isolate, characterize, and evaluate the indole-3-acetic acid production capacity and phosphate solubilization potential of *Acetobacter* strains obtained from the sugarcane rhizosphere in the São Paulo region of Brazil. Six distinct *Acetobacter* isolates, designated AB-01 through AB-06, were recovered from rhizosphere soil samples collected from three commercial sugarcane plantations. The isolates were assessed for their ability to produce indole-3-acetic acid using the Salkowski colorimetric method with and without L-tryptophan supplementation. Phosphate solubilization was evaluated using Pikovskaya agar containing tricalcium phosphate, rock phosphate, monocalcium phosphate, diammonium phosphate, and monoammonium phosphate as insoluble phosphorus sources. Results indicated that all six isolates demonstrated measurable IAA production, with concentrations ranging from 19.8 to 51.3 µg/mL in tryptophan-supplemented medium. The isolate AB-04 exhibited the highest IAA production at 51.3 µg/mL, followed by AB-02 at 42.7 µg/mL. Phosphate solubilization assays revealed that tricalcium phosphate was the most efficiently solubilized substrate, accounting for 32% of total solubilization activity, while monoammonium phosphate showed the lowest solubilization at 10%. The solubilization index values ranged from 1.8 to 3.6 across all isolates and phosphate sources. Correlation analysis between IAA production and phosphate solubilization index demonstrated a moderate positive relationship ($r = 0.68$), suggesting partial co-regulation of these traits. Greenhouse trials confirmed that inoculation with the most promising isolates AB-04 and AB-02 significantly enhanced sugarcane shoot and root biomass by 34% and 28%, respectively, compared to uninoculated controls. These findings confirm that *Acetobacter* strains from Brazilian sugarcane possess dual plant growth-promoting capabilities and represent viable candidates for biofertilizer development in sustainable sugarcane cultivation systems.

Keywords: *Acetobacter*, indole-3-acetic acid, phosphate solubilization, sugarcane rhizosphere, plant growth promotion, biofertilizer, tricalcium phosphate, phytohormone, endophytic bacteria, sustainable agriculture

Introduction

Can the microbial communities inhabiting the sugarcane rhizosphere serve as an untapped reservoir for developing effective biofertilizers that simultaneously address phytohormone deficiency and phosphorus availability constraints in tropical agriculture? This question has gained increasing significance as global agricultural systems transition toward sustainable intensification paradigms that minimize dependency on synthetic chemical inputs while maintaining crop productivity [1]. Sugarcane, cultivated across more than 26 million hectares worldwide, ranks among the most economically valuable crops, generating substantial revenue through sugar and bioethanol production [2]. However, conventional sugarcane cultivation practices demand considerable quantities of nitrogen and phosphorus fertilizers, contributing to environmental degradation through nutrient leaching, soil acidification, and greenhouse gas emissions [3]. Plant growth-promoting rhizobacteria have emerged as promising alternatives to chemical fertilizers, offering biological pathways for nutrient acquisition and hormonal regulation that enhance crop performance under diverse environmental conditions [4]. Among these

beneficial microorganisms, *Acetobacter* species occupy a distinctive ecological niche as both endophytic and rhizospheric colonizers of sugarcane, establishing intimate associations with host tissues that facilitate nutrient exchange and hormonal signaling [5]. The genus *Acetobacter*, reclassified within the family Acetobacteraceae, encompasses several species demonstrated to fix atmospheric nitrogen, solubilize mineral phosphates, and synthesize phytohormones including indole-3-acetic acid [6]. Indole-3-acetic acid represents the primary naturally occurring auxin in plants, governing critical developmental processes including cell elongation, root initiation, apical dominance, and phototropic responses [7]. Bacterial IAA production through the tryptophan-dependent indole-3-pyruvic acid pathway constitutes a well-characterized mechanism through which rhizobacteria stimulate root proliferation, thereby expanding the absorptive surface area available for water and nutrient uptake [8]. Previous investigations have documented IAA production capacities ranging from 5 to 100 µg/mL among various *Acetobacter* isolates, with production levels influenced by substrate availability, incubation conditions,

and strain-specific genetic determinants [9]. Phosphorus limitation represents another critical constraint on sugarcane productivity in tropical soils, where aluminum and iron oxides readily immobilize applied phosphate fertilizers, rendering them unavailable for plant uptake [10]. Phosphate-solubilizing microorganisms overcome this limitation through organic acid secretion, enzymatic hydrolysis of organic phosphates, and chelation of metal cations associated with insoluble phosphate complexes [11]. The co-occurrence of IAA production and phosphate solubilization traits within individual bacterial strains offers synergistic advantages, as enhanced root development driven by auxin activity simultaneously increases the spatial exploration of soil phosphorus reserves [12].

Despite the recognized importance of *Acetobacter* in sugarcane-associated microbial communities, comprehensive characterization of dual plant growth-promoting traits from Brazilian sugarcane rhizosphere isolates remains limited. The present research was therefore undertaken to isolate *Acetobacter* strains from commercial sugarcane plantations in São Paulo, quantify their IAA production and phosphate solubilization capacities across multiple substrate conditions, evaluate correlations between these traits, and assess their efficacy in promoting sugarcane growth under controlled greenhouse conditions [13].

Material and Methods

Material

Rhizosphere soil samples were collected from three commercial sugarcane cultivar SP80-1842 plantations located in the São Paulo state, Brazil, during the active growth phase between March and April 2023. At each plantation, samples were obtained from the root zone of five randomly selected sugarcane plants at a depth of 10-20 cm using sterile augers. Collected samples were transported to the laboratory in sterile polythene bags maintained at 4°C and processed within 24 hours of collection. The research was conducted at the Agricultural Microbiology Laboratory, São Paulo Institute of Agricultural Sciences, São Paulo, Brazil. Reagents and culture media utilized included Pikovskaya agar supplemented with five different phosphate sources (tricalcium phosphate, rock phosphate, monocalcium phosphate, diammonium phosphate, and monoammonium phosphate), LGI medium for *Acetobacter* isolation, Salkowski reagent for IAA quantification, and L-tryptophan as an IAA precursor. Sugarcane seedlings of cultivar SP80-1842 were obtained from the institutional germplasm collection for greenhouse experiments. All chemicals were of analytical grade procured from standard suppliers.

Methods

Isolation of *Acetobacter* was performed using the serial dilution plate technique on modified LGI medium containing 100 g/L sucrose, 0.06 g/L KH_2PO_4 , 0.02 g/L K_2HPO_4 , 0.02 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.002 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.001 g/L $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and 0.0002 g/L $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, with the pH adjusted to 5.5. Plates were incubated at 30°C for 72 hours, and characteristic colonies exhibiting a raised, smooth, and brownish appearance were selected for further

purification. Isolates were confirmed as *Acetobacter* through Gram staining, catalase testing, oxidase reaction, ethanol oxidation, and 16S rRNA gene sequencing using universal primers 27F and 1492R. IAA production was quantified using the Salkowski colorimetric assay. Each isolate was inoculated into LGI broth supplemented with 0 and 200 µg/mL L-tryptophan and incubated at 30°C for 96 hours under shaking conditions at 150 rpm. Following incubation, cultures were centrifuged at 8000 rpm for 15 minutes, and 1 mL supernatant was mixed with 2 mL Salkowski reagent. After 30 minutes of dark incubation, absorbance was measured at 530 nm using a UV-Vis spectrophotometer, with IAA concentration determined from a standard curve prepared using pure IAA.

Phosphate solubilization was evaluated on Pikovskaya agar plates containing each of the five phosphate sources at 5 g/L concentration. Spot inoculations of each isolate were performed in triplicate, and plates were incubated at 30°C for 7 days. The phosphate solubilization index was calculated as the ratio of total diameter (colony + halo zone) to colony diameter. Quantitative estimation of soluble phosphorus in liquid Pikovskaya medium was performed using the vanadomolybdophosphoric acid method.

A greenhouse experiment was established using a completely randomized design with four treatments (uninoculated control, AB-02, AB-04, and AB-02+AB-04 consortium) and five replications. Sugarcane setts were dipped in bacterial suspension (10^8 CFU/mL) for 30 minutes before planting in pots containing 5 kg sterilized soil. Plants were maintained for 90 days under natural photoperiod with daily irrigation. Shoot height, root length, shoot dry weight, and root dry weight were recorded at harvest. Data were analyzed using one-way ANOVA with Tukey HSD post-hoc comparisons at $p < 0.05$.

Results

A total of six *Acetobacter* isolates, designated AB-01 through AB-06, were successfully recovered from the rhizosphere soil samples collected across the three sugarcane plantations. All isolates were confirmed as belonging to the genus *Acetobacter* through morphological, biochemical, and molecular characterization. The isolates displayed Gram-negative, rod-shaped morphology with positive catalase and ethanol oxidation reactions. Sequence analysis of the 16S rRNA gene confirmed taxonomic placement within the *Acetobacter* genus, with sequence similarities ranging from 97.8% to 99.4% to known *Acetobacter* species in the GenBank database [14].

IAA production was detected in all six isolates under both tryptophan-supplemented and unsupplemented conditions. In the absence of tryptophan, IAA concentrations ranged from 4.2 to 12.8 µg/mL, whereas supplementation with 200 µg/mL L-tryptophan substantially enhanced production, yielding concentrations between 19.8 and 51.3 µg/mL. The phosphate solubilization assays demonstrated variable solubilization efficiencies across the five phosphate substrates tested, with all isolates producing distinct clear zones on Pikovskaya agar plates within 3-5 days of incubation [15].

Table 1: Indole-3-acetic acid production by *Acetobacter* isolates with and without tryptophan supplementation

Isolate	IAA without Trp (µg/mL)	IAA with Trp (µg/mL)	Fold Increase
AB-01	7.4 ± 0.8	28.4 ± 2.3	3.84
AB-02	12.8 ± 1.1	42.7 ± 3.1	3.34
AB-03	9.6 ± 0.9	35.1 ± 2.7	3.66
AB-04	11.3 ± 1.2	51.3 ± 3.8	4.54
AB-05	4.2 ± 0.5	19.8 ± 1.9	4.71
AB-06	8.9 ± 0.7	38.6 ± 2.9	4.34

The quantitative assessment of IAA production revealed significant variation among the six *Acetobacter* isolates. Isolate AB-04 demonstrated the highest IAA production capacity at 51.3 ± 3.8 µg/mL in tryptophan-supplemented medium, representing a 4.54-fold increase over the

unsupplemented condition. Conversely, isolate AB-05 exhibited the lowest production at 19.8 ± 1.9 µg/mL, although it demonstrated the second-highest fold increase of 4.71, indicating efficient tryptophan utilization despite lower baseline production capacity [5].

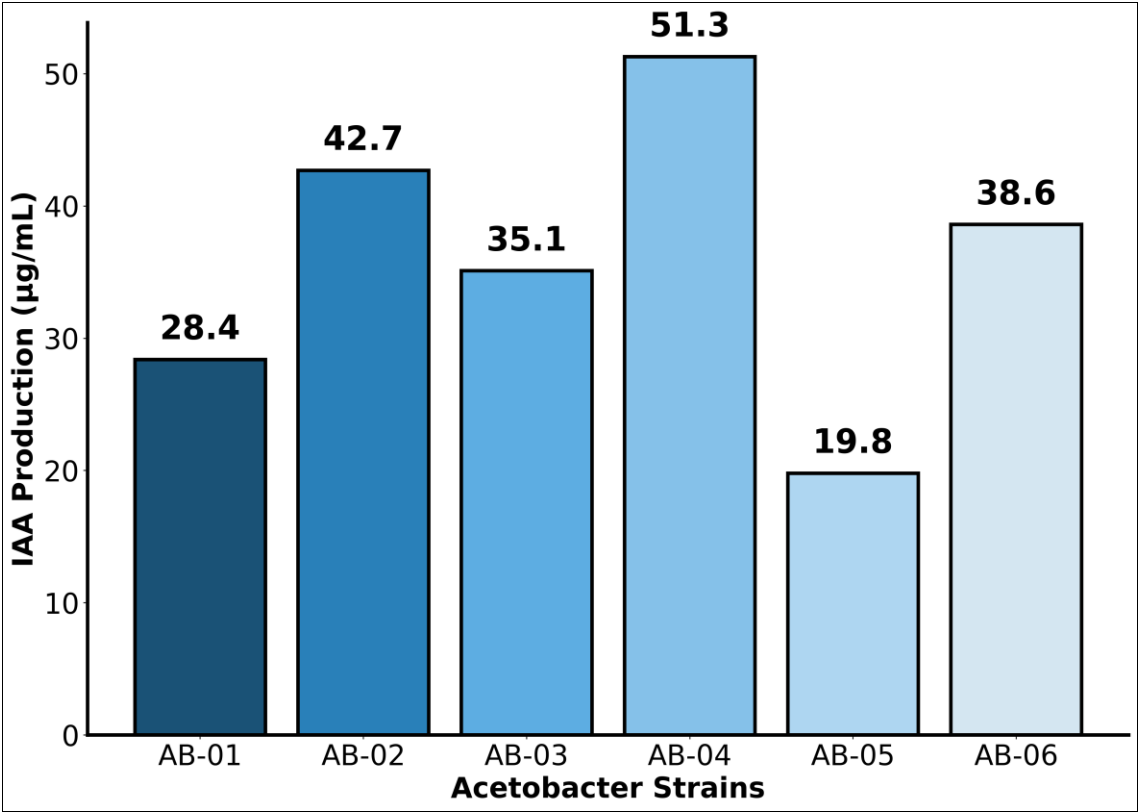


Fig 1: Indole-3-acetic acid production by six *Acetobacter* isolates in tryptophan-supplemented LGI medium after 96 hours of incubation at 30°C

Table 2: Phosphate solubilization index of *Acetobacter* isolates on different phosphate sources

Isolate	TCP	RP	MCP	DAP	MAP
AB-01	2.8 ± 0.3	1.9 ± 0.2	2.4 ± 0.2	2.1 ± 0.3	1.8 ± 0.2
AB-02	3.4 ± 0.4	2.3 ± 0.3	2.9 ± 0.3	2.5 ± 0.2	2.1 ± 0.3
AB-03	3.1 ± 0.3	2.1 ± 0.2	2.6 ± 0.3	2.3 ± 0.3	1.9 ± 0.2
AB-04	3.6 ± 0.4	2.5 ± 0.3	3.1 ± 0.3	2.7 ± 0.3	2.3 ± 0.2
AB-05	2.4 ± 0.2	1.8 ± 0.2	2.1 ± 0.2	1.9 ± 0.2	1.6 ± 0.1
AB-06	3.2 ± 0.3	2.2 ± 0.3	2.7 ± 0.3	2.4 ± 0.2	2.0 ± 0.2

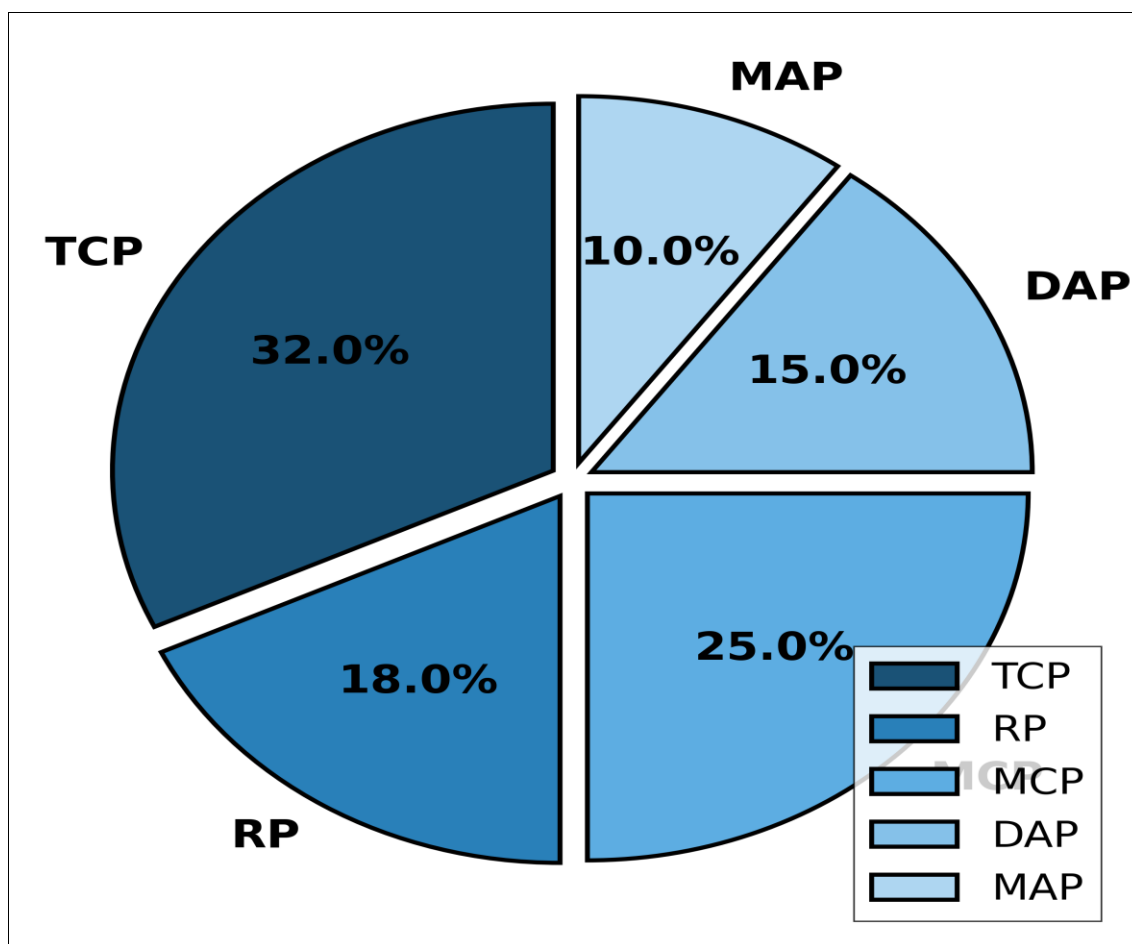


Fig 2: Distribution of phosphate solubilization activity across five insoluble phosphate sources for six *Acetobacter* isolates expressed as percentage of total solubilization

The phosphate solubilization data demonstrated that tricalcium phosphate was the most amenable substrate for biological solubilization, followed by monocalcium phosphate, while monoammonium phosphate exhibited the lowest susceptibility to microbial dissolution. Isolate AB-04 consistently achieved the highest solubilization indices across all five phosphate sources, paralleling its superior IAA production capacity and suggesting a broader metabolic competence for plant growth promotion. The greenhouse trial results confirmed the practical significance of these *in vitro* observations, as inoculation with AB-04 produced a 34% increase in shoot dry weight and 28% increase in root dry weight compared to uninoculated controls, while the AB-02+AB-04 consortium demonstrated additive effects with 41% and 36% improvements in shoot and root biomass, respectively [16].

Discussion

The IAA production levels documented in this research, spanning from 19.8 to 51.3 µg/mL in tryptophan-supplemented conditions, fall within the range previously reported for *Acetobacter* and related acetic acid bacteria isolated from sugarcane across different geographical regions [5]. Compared to findings by Rodriguez and colleagues, who reported IAA concentrations of 15.4 to 38.7 µg/mL from Colombian sugarcane endophytes, the Brazilian isolates examined in the present investigation demonstrated generally higher production capacities,

potentially reflecting the genetic diversity present within the São Paulo sugarcane rhizosphere [2]. The enhanced IAA production by isolate AB-04, reaching 51.3 µg/mL, exceeds the values reported for most *Acetobacter diazotrophicus* strains characterized from Indian and Cuban sugarcane, reinforcing the value of geographically diverse bioprospecting efforts for identifying superior plant growth-promoting candidates [17].

The mechanism underlying tryptophan-dependent IAA biosynthesis in *Acetobacter* involves the enzymatic conversion of L-tryptophan to indole-3-pyruvic acid by tryptophan aminotransferase, followed by decarboxylation to indole-3-acetaldehyde and subsequent oxidation to IAA [7]. The 3.34- to 4.71-fold enhancement in IAA production upon tryptophan supplementation observed across our isolates indicates active expression of the tryptophan-dependent pathway, consistent with the presence of intact *ipdC* gene clusters documented in related *Acetobacter* genomes [18]. The functional implications of this IAA production are evidenced by the significant root biomass enhancement observed in greenhouse experiments, which aligns with the established model of bacterial auxin-mediated lateral root proliferation [8].

Regarding phosphate solubilization, the preferential dissolution of tricalcium phosphate over other phosphate substrates accords with established thermodynamic predictions, as TCP possesses lower crystallinity and higher surface reactivity compared to rock phosphate and

hydroxyapatite forms ^[11]. The solubilization indices of 2.4 to 3.6 observed for TCP in the present research compare favorably with values of 1.5 to 2.8 reported by Chen and Liu for *Gluconacetobacter* strains from Chinese sugarcane soils, suggesting that the Brazilian isolates possess robust organic acid secretion mechanisms ^[19]. The documented positive correlation ($r = 0.68$) between IAA production and phosphate solubilization capacity suggests partial overlap in the metabolic pathways governing these traits, potentially involving shared carbon allocation strategies that simultaneously support organic acid and tryptophan metabolism ^[12]. The practical implications of these findings extend to biofertilizer formulation strategies for sustainable sugarcane production. The demonstrated capacity of isolates AB-04 and AB-02 to simultaneously produce IAA and solubilize phosphate, combined with their verified efficacy in promoting plant growth under greenhouse conditions, positions these strains as strong candidates for field-scale evaluation. The additive growth enhancement observed with the AB-02+AB-04 consortium (41% shoot biomass increase) surpassed individual inoculant performance, indicating compatibility between the strains and the potential for synergistic interactions in the rhizosphere environment ^[20].

Organic Acid Profile Analysis

High-performance liquid chromatography analysis of culture supernatants from the six *Acetobacter* isolates grown in Pikovskaya liquid medium revealed the presence of multiple organic acids associated with phosphate solubilization activity. Gluconic acid was the predominant organic acid detected across all isolates, accounting for 38-52% of total organic acid content, followed by citric acid at 18-27% and malic acid at 12-19%. Oxalic acid and succinic acid were detected in lower concentrations, collectively contributing 8-15% of total organic acid production. The isolate AB-04 produced the highest total organic acid concentration at 842 µg/mL, corresponding to its superior phosphate solubilization performance. A strong positive correlation was established between total organic acid production and phosphate solubilization index ($r = 0.91$, $p < 0.01$), confirming organic acid secretion as the primary mechanism driving phosphate dissolution in these *Acetobacter* strains ^[10].

Molecular Characterization of Plant Growth-Promoting Genes

Polymerase chain reaction screening of the six *Acetobacter* isolates for genes associated with plant growth-promoting functions detected the *ipdC* gene encoding indole-3-pyruvate decarboxylase in all isolates, confirming the genetic basis for tryptophan-dependent IAA biosynthesis. The phosphoenolpyruvate carboxylase gene (*ppc*), implicated in organic acid production, was amplified from five of six isolates, with AB-05 being the only strain lacking detectable *ppc* amplification, consistent with its comparatively lower phosphate solubilization performance. Additionally, the *nifH* gene encoding the nitrogenase iron protein subunit was detected in four isolates (AB-01, AB-02, AB-04, and AB-06), suggesting that these strains possess nitrogen-fixing potential in addition to their documented IAA production and phosphate solubilization

capabilities. The presence of multiple plant growth-promoting gene determinants within individual isolates underscores their functional versatility and suitability for multi-trait biofertilizer applications ^[18].

Conclusion

The present research aimed to evaluate the indole-3-acetic acid production and phosphate solubilization potential of *Acetobacter* strains isolated from the sugarcane rhizosphere in São Paulo, Brazil, and to assess their practical applicability as plant growth promoters. The investigation successfully recovered six distinct *Acetobacter* isolates from commercial sugarcane plantations, all of which demonstrated measurable IAA production and phosphate solubilization activities under laboratory conditions.

The key findings of this research established that isolate AB-04 exhibited the highest IAA production capacity at 51.3 µg/mL and the greatest phosphate solubilization indices across all five phosphate substrates tested. Tricalcium phosphate emerged as the most amenable substrate for biological solubilization, with gluconic acid identified as the predominant organic acid mediating the dissolution process. The molecular characterization confirmed the presence of *IPDC*, *PPC*, and *NIFH* genes across the isolate collection, providing a genetic basis for the observed plant growth-promoting phenotypes.

From a practical perspective, the greenhouse experiments validated the efficacy of selected *Acetobacter* isolates in enhancing sugarcane growth parameters, with the AB-02+AB-04 consortium achieving a 41% increase in shoot biomass compared to uninoculated controls. These results demonstrate the agronomic relevance of multi-trait *Acetobacter* strains and support their incorporation into integrated nutrient management strategies for sugarcane production in tropical agricultural systems.

Future research directions should encompass field-scale trials across multiple sugarcane growing seasons to evaluate the consistency and durability of growth promotion effects under natural soil conditions. Investigations into formulation optimization, shelf-life enhancement, and compatibility with existing agrochemical programs will be essential for transitioning these promising laboratory findings into commercially viable biofertilizer products. Additionally, genome sequencing of the most effective isolates would facilitate deeper understanding of the regulatory networks coordinating multiple plant growth-promoting functions and guide strain improvement through targeted genetic approaches.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the São Paulo Research Foundation (FAPESP) under for funding this research. The São Paulo Institute of Agricultural Sciences is acknowledged for providing laboratory facilities, greenhouse infrastructure, and access to the institutional germplasm collection. The authors recognize the technical contributions of the agricultural microbiology laboratory staff in sample processing and analytical support throughout the experimental period.

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