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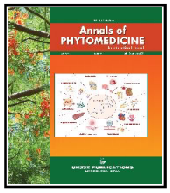

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Unlocking the potential of indigenous plant growth-promoting bacteria for sustainable agricultural practices

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Abstract

The current research targeted to characterize the plant growth-promoting (PGP) traits of bacteria, isolated from soil samples, collected from organic and inorganic fertilized areas in Namakkal District, Tamil Nadu, India. A total of 60 bacterial strains were isolated, 33 from organic soil and 27 from inorganic soil. Biochemical assays were used to identify and characterize the bacterial isolates to evaluate their PGP properties, such as the generation of hydrogen cyanide (HCN), ammonia, phosphate solubilization, indole-3-acetic acid (IAA), cellulase, amylase, protease activity, and nitrogen-fixing capacity. Among the isolates, *Alcaligenes faecalis* from organic soil exhibited strong PGP properties, including the highest phosphate solubilization and IAA production, along with ammonia and cellulase activity. It also demonstrated the ability to promote seed germination, with an 85% germination rate observed during testing. Further, cytotoxicity analysis using VERO cells indicated that *A. faecalis* had minimal cytotoxicity, with 95.8% cell viability, and ecotoxicity assessment using earthworms revealed no significant adverse effects. The potential isolate was identified through 16S rRNA gene sequencing, confirming its identity as *A. faecalis*. The results of this investigation indicate that *A. faecalis* isolated from organic soil holds great potential as a biofertilizer, offering enhanced plant growth and improved soil health. Its minimal cytotoxic and ecotoxic effects further highlight its safety for agricultural applications. These results evidenced the use of *A. faecalis* as an eco-friendly alternative to chemical fertilizers, contributing to sustainable agriculture and environmental preservation.

1. Introduction

Modern agriculture has revolutionized food production, allowing it to keep pace with the growing global population. Technological advancements, including synthetic fertilizers, pesticides, and herbicides, have significantly enhanced crop yields and farming efficiency (Dhanapal *et al*., 2024). A primary concern is the extensive use of chemical inputs, which has led to adverse health effects for both farm workers and consumers, including increased risks of cancers and endocrine disorders (Akshaya *et al*., 2021). The interplay between these chemicals and soil fertility is complex; while they may boost short-term productivity, the long-term ramifications often include deteriorating soil health and compromised plant growth (Thirumalaivasan *et al*., 2024).

Prolonged reliance on chemical inputs has resulted in severe ecological consequences. Soil acidification, nutrient imbalances, depletion of organic matter, and water pollution are all linked to such practices. Furthermore, the development of resistance among pests and pathogens, detrimental effects on soil flora and microbial communities, and heavy metal contamination have emerged as critical issues. These factors contribute to reduced soil fertility and agricultural productivity, leading to bioaccumulation and biomagnification through the food chain (Dhanushkodi *et al*., 2024).

A multifaceted approach is essential to mitigate these challenges and improve agricultural resilience. This includes the adoption of biofertilizers and biopesticides, which utilize beneficial micro-organisms to enhance soil fertility and pest management (Vadakkan *et al*., 2024). Conservation agriculture practices that emphasize minimal soil disturbance, crop rotation, and the maintenance of ecosystem health can also play a vital role in sustaining agricultural productivity (Lal *et al*., 2023). Organic farming practices stand out in this regard by avoiding synthetic chemicals altogether, promoting biodiversity, enhancing soil health, and supporting the ecological balance within agricultural systems (Minhas *et al*., 2017).

Organic farming, in particular, has gained traction as a holistic agricultural strategy that yields numerous benefits for environmental sustainability and human well-being (Rani *et al*., 2023). By avoiding synthetic fertilizers and pesticides, organic practices contribute to

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healthier soils and reduce environmental pollution. They foster natural pest control mechanisms through enhanced biodiversity, leading to ecosystems that are more resilient and capable of self-regulation. Furthermore, organic farming provides food free from harmful chemical residues, which is critical for safeguarding human health and fostering local ecosystems (Prabhu *et al*., 2024).

The synergistic relationship between organic farming and PGPB exemplifies a holistic approach to sustainable agriculture and highlights the interconnectedness of soil health, ecosystem resilience, and human prosperity (Seetharamu *et al*., 2023). Recognizing the potential of these microorganisms to improve agricultural practices, this study aims to isolate and characterize PGP bacteria from organic fertilized soils and compare their capabilities with those from inorganic fertilized soils. By evaluating the PGPB traits of these isolates, along with their potential for enhancing seed germination efficiency and their ecotoxicity and cytotoxicity profiles, this research aims to contribute to the development of effective strategies for sustainable agriculture and soil health management.

2. Materials and Methods

2.1 Sampling locations

Soil samples (bulk soil), were collected in December 2022, from the cultivated fields of Vallipuram (11.2209° N, 78.1020°E), Mohanur (11.0604°N, 78.1388°E), Rasipuram (11.4615°N, 78.1855°E), Paramathivelur (11.1121° N, 78.0039°E) and Kabilarmalai (11.1467°N, 77.9440°E), villages of Namakkal District, Tamil Nadu, India. Both organic and inorganic bulk soil samples were collected from agricultural fields at the specified sampling sites. A total of ten soil samples (n=10) were obtained, with two samples (n=2) from each village, one from an organically fertilized field and another from an inorganically fertilized field. The designation of fields as organic was confirmed by the landowner consent based on the fact that only organic farming practices had been employed in the area for the past five years. Soil samples were taken aseptically at a depth of 30 cm using sterile polypropylene bags. They were immediately transported to the laboratory under refrigerated conditions (4°C) and stored at -20°C until further analysis. Triplicate samples were randomly collected from each site and pooled for analysis, following the method described by Gao *et al*. (2019).

2.2 Isolation of bacteria from soil samples

The initial inoculum, consisting of 1 g of soil sample, was aseptically transferred into 100 ml of physiological saline. A series of five 10-fold serial dilutions was then prepared using physiological saline. From each dilution, 1 ml and 01. ml of the aliquot was inoculated onto Nutrient Agar (NA) plates using the pour and spread method, respectively, with each sample plated in triplicate. The plates have been incubated for 24 to 48 h at 28 ± 2°C, as per Li *et al*. (2021). Based on unique morphological traits, bacterial colonies were separated and kept separately on NA slants at 4°C to facilitate further identification. Physical traits and biochemical testing were used to identify the isolates, following the guidelines provided in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

2.3 Qualitative assessment of PGP traits in bacterial isolates

Following the previous trials, all bacterial isolates (27 from inorganic soil and 33 from organic soil) were qualitatively assessed for a range of plant growth-promoting (PGP) characteristics. The most promising isolates from both soil types were then identified for further study.

2.3.1 Indole acetic acid (IAA) production

The method described by Sheng *et al*. (2008) was used to measure the IAA production by the bacterial isolates. The isolates were cultivated for 7 days at 28°C with constant shaking at 125 rpm in a nutrient broth enriched with 0.2% L-tryptophan. The cultures were centrifuged for 20 min at 10,000 rpm after incubation. After mixing 1 ml of the supernatant with 2 ml of Salkowski's reagent and incubating for 30 min in the dark, IAA production was detected. The mixture started to become pink, which was an indication of IAA formation.

2.3.2 Ammonia

Peptone water was used to assess the bacterial isolates' ability to produce ammonia (NH_3). Upon inoculating 10 ml of peptone water into each tube, freshly produced cultures were cultured for 48-78 h at 28°C. Following incubation, each tube received 0.5 ml of Nessler's reagent resulting inthe formation of a brown-to-yellow color suggesting a favorable outcome for ammonia synthesis.

2.3.3 Hydrogen cyanide

Hydrogen cyanide (HCN) generation has been monitored on every bacterial isolate. Nutrient Agar (NA) plates enriched with 1.4 g/l glycine were streaked with the cultures. Whatman No. 1 filter paper strips inside each Petri dish cover after they had been soaked in 0.5% picric acid and 2% sodium carbonate. Following a 4-day incubation period at 28°C, the plates were parafilm-sealed. The filter paper strips were incubated according to Kumar *et al*. (2020), and color changes were observed to determine HCN generation. The color changes ranged from yellow (-) to light brown (++), brown (+++), and dark brown (++++).

2.3.4 Protease

According to Gaur *et al*. (2014), skim milk agar (SMA) plates were used to test all bacterial isolates for proteolytic activity. The isolates were placed on SMA plates and allowed to incubate for 3 days at 28°C. The development of a clear zone around the bacterial colonies served as a sign of protease activity.

2.3.5 Phosphate solubilization (PS)

Pikovskaya's agar plates containing 2% tri-calcium phosphate were used to inoculate the bacterial cultures to measure the solubility of insoluble phosphate. The plates were incubated for seven days at 28°C. The formation of a clear zone around the bacterial colonies was indicative of phosphate solubilization, as reported by (Lelapalli *et al*., 2021).

2.3.6 Nitrogen fixing capacity (NF)

Bromothymol blue (BTB) was used as an indicator to qualitatively evaluate the nitrogen fixation by the bacterial isolates on Norris Glucose Nitrogen Free Medium (NGNFM) agar. For 7 days, the agar plates were incubated at 28°C after the isolates were inoculatedinto them. Following that, using the protocol described by Bashir *et al*. (2013), the color shift of the bacterial colonies was documented.

2.3.7 Cellulase

To evaluate cellulolytic activity qualitatively, the isolated bacteria were cultured in carboxymethyl cellulose (CMC) peptone broth overnight before being streaked onto CMC agar plates. Following an overnight incubation period, the CMC agar plates were immersed in a 0.1% Congo red solution and shaken intermittently for 15 min. A 1M NaCl solution was used to wash the plates after they had been rinsed with water and the bacterial colonies' appearance of a clearing zone suggested cellulolytic activity.

2.3.8 Amylase

On starch agar plates, the separated bacterial colonies were cultivated and incubated for 72 h at 28°C. 3 ml of 1% iodine solution was then added to each plate after incubation, the colonies' produced clean zone suggested the production of amylase.

2.3.9 Siderophore (SP)

Each isolate was inoculated into 20 ml of nutrition broth (NB), and the mixture was agitated for 48 h at 30°C and 200 rpm. Following incubation, 1 ml of the bacterial solution was centrifuged, cleaned with sterile deionized water, and then reconstituted in 200 µl of sterile deionized water. 10 µl of this suspension was added onto a chrome azurol S(CAS) plate. Iron carriers were detected by a color shift from blue to pale orange.

2.4 Evaluation of the potential isolates for further PGP traits

Ecotoxicity, cytotoxicity, and seed germination were evaluated for the promising isolates found in the screening studies.

2.4.1 Seed germination test

For the seed germination test, unprimed bean seeds were used. After two min of surface sterilization with a 2% sodium hypochlorite solution, the seeds were placedin a min of 70% ethanol. The seeds were then immersed for 30 min in a bacterial solution (10^{-7} CFU/ml) after being sterilized, with sterile distilled water serving as the control. The seeds were soaked, then taken out of the solution and put on autoclaved Whatman filter paper No. 1. Inside a Petri dish, the paper was wet with sterile distilled water. Twenty seeds were present in triplicate on each petri plate. For 7 days, the bean seed plates were incubated at 25 ± 2°C. The seeds were observed throughout the incubation period, and Konappa *et al*. (2020) technique was used to compute the germination index or percentage of germination.

2.4.2 Cytotoxicity

About 50 µl aliquots of Vero cell suspension (3 x 10^5 cells/ml) in growth media were transferred onto 96-well microplates, and they were incubated at 37°C for 24 h. Separate additions of the putative plant growth-promoting (PGP) isolates were made at serial doses of 100, 50, 25, 12.5, and 6.25 µg/ml. After that, the plates were incubated for 24 h at 37°C in a 5% CO_2 environment. After that, the cells were treated with 50 µl/well of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) for 1.5 to 3 h. Following incubation, the medium was withdrawn, and each well received 100 µl of DMSO to dissolve the insoluble purple formazan crystals. This was done after thoroughly mixing each well. Every experiment was carried out in triplicate, and the optical density (OD) of each well was measured at 570 nm using a microplate reader to determine the vitality of the cells. Negative control was provided by a 0.5% DMSO solution. Additionally, an inverted phase contrast microscope was used to investigate morphological changes in Vero cells during a 24 h incubation period with the tested doses (Freshney, 2015). The following formula was used to determine cell survival:

Cell viability % = (Test OD/Control OD) × 100

Cytotoxicity % = 100 – Viability %

2.4.3 Ecotoxicity

The toxicity study on earthworms was conducted according to the acute toxicity method outlined by the OECD (Organization for Economic Co-operation and Development) Guideline207 (1984). Well-developed clitellate adult worms (*Eisenia foetida*) were selected, fasted for 3 h, and then washed and dried before testing. The earthworms were placed in containers lined with sterile Whatman filter paper No. 1, which was impregnated with 1 ml of each potential isolate. The containers were incubated in the dark at 20 ± 1°C for 72 h, with sterile deionized water serving as the control. Morphological and behavioral changes, as well as weight and mortality rates, were monitored and photographed every 24 h.

2.5 Molecular identification of the potential isolate

Among the two strains tested in the above experiments, one isolated from organic soil and the other from inorganic soil the unknown strain from the organic soil exhibited greater efficiency. This strain was identified at the species level through 16S rRNA gene sequencing and phylogenetic analysis. The analysis was conducted at Barcode Bioscience in Bangalore, Karnataka, using the Sanger 16S rRNA sequencing method (Chen *et al*., 2014) and MEGA X software for phylogenetic analysis (Kumar *et al*., 2018).

2.6 Chi-square analysis

Statistical analysis of data on the PGP characteristics between the groups was performed using SPSS Version 23.0. Descriptive analysis involved frequencies and percentages for categorical variables. Inferential analysis included the Chi-square test for categorical data, which analysed the relationship between soil type and their PGP traits, with a significance level set at $p< 0.05$.

3. Results

3.1 Isolation and identification of bacterial strains

A total of 10 soil samples, 5 each from organic and inorganic fertilized soils, were collected and subjected to bacterial isolation. Among these samples, 33 bacterial strains were isolated from organic soil samples, while 27 were from inorganic soil samples. Among the 33 organic soil isolates, 7 different bacterial genera were identified by biochemical test. *Bacillus* sp. was found to be predominant (39.39%), followed by *Pseudomonas* sp. (18.18%), *Rhizobium* sp. and *Azotobacter* sp. (15.15%), *Azospirillum* sp. (6.06%), and *Klebsiella* sp. (3.03%). However, a single isolate, which was not confirmed through biochemical tests, was labeled as unidentified, accounting for 3.03%.

3.2 Molecular identification of the isolate

Utilizing 16S rRNA sequencing for molecular identification, the strain identified as *A. faecalis* was shown to have a 98% identity to strain KT748638.1 (Figure 1). This strain was derived from organic soil.

By demonstrating a firmlinkage with other beneficial bacteria, a phylogenetic study provided further support for this diagnosis. In line with the established roles of related strains in boosting soil fertility and improving plant health through nitrogen fixation, phosphate solubilization, and biocontrol of plant pathogens, this close phylogenetic relationship implies that *A. faecalis* may have a major role in promoting plant growth. This research demonstrates how it may be used in biotechnology and sustainable agriculture.

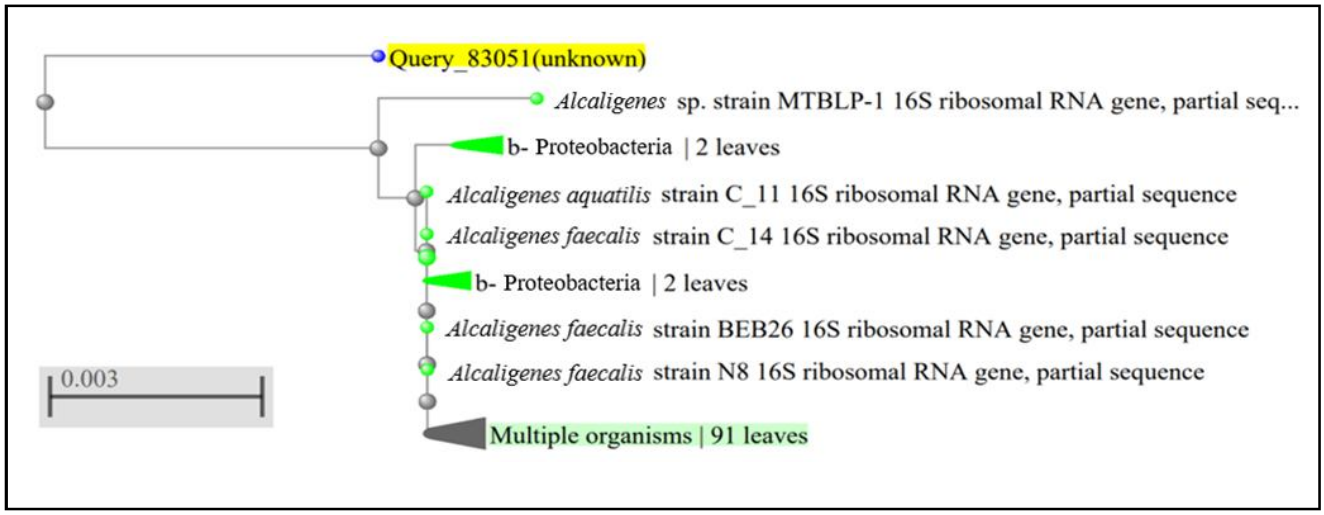

Figure 1: Phylogenetic analysis of *A. faecalis*.

3.3 Assessment of plant growth-promoting traits in organic soil isolates

In the present study, 9 PGP traits were investigated. About the 33 bacterial strains isolated from organic soil, NH_3 production, PS, and cellulase activity were recorded 100% in *Klebsiella* sp., *Azospirillum* sp., and the unidentified isolate. The findings demonstrated that the unidentified isolate was found to be positive for all the PGP traits, recording 100% activity. *Klebsiella* sp. exhibited the second most PGP traits accounting for 55.56%, followed by *Azospirillum* sp. (50%), *Bacillus* sp. (47.81%), *Rhizobium* sp. (40%), *Pseudomonas* sp. (38.86%), and the least was recorded by *Azotobacter* sp. (37.78%).

3.4 Unique traits of the isolate

The isolate distinguished itself by demonstrating increased activity across many biochemical markers, one of its distinctive and adaptable features that promote plant development. Figure 2 shows that there was a notable rise in the generation of siderophore production, ammonia (NH_3), hydrogen cyanide (HCN), and indole-3-acetic acid (IAA). Enzymatic activities were also increased. These characteristics point to its possible use in agricultural applications by indicating its strong capacity to stimulate plant development via processes such as nutrient solubilization, pathogen suppression, and improved root growth.

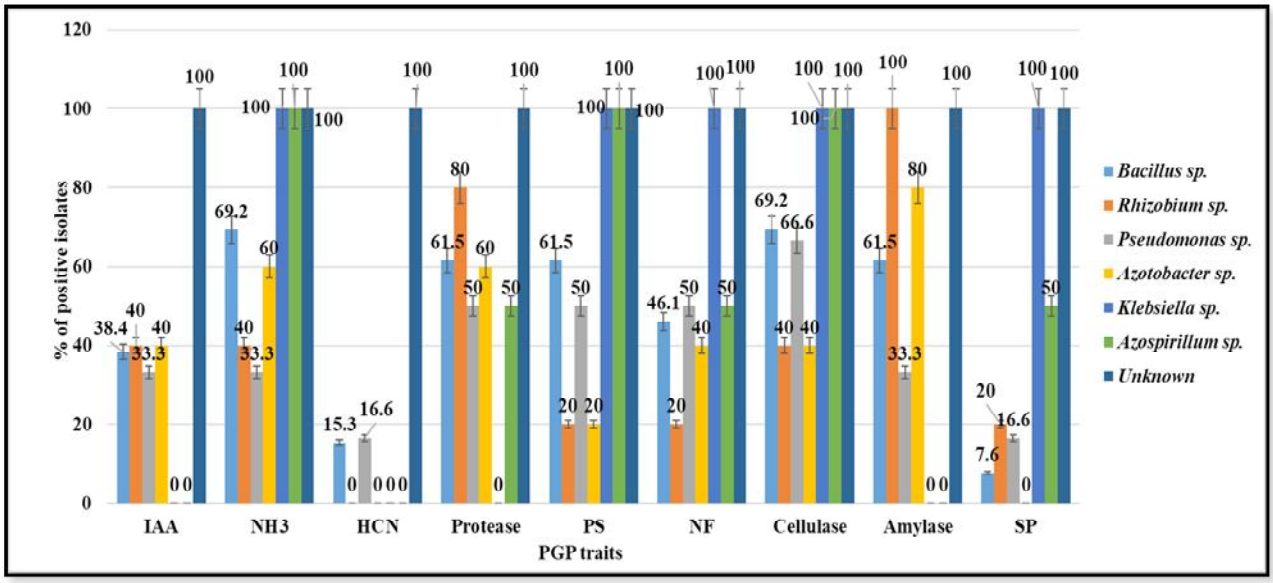

Figure 2: Identification of PGP traits of bacterial strains isolated from organic soil.

3.5 Bacterial profiles in inorganic soil isolates

The present investigation uncovered a varied bacterial composition by the isolation of plant growth-promoting bacteria (PGPB) from chemically fertilized (inorganic) soils. Biochemical studies revealed six bacterial genera among the 27 isolated strains. With 40% of the isolates, *Pseudomonas* sp. was the most common strain. It was closely followed by *Rhizobium* sp. (38.89%), *Azotobacter* sp. (37.02%), *Azospirillum* sp. (33.33%), *Bacillus* sp. (32.25%), and *Flavobacterium* sp. (22.22%). There are variations in the microbial communities of organic and inorganic agricultural systems, as seen by this distribution's contrast with the bacterial profiles found in organic soils, where *Pseudomonas* sp. dominance was less noticeable.

3.6 Enzymatic capabilities of inorganic soil isolates

Among the 6 genera, *Bacillus* sp. displayed versatile enzymatic capabilities with moderate IAA production (44%), substantial NH_3 production (55.5%), and notable protease activity (44.4%). *Rhizobium* sp. exhibited exceptional IAA production (100%) and cellulase activity (100%), while *Pseudomonas* sp. displayed varied enzymatic activities, including IAA (70%), NH_3 (20%), protease (50%), cellulase (50%), and amylase (50%). *Azotobacter* sp. displayed significant NH_3 production (66.6%) and cellulase activity (100%). Conversely, *Flavobacterium* sp. demonstrated 100% IAA and HCN production, and *Azospirillum* sp. exhibited a balanced enzymatic profile (IAA and NH_3-50%, protease-100%, cellulase and amylase-50%) (Figure 3).

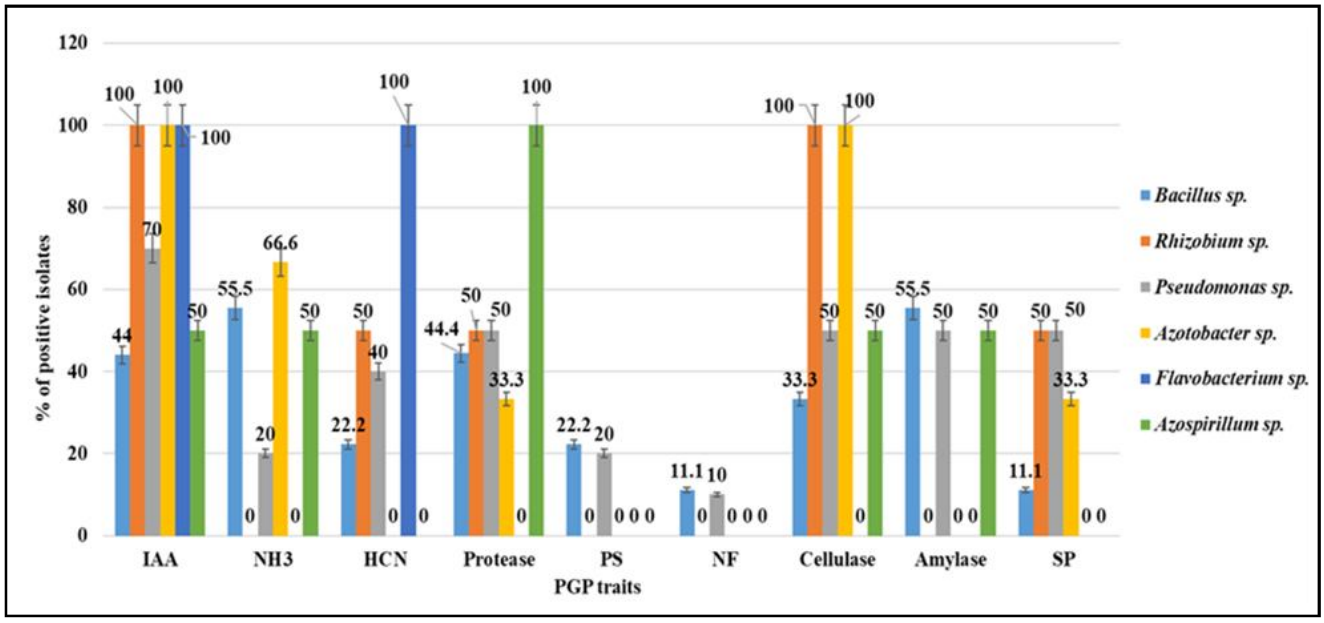

Figure 3: Identification of PGP traits of bacterial strains isolated from inorganic soil.

3.7 Comparison of PGP traits in organic and inorganic soil isolates

Pseudomonas sp., accounting for 38.75% of all isolates, was found to be the most prevalent bacterial strain recovered from inorganic soil. After following *Flavobacterium* sp., this had the lowest prevalence at 25%, *Bacillus* sp. (36.03%), *Rhizobium* sp., *Azotobacter* sp., and *Azospirillum* sp. (each representing 37.5%). The bacterial strains from inorganic soil samples showed less plant growth-promoting (PGP) characteristics than those from organic soil samples; *Pseudomonas* sp. had the highest PGP activity at 40%. As an illustration of the higher microbial functioning of organic soil in supporting beneficial bacteria for plant development, the unidentified strain from organic soil showed 100% PGP activity.

3.8 Seed germination and cytotoxicity assessment

The seed germination experiment revealed that the unidentified isolate from organic soil achieved anotable 85% germination rate, compared to 60% for *Pseudomonas* sp. and only 30% in the control (Figure 4). Cytotoxicity assessments using Vero cell lines demonstrated that the unidentified organic isolate exhibited minimal cytotoxicity, with viability of 95.8% at 6.25 µg, while *Pseudomonas* sp. displayed 81.6% viability (Figure 5). The low cytotoxicity of the unidentified isolate positions it as a safer option for agricultural applications.

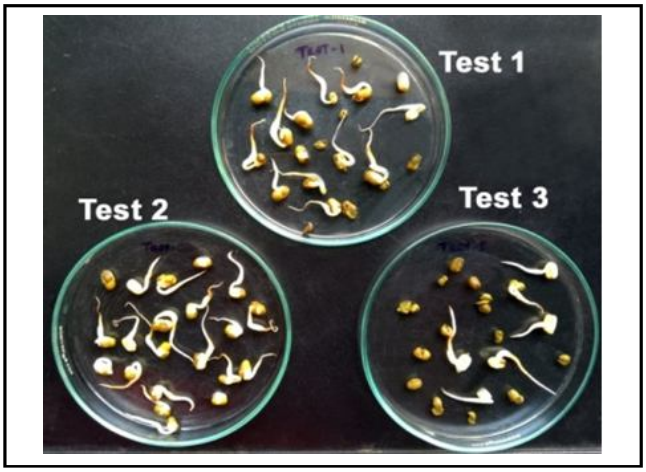

Figure 4: Determination of efficacy of organic and inorganic soil isolates on seed germination test (Green beans), Test 1- Green beans were treated with chemical fertilizer soil's isolate; Test 2 - Green beans were treated with organic soil isolate was tested for seed germination; Test 3 - Control without any treated isolate.

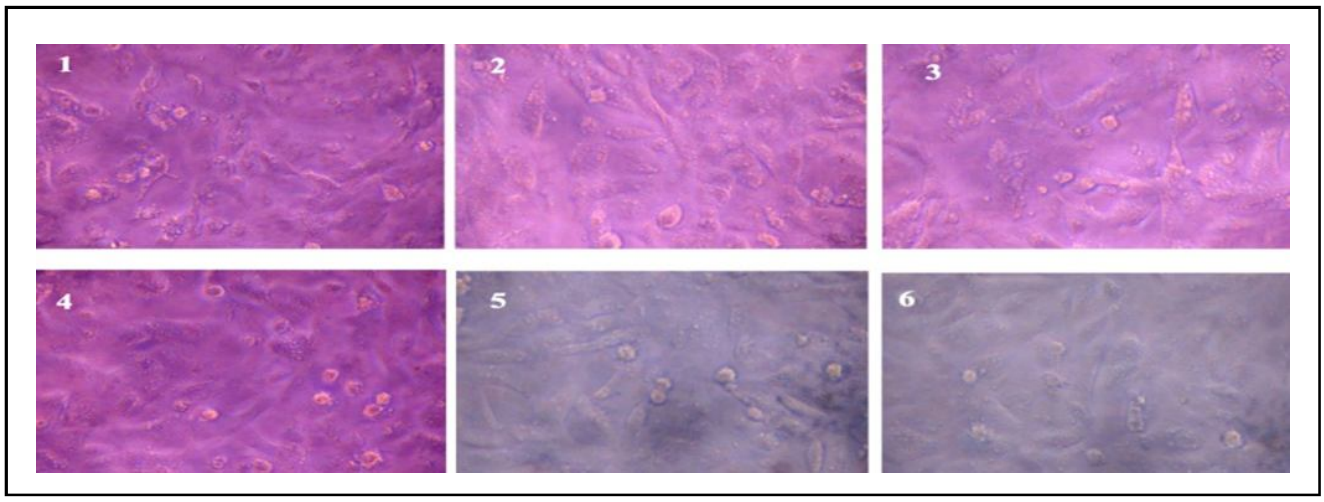

Figure 5: Determination of cytotoxicity of PGPR isolates on VERO cell lines. Plate 1 and 2 VERO cell line treated with organic soil isolate's cell (unknown) (1:100 µg, 2:6.25 µg); Plate 3 and 4. VERO cell line treated with inorganic soil isolate (*Pseudomonas* sp.), (3:100 µg, 4:6.25 µg); Plate 5. VERO Control cells; Plate 6. VERO cells with DMSO control.

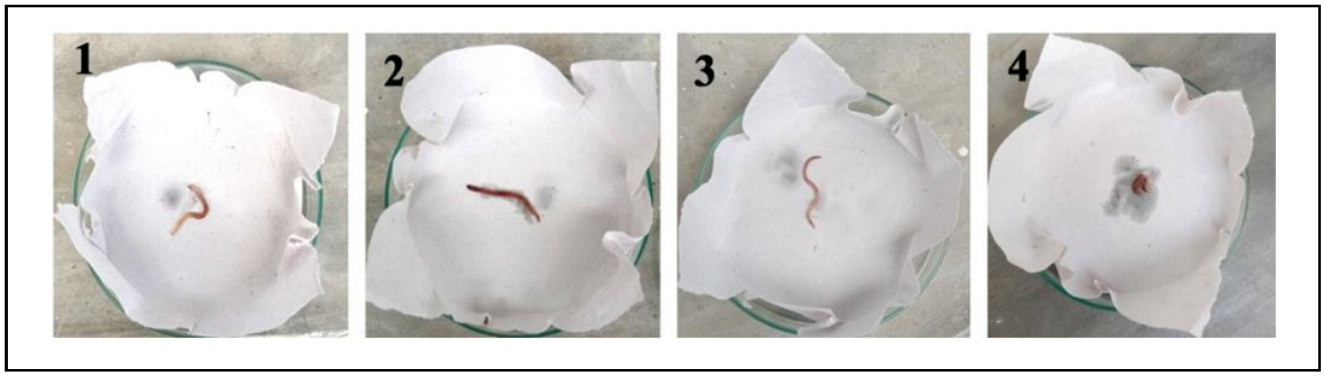

Figure 6: Determination of acute toxicity of PGPR isolates in earthworms (*Eisenia foetida*) 1. Earthworms treated with organic soil isolate (Unknown isolate), 2. Earthworms treated with chemical fertilizer used soil isolate (*Pseudomonas* sp.), 3. Control (Without being treated with isolate), 4. After 48 h, the formation of coiling while increasing the concentration of chemical fertilizer used soil isolate.

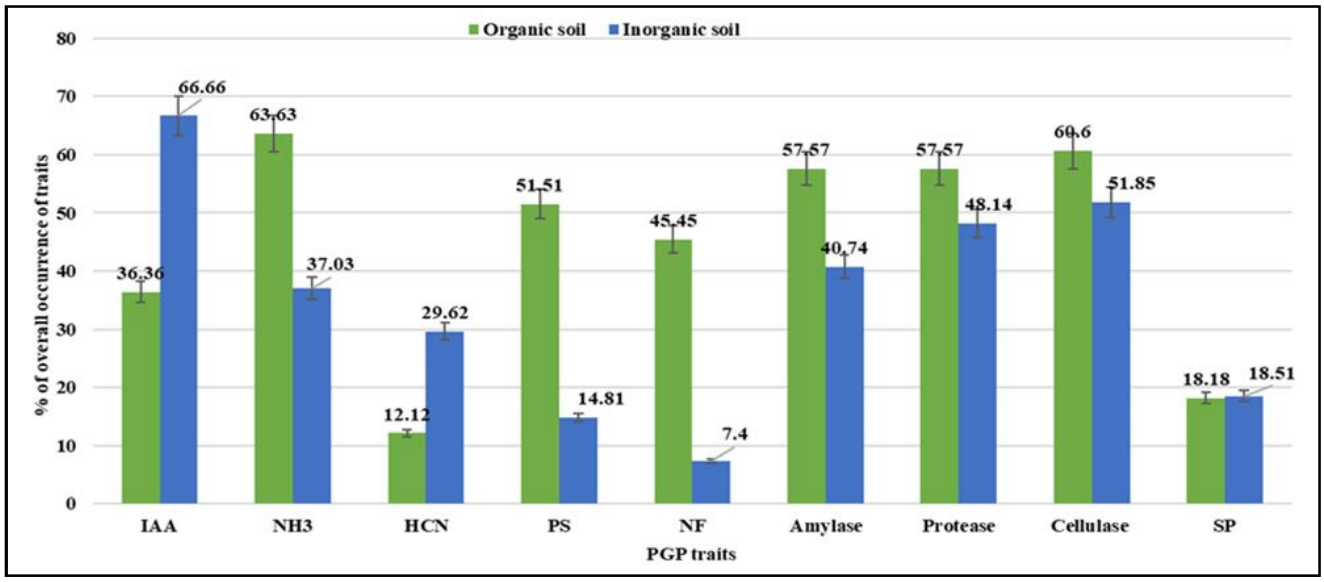

Figure 7: Percentage of overall occurrence of PGP traits in organic and inorganic soil groups.

Table 1: Comparative analysis of microbial characteristics in organic and inorganic soil using Chi-square test

PGP traits	Present (+) /Absent (-)	Soil type and % of occurrence				χ^2 value	'p' value	Sig*
		Organic (n=33)		Inorganic (n=27)				
		n	%	n	%			
IAA	+	12	36.36	18	66.66	18.028	0.00002	S
	-	21	63.64	9	33.34			
NH_3	+	21	63.63	10	37.03	14.581	0.00013	S
	-	12	36.37	17	62.97			
HCN	+	4	12.12	8	29.62	9.764	0.00177	S
	-	29	87.88	19	70.38			
PS	+	17	51.51	4	14.81	29.308	<0.00001	S
	-	16	48.49	23	85.19			
NF	+	15	45.45	2	7.40	37.526	<0.00001	S
	-	18	54.55	25	92.6			
Amylase	+	19	57.57	11	40.74	5.7806	0.016204	S
	-	14	42.43	16	59.26			
Protease	+	19	57.57	13	48.14	2.007	0.156551	NS
	-	14	42.43	14	51.86			
Cellulase	+	20	60.6	14	51.85	1.647	0.199252	NS
	-	13	39.4	13	48.15			
SP	+	6	18.8	5	18.51	0	1	NS
	-	27	81.2	22	81.49			

n - Frequency, % - Percentage, *S - statistically significant (p<0.05), NS - statistically insignificant

3.9 Earthworm toxicity assessment

According to the toxicity evaluation of the possible isolates against the earthworm *Eisenia foetida*, *Pseudomonas* sp. displayed a greater effect on the worms than the unknown isolate from organic soil. *Pseudomonas* sp. produced coiling and mucous production in the earthworms after 48 h of exposure, but the organic soil isolate had very few negative effects (Figure 6). In contrast to the more detrimental effects shown by *Pseudomonas* sp., this shows that the unidentified organic soil isolate is less toxic and could be safer for use in ecologically sustainable applications.

3.10 Overall occurrence of PGP traits and Chi-square analysis

The bacterial isolate from the organic group exhibited a higher percentage of NH_3, PS, NF, amylase, protease, and cellulase compared to the inorganic group, indicating a pronounced association between organic soil and the occurrence of these attributes (Figure 7 and Table 1). Chi-square test of independence conducted to examine the relationship between the overall occurrence of various PGP traits in bacterial isolates and the soil groups (organic and inorganic). The resultswere statistically significant, with p-value < 0.05 with reference to IAA, NH_3, HCN, PS, NF, and amylase.Whereas, PGP traits such as protease, cellulase, and SP did not significantly differ between the groups.

4. Discussion

In this study, the efficiency and characteristics of plant growth-promoting bacteria (PGPB) isolated from inorganic and organic fertilized soils were compared and shown to vary significantly. When compared to bacterial isolates from inorganic soils, those from organic soils had greater plant growth-promoting (PGP) capacities, as seen by increased synthesis of siderophores, ammonia (NH*f*), hydrogen cyanide (HCN), and indole-3-acetic acid (IAA). These results are consistent with another study by Bonanomi *et al.* (2020) that shows how organic soils are beneficial for supporting microbial communities that improve plant development.

Results of the present study align with previous research particularly that conducted in the Namakkal region, where it has been shown that bacterial consortia promote plant growth (Sundaram *et al*., 2018). The similarities between our results and those reported by Sathya *et al.* (2024) from pepper-cultivated regions in the Namakkal region further illustrate the importance of microbial communities in organic farming systems.Additionally, studies conducted in other regions, like Sherpa *et al*. (2021) in Sikkim, indicate that organic soils are a rich source of a variety of advantageous bacterial strains. The PGP potential of *A. faecalis* is further highlighted by earlier studies by Divyanshu *et al*. (2022) and Jia *et al*. (2022), which corroborate our findings on the strain's function in promoting plant growth.

Taurian *et al*. (2010) discussed the significance of these features for enhancing plant nutrition, and our investigation reveals that the isolates from organic soil, especially *Pseudomonas* sp., possess the same capabilities. Similar results have been reported by Ahmad *et al*. (2008) and Sharma *et al*. (2011), who identified several PGP features in *Pseudomonas* species that originated from the rhizospheric soil of rice, wheat, and barley. This research indicates that organic soil conditions- which are often less modified by chemical fertilizers are more favorable for the establishment of practical PGP traits.

Conversely, *Pseudomonas* sp. from organic soil exhibited only 40% of the activity described in the unidentified isolate, suggesting that PGP properties were lower in bacterial isolates isolated from chemically fertilized (inorganic) soils. This trend was also seen by Reid *et al*. (2021) in their study of PGP fungus isolates from chemically treated soils. Microbial life may still exist in inorganic soils, but it seems that the diversity and functional potential of these communities are less abundant than in organic soils. These results corroborate a broader understanding that microbial diversity and functionality are often decreased in traditional agricultural systems that rely on chemical inputs.

Our findings provide new insights into the cytotoxicity of isolates of PGP. The cytotoxicity of microbial isolates *Pseudomonas* sp. in particular has been documented, but few studies have looked at the cytotoxic effects of PGPB on Vero cells, which makes our findings unique in this context.

Sharma *et al*. (2018) and Soltani *et al*. (2021) have documented microbial cytotoxicity against a range of cell lines; however, few studies have explicitly addressed Vero cells. Jeyaprakasam *et al*. (2021) reported that *Pseudomonas* sp. may be more hazardous because of its metabolic byproducts, based on their documentation of contact toxicity in related bacterial strains.

Toxicity studies conducted on *Eisenia foetida* also revealed significant differences between the isolates from organic and inorganic soils. According to this, the unidentified strain might not pose as much of an ecological risk in agricultural settings, which is consistent with the growing interest in microbial inoculants that promote plant growth without posing a threat to the environment (Rahnama *et al*., 2023). Our findings highlight how important it is to choose microbial strains that exhibit both high performance and minimal environmental toxicity.

According to Rahnama *et al*. (2023), crops treated with PGP isolates from organic sources had higher germination rates and more plant vigor. These isolates also perform better than those from chemically fertilized soils. Sherpa *et al*. (2021) described that, significantly compared to inorganic farming; organic treatment enhanced a greater number of specific bacterial taxa. The isolates from organic soils had a higher functional capacity and lower toxicity, which highlights the benefits of organic farming systems in boosting microbial diversity and plant-microbe interactions. Future research on the use of these beneficial bacteria in sustainable agriculture is warranted, with a focus on lowering environmental risks and boosting crop productivity.

5. Conclusion

In conclusion, this study highlights the potential of indigenous plant growth-promoting bacteria (PGPB) from organic and inorganic soils in sustainable agriculture. The findings indicate that PGPB from organic soils exhibit superior growth-promoting traits, enhancing plant growth and soil health. Additionally, the safety assessments of these isolates support their viability for agricultural use. Integrating PGPB into farming practices can improve nutrient availability and reduce reliance on synthetic inputs. This research reinforces the role of PGPB in fostering resilient and productive agricultural systems.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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