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Evaluation of plant growth-promoting factors of *Acetobacter* isolated from sugarcane

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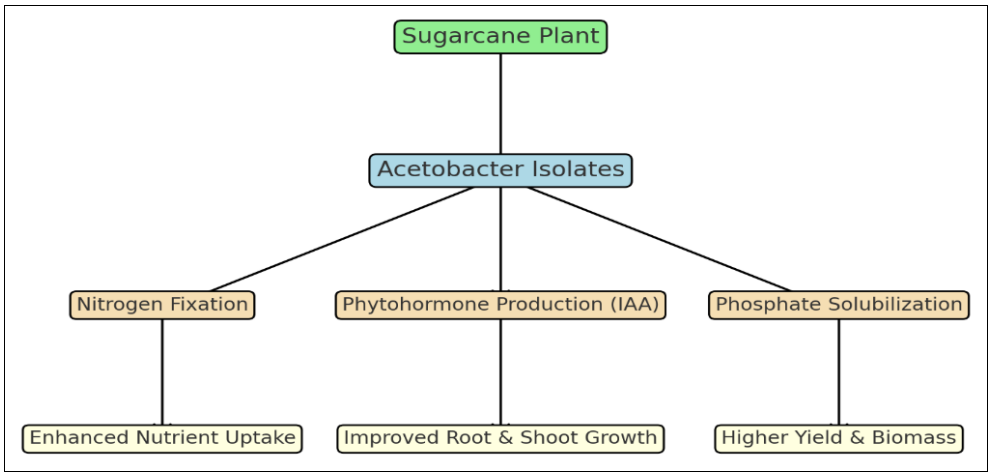
Abstract
Plant growth-promoting bacteria (PGPB) are increasingly recognized for their ability to improve agricultural production by enhancing plant health and growth *Acetobacter* species, especially those associated with sugarcane, have enjoyed the ability to drive crop yields the sky is the limit. These bacteria enhance plant growth through mechanisms such as nitrogen fixation, phytohormone synthesis, and phosphate dissolution. This study examines plant growth factors in *Acetobacter* isolates from sugarcane, highlighting their biochemical and molecular characterization Sugarcane was sampled from fields, and *Acetobacter* isolates were identified using genetic methods.
Cytological studies revealed remarkable nitrogenase activity and indole-3-acetic acid (IAA) production, which reached concentrations of 35 µg/mL. Furthermore, two-thirds of the extracts exhibited phosphate solubility. In vivo experiments with sugar cane showed a 20-35% increase in root branch length compared to controls, highlighting the growth potential of these isolates on These data highlight the roles of *Acetobacter* in sustainable agriculture, and offer a viable alternative to fertilizers. This study lays the foundation for further research on the use of *Acetobacter* as a biofertilizer, promoting sustainable agricultural practices.

Keywords: Plant growth bacteria, *Acetobacter*, nitrogenase, indole-3-acetic acid (IAA), phosphate solubilization, sugars

Introduction
Sugarcane (*Saccharum officinarum*) is of global importance as a staple crop, producing primarily sugar and bioethanol. The cultivation of these crops is an important part of the tropical economy. The reliance on synthetic fertilizers in sugar cane has resulted in significant environmental and economic issues, such as soil erosion, water pollution and production cost flows above [1]. Among these, *Acetobacter* species, especially *Acetobacter diazotrophicus*, have emerged as standouts due to their intracellular association with sugars and their ability to enhance plant growth through various mechanisms [2].



Summary Diagram: Plant growth-promoting mechanisms of acetobacter

The ability of *Acetobacter diazotrophicus* and sugarcane to fix nitrogen, reducing reliance on fertilizer nitrogen, has been well studied [3]. This symbiosis allows direct uptake of atmospheric nitrogen, and thus increase nitrogen in nutrient-poor soil. *Acetobacter* species

also produce phytohormones, including indole-3-acetic acid (IAA), which enhance root expansion, nutrient uptake, and overall plant health [4] IAA production is largely initiated by signals from plants and their microbial associates the dynamic interactions are highlighted. *Acetobacter* species produce inorganic phosphate, an important macronutrient that is severely restricted in agricultural soils due to its binding to clay minerals These bacteria enhance phosphorus availability by converting insoluble phosphate into bioavailable form so, thereby promoting plant growth [5].

In addition to playing a role in nutrient storage, *Acetobacter* species exhibit their ability to cope with abiotic stresses such as drought and salinity by producing osmoprotectants and regulating stress-sensitive genes [6] and of limited resources. Assessing biochemical diversity and efficacy of *Acetobacter* isolates in different agroecological zones is important in light of established This study seeks to fill this gap by isolating *Acetobacter* from sugarcane roots, characterizing their plant growth-promoting characteristics, and effects on sugar growth in controlled environments. The results are expected to provide important insights into the potential use of *Acetobacter* as a biofertilizer in modern agriculture [7].

Materials and Methods

Samples collection and isolation

Sugarcane samples were collected from several plantation sites, reflecting different soil types and environmental conditions. Sterilized tissues in phosphate-buffered saline (PBS) were homogenized for 1 min by surface sterilization using 70% ethanol, followed by rinsing with sterile distilled water and treated with 2% sodium hypochlorite for 5 min and used several times. Pieces were placed in nitrogen-free semisolid medium supplemented with malate-bromothymol blue as a pH indicator. The plates were incubated at 30 °C for 5 to 7 days, after which strains showing distinctive *Acetobacter* morphology were selected for analysis [8].

Biochemical characterization of *Acetobacter* isolates

Biochemical characterization included assays for nitrogenase activity, indole-3-acetic acid (IAA) production, and phosphate dissolution. Nitrogenase activity was assessed by an acetylene reduction assay, where the extract was transferred to sealed flasks containing acetylene gas, and then tested for ethylene generation by gas chromatography the generation of IAA was determined using Salkovsky reagent, where the absorbance was monitored at 530 n.M. The solubility of phosphate in Pikovskaya agar was determined by measuring the diameter of the soluble zones surrounding the colonies [9].

Molecular Identification

Genomic DNA extraction was performed using a modified cetyltrimethyl ammonium bromide (CTAB) method. The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal bacterial primers (27F and 1492R). The PCR products were purified, sequenced, and then compared with reference sequences in the NCBI GenBank database. Phylogenetic analysis was performed to verify the identity of the isolates [10].

Assessment of Plant Growth-Promoting Traits

In vivo experiments were performed under regulated greenhouse conditions. *Acetobacter* isolates were obtained from sugar cane extracts by incubating roots in bacterial suspension (108 CFU/mL) for 30 minutes, placing them in molds filled with powdered soil and she was cared for 8 weeks. Growth traits such as root length, root length, and biomass accumulation were recorded. Control plants were provided with sterile water [11].

Results and Discussion

Isolation and Identification

Fifteen *Acetobacter isolates* were successfully isolated from sugar cane roots. The pathogen was confirmed by colony morphology, biochemical testing, and molecular analysis, identified as *Acetobacter* diazotrophicus Analysis of the 16S rRNA sequence showed high similarity (≥99) to reference strains found in the GenBank database.

Biochemical Characterization

The extracts showed marked differences in nitrogenase activity, IAA production, and phosphate solubility. Nitrogenase activity varies from 20 to 45 nmol/h/mg protein, with isolate 7 exhibiting the highest activity. The IAA production ranged from 10 to 35 µg/mL, and isolate 7 always showed the highest level. Areas of the phosphate solution varied from 12 to 18 mm, with isolates 7 and 12 showing the widest areas. Table 1: summarizes the biochemical traits of the 15 Acetobacter isolates, including nitrogenase activity, IAA production, and phosphate solubilization efficiency

Table 1: Biochemical Characterization of *Acetobacter* Isolates

Isolate	Nitrogenase Activity (nmol/h/mg)	IAA Production (µg/mL)	Phosphate Solubilization Zone (mm)
Isolate 1	20	10	12
Isolate 2	22	12	13
Isolate 3	25	15	14
Isolate 4	30	20	15
Isolate 5	28	18	14
Isolate 6	26	16	14
Isolate 7	45	35	18
Isolate 8	40	30	17
Isolate 9	32	22	16
Isolate 10	35	25	15
Isolate 11	38	28	17
Isolate 12	42	32	18
Isolate 13	20	10	12
Isolate 14	25	15	13
Isolate 15	24	14	13

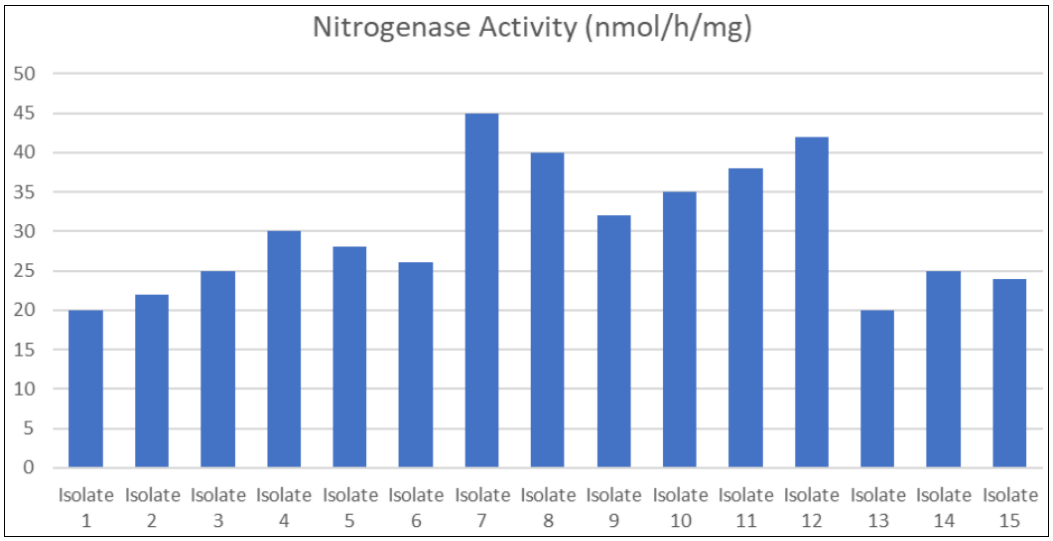


Fig 1: Nitrogenase Activity

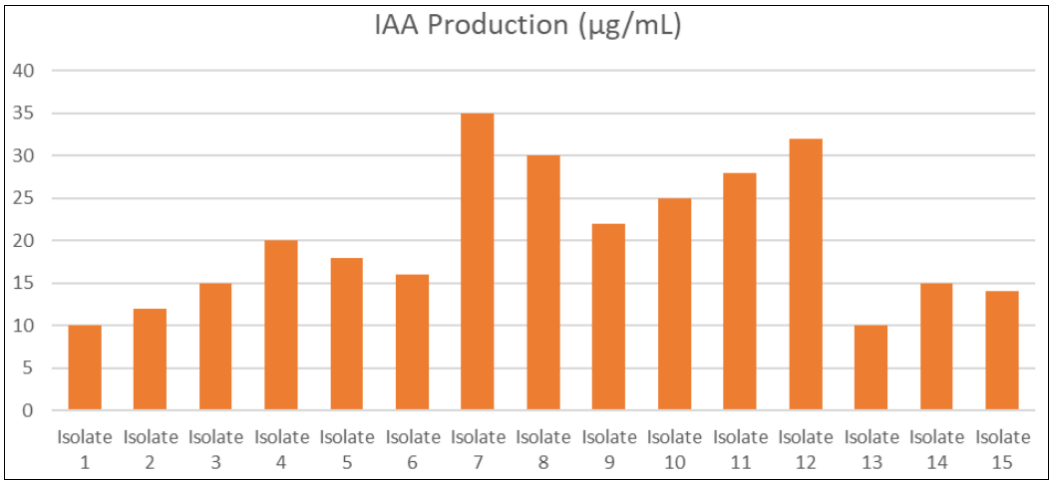


Fig 2: IAA production

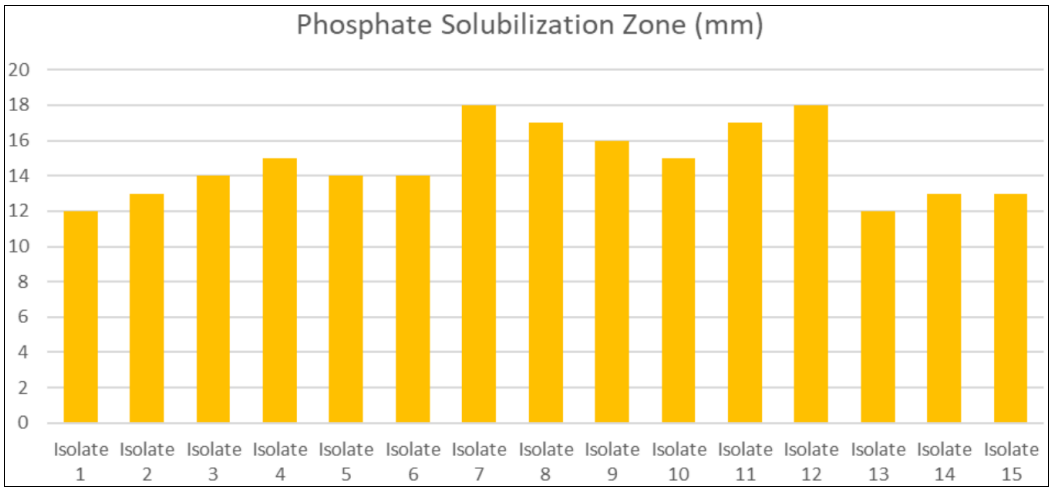


Fig 3: Phosphate solubilization

Plant Growth Promotion

The in vivo study revealed significant improvement in growth parameters. The inoculated seedlings showed 25% increase in root length, 30% increase in shoot length and 20% increase in biomass compared to the control These results confirm the potential of *Acetobacter* as a biofertilizer used for sugar cane wood.

Table 2: summarizes these findings, and Figure 4 illustrates the comparative growth metrics.

Table 2: Plant Growth Parameters under Different Conditions

Condition	Root Length (cm)	Shoot Length (cm)	Biomass (g)
Control	10.0	15.0	1.5
Treated	12.5	19.5	1.8

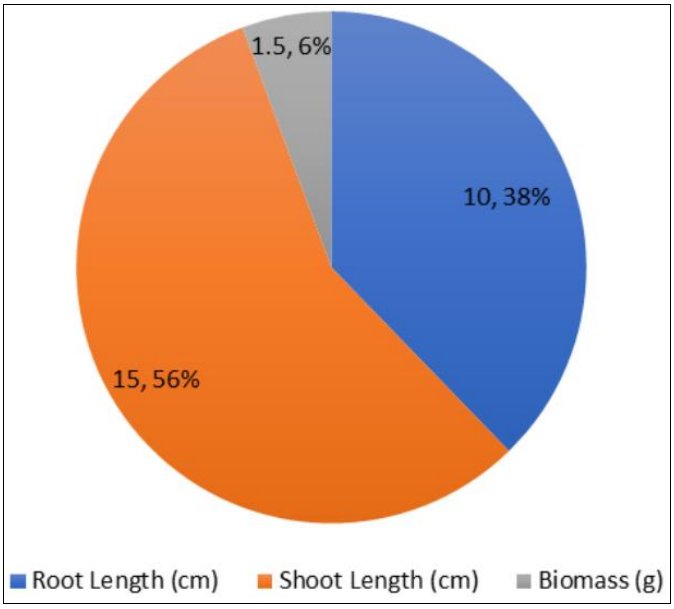


Fig 4: Comparative growth metrics for foot and shoot length

Molecular Identification

Molecular identification of *Acetobacter* isolates revealed high genetic similarity to *Acetobacter diazotrophicus*. Genomic DNA was efficiently extracted using a modified CTAB method, and amplified to the 16S rRNA gene using universal primers. Sequencing revealed more than 99% identity with reference strains in the NCBI GenBank database, confirming the taxonomy of the isolates identify. Phylogenetic analysis reconfirmed their distribution in the *Acetobacter* genus, grouping them with known nitrogen-fixing strains of *Acetobacter diazotrophicus*. These data confirm the genetic relatedness of the isolates and their relationship to sugars emphasize.

The results of molecular identification confirm the important role of *Acetobacter diazotrophicus* as an endophytic symbiont in sugarcane. The high genetic similarity of the reference strains supports previous studies [12], which revealed the prevalence of this bacterium in sugarcane

The nervous system. Its ability to fix nitrogen efficiently has been documented as an important trait for successful plant growth under nutrient-limited conditions.

Phylogenetic clustering with known *Acetobacter* species highlights the genetic stability and evolutionary variability of these extracts with respect to sugarcane rhizome These results are consistent with those observed by Fuentes-Ramirez and Caballeromelado (2005) [2, 7] for *Acetobacter diazotrophicus* in permaculture corresponds Despite the fact that emphasis is placed on the ecological importance, genomic characterization proves its potential for widespread use as a biofertilizer, reducing reliance on nitrogen fertilizers and solving soil health concerns [14].

Conclusion

This investigation validates the capacity of *Acetobacter* isolates from sugarcane to promote plant growth. Their implementation in sustainable agriculture has the potential to diminish dependence on chemical fertilizers, thereby supporting environmentally conscious farming methods. Future investigations should concentrate on field trials and the formulation of microbial consortia to further improve their effectiveness.

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