



RESEARCH ARTICLE

Plant growth-promoting rhizobacteria from vegetablecultivated soils in Namakkal district: Isolation, characterization and biocontrol potential

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Abstract

This study investigated plant growth promoting rhizobacteria (PGPR) isolated from soil samples collected across 5 villages in Namakkal district, a region known for cereal and vegetable cultivation, with the aim of evaluating their plant growth promoting (PGP) traits and biocontrol potential as sustainable alternatives to agrochemicals. A total of 21 bacterial isolates were obtained and screened for key PGP traits, including indole-3-acetic acid (IAA) and ammonia (NH₃) production, hydrogen cyanide (HCN) production, phosphate solubilization (PS), nitrogen fixation (NF) and enzymatic activities (amylase, protease and cellulase), while antagonistic activity against*Xanthomonas* sp. was also assessed. Identification by 16S rRNA sequencing revealed that an unclassified isolate belonged to *Bacillus paramycoides*, which exhibited all positive PGP traits and demonstrated strong antagonistic activity against*Xanthomonas* sp. Molecular docking further showed that IAA had a binding affinity of -6.6 kcal/mol with the *Xanthomonas* sp. (6K62) protein, indicating antimicrobial potential. These findings highlight the application of PGPR, particularly *B. paramycoides*, in improving soil fertility, enhancing plant growth and providing biocontrol against phytopathogens, thereby reducing reliance on chemical fertilizers and pesticides.

Keywords: antagonistic activity; Bacillus paramycoides; IAA; molecular docking; rhizosphere; Xanthomonas sp

Introduction

India is among the fastest growing countries globally in terms of population and economy. By 2050, the population is projected to reach 1.5 billion, with an estimated demand of nearly 300 million metric tonnes of food grains, almost double the current production. To meet this demand, agricultural productivity must significantly improve, yet expansion of cultivated land is constrained by various physical (soil texture, environmental (temperature, moisture) and social factors (agricultural practices, land-use patterns) (1). Consequently, farmers have become increasingly dependent on chemical fertilizers and pesticides, which, despite boosting yields, have caused adverse ecological impacts such as soil and groundwater contamination, loss of beneficial microbes, pest resistance and accumulation of residues in food crops that pose risks to human health (2, 3). Excessive reliance on synthetic pesticides has further contributed to environmental pollution and disruption of soil microbial balance, reinforcing the need for sustainable alternatives (4).

PGPR offer a promising eco-friendly strategy for sustainable agriculture by enhancing soil fertility, nutrient uptake and plant growth, while also suppressing phytopathogens (5, 6). The rhizosphere, a nutrient rich zone surrounding plant roots, harbors diverse microbial

communities sustained by plant root exudates, including sugars, amino acids, lipids and secondary metabolites. These microbes contribute directly through phytohormone synthesis (e.g., auxins, gibberellins), phosphorus and zinc solubilization and NF, or indirectly by producing antifungal metabolites such as antibiotics, hydrogen cyanide, siderophores and hydrolytic enzymes like cellulases, chitinases, proteases and amylases (7, 8). Genera such as Pseudomonas, Bacillus, Rhizobium and Azospirillum are widely recognized for their multifaceted plant growth promoting functions (9, 10). Additionally, PGPR enhance plant defense by inducing systemic resistance and stimulating defense-related enzymes such as peroxidase (POX), phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) (11). Synergistic use of multiple beneficial microbes has also been shown to provide broader-spectrum biocontrol and more consistent suppression of plant diseases (12, 13).

Although numerous studies have reported antagonistic activity of PGPR against phytopathogens, the underlying molecular mechanisms remain poorly understood. Computational approaches such as molecular docking provide valuable insights into ligand pathogen interactions by predicting binding affinities and active site orientations, thereby facilitating the identification of potential inhibitory mechanisms (14, 15).

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In this context, the present study focuses on the isolation, identify and characterization of PGPR from vegetable cultivated soils of Namakkal District, with particular emphasis on *Bacillus paramycoides*. This species demonstrated multiple PGP traits and antagonistic activity against *Xanthomonas* sp., a major phytopathogen responsible for bacterial spot in tomato, which threatens agricultural productivity and food security. We hypothesize that PGPR isolates, especially *B. paramycoides*, can simultaneously enhance plant growth and provide biocontrol against phytopathogens and that molecular docking of IAA against *Xanthomonas* sp. will help elucidate the mechanistic basis of inhibition.

Materials and Methods

Collection of soil samples

Soil samples were collected from 5 agricultural fields located in different villages of Namakkal District, Tamil Nadu, India (GPS coordinates: 11.2194° N, 78.1678° E). A single representative sample was obtained from each site (n = 5). Samples were collected aseptically in sterile polypropylene bags at a depth of 20 cm, a level chosen to target rhizosphere associated microbes while avoiding surface contaminants. The samples were transported to the laboratory at 4 °C and stored at -20 °C until further use. Prior to analysis, duplicate subsamples were randomly taken from each location to ensure representativeness.

Isolation of bacteria

One gram of soil was aseptically suspended in 100 mL of sterile physiological saline and serially diluted up to 10^5 . Aliquots (1 mL) from 10^3 and 10^5 dilutions were plated in triplicate on nutrient agar (NA) using the pour plate method, followed by incubation at 28 ± 2 °C for 24-48 hrs. Morphologically distinct colonies were purified and maintained on NA slants at 4 °C. Preliminary identification was carried out based on cultural and biochemical characteristics described in Bergey's Manual of Determinative Bacteriology (16).

Invitro screening of PGP traits

IAA production capability test

Isolates were inoculated into 5 mL sterile nutrient broth supplemented with L-tryptophan (3 mg/mL) and incubated at 28 °C for 5 days. After centrifugation (10000 rpm, 10 min), the supernatant was mixed with one drop of orthophosphoric acid and 2 mL of Salkowski's reagent (50 mL of 35 % perchloric acid + 1 mL of 0.5 M FeCl₃). Development of a pink color indicated IAA production (17).

NH₃ production capability test

Isolates were grown in peptone water at 35 ± 2 °C for 48 hrs. Following incubation, 0.5 mL of 0.5 % Nessler's reagent was added; a yellow to brown color confirmed NH₃ production (18).

HCN production capability test

Cultures were streaked on NA plates supplemented with glycine. Filter papers soaked in 0.5 % picric acid solution (in 2 % sodium carbonate) were placed in the lids and color change from orange to red indicated HCN production (19).

Nitrogen fixation capability test

Isolates were inoculated on nitrogen free minimal medium (NFMM) composed of (g/L): 1.0 K₂HPO₄, 1.0 CaCl₂, 0.5 NaCl, 0.25

MgSO₄·7H₂O, 0.01 FeSO₄·7H₂O, 0.01 Na₂MoO₄·2H₂O, 0.01 MnSO₄·5H₂O and glucose (7 g/L) as carbon source (pH 7.0). The medium was solidified with 2 % agar and contained 0.5 % bromothymol blue as an indicator. After 3-10 days, a color shift from green to blue signified nitrogen-fixing ability (20).

Phosphate solubilization capability test

Isolates were spot inoculated on modified Pikovskaya's agar containing tricalcium phosphate and incubated at 30 °C for 5-7 days. Clear halo zones around colonies indicated solubilization (20). The solubilization index (SI) was calculated as:

Enzyme production capability test

Protease activity was determined on skim milk agar, where clear zones around colonies after 48 hrs indicated proteolysis. Cellulase activity was assayed on carboxymethyl cellulose (CMC, 10 g/L) agar plates; clear halos after Congo red staining indicated cellulolytic activity. Amylase activity was evaluated on starch agar plates by flooding with iodine solution after incubation, with transparent halos denoting positive activity (21).

Molecular identification (16S rRNA sequencing)

Genomic DNA was extracted from selected isolates showing strong PGP traits. The 16S rRNA gene was amplified using universal primers, purified and sequenced commercially (Barcode Biosciences, Bengaluru, India). Sequences were analyzed using NCBI-BLAST, aligned using ClustalW 1.74 and a phylogenetic tree was constructed using MEGA 11 software to determine taxonomic affiliation.

Antagonistic activity against Xanthomonas sp.

The most promising isolate was cultured in LB broth at 30 °C for 72 hrs with shaking. Cell-free supernatants were obtained by centrifugation (10000 rpm, 10 min) and filtration through a 0.45 μ m membrane. Antibacterial activity was evaluated by the agar well diffusion method on Mueller Hinton agar plates inoculated with ~10° CFU/mL of *Xanthomonas* sp. Wells (0.6 cm) were filled with 50 μ L of filtrate and plates were incubated at 30 °C for 48 hrs. Zones of inhibition were measured to assess antagonism (20).

Molecular docking analysis

Molecular docking of IAA with *Xanthomonas* sp. receptor protein (PDB ID: 6K62) was carried out using PyRx 0.8 with AutoDock Vina. The receptor was energy-minimized and prepared by adding hydrogen atoms. Ligand structures were built and optimized using Chimera 1.16. Docking was performed using a defined grid box and binding affinities were calculated. Protein-ligand interactions were visualized with Flare Visualizer 9.0.0. Drug likeness was assessed using Lipinski's Rule of Five.

Statistical analysis

Data were analyzed using one-way ANOVA (Excel 2015) to evaluate differences in PGP traits among bacterial isolates. The sum of squares (SS), degrees of freedom (DF), mean squares (MS), F-values and P-values were calculated. A significance level of p < 0.05 was applied to determine statistical significance.

Results and Discussion

Soil sample collection

Soil microorganisms are widely recognized for their crucial role in plant health, nutrient cycling and overall crop productivity. The cultivated soils of Namakkal district, predominantly supporting cereals such as paddy, maize and cassava, along with cotton, sugarcane and vegetable crops, were selected for microbial isolation in this study. These agroecosystems are characterized by intensive use of fertilizers and pesticides to maintain high yields, which often deteriorates soil fertility and microbial balance. Under such conditions, PGPR provide an ecologically sustainable alternative by directly enhancing nutrient availability and indirectly suppressing phytopathogens through antagonistic activity. The rhizospheric soils from Namakkal harbored diverse PGPR with potential applications as biofertilizers, supporting sustainable agriculture under similar agroclimatic conditions (22).

Bacterial isolation from rhizospheric soil

A total of 21 bacterial isolates were recovered from soil samples collected across 5 villages of Namakkal. Based on biochemical assays, the isolates were classified into 6 genera: *Bacillus*, *Pseudomonas*, *Rhizobium*, *Azotobacter*, *Azospirillum* and one unclassified isolate. *Bacillus* sp. and *Azotobacter* sp. were predominant, in agreement with previous findings where *Bacillus* and *Azotobacter* are frequently recovered as dominant PGPR in cereal and vegetable soils due to their high survival adaptability in diverse environments (23, 24). The presence of multiple genera highlights the rhizosphere as a dynamic microbial habitat shaped by root exudates and nutrient availability.

Characterization of soil bacterial isolates for various PGP attributes

Production of IAA

Among the 21 isolates, 17 (80.9%) produced IAA, a key auxin known to regulate root elongation, lateral root initiation and plant biomass accumulation. The high frequency of IAA-producing isolates indicates that the Namakkal soils harbor bacteria capable of modulating root architecture and enhancing nutrient absorption efficiency. IAA-producing PGPR are known to stimulate adventitious root development, improve water uptake and enhance crop stress tolerance. Similar results were reported in early works, who observed that IAA producing PGPR significantly enhanced maize root length and shoot biomass under nutrient-limited conditions (25). The predominance of IAA producers in this study suggests that they could be valuable inoculants for crops cultivated in Namakkal, particularly in soils experiencing nutrient depletion due to agrochemical overuse.

Production of NH₃

Eight isolates (38.0 %) were positive for NH_3 production. NH_3 production is an important indirect PGP trait as it increases nitrogen availability in the rhizosphere and contributes to plant nutrition. Isolates that released NH_3 into the medium demonstrated their potential to act as supplementary nitrogen sources, supporting crop growth in nitrogen deficient soils. These findings are consistent with a study, which demonstrated that NH_3 producing PGPR improved nitrogen uptake and enhanced the growth of wheat under nitrogen limited conditions (26). The moderate frequency of NH_3 producers observed here indicates that while not all isolates contribute significantly to nitrogen

enrichment, selected strains could be utilized in biofertilizer formulations to complement synthetic nitrogen fertilizers.

Production of HCN

Two isolates (9.5 %) tested positive for HCN production. Although low in frequency, these isolates are important due to their potential role in biocontrol. HCN is a volatile compound that interferes with the electron transport chain of soilborne pathogens, thereby reducing disease incidence. Previous studies have emphasized the contribution of HCN-producing PGPR in suppressing pathogens such as *Fusarium oxysporum* and *Rhizoctonia solani* (27-29). In the present study, the recovery of HCN producers suggests a potential role in integrated biocontrol strategies, though their low prevalence indicates that they may be more effective when combined with other antagonistic traits.

Production of PS

PS was observed in 52.3 % of the isolates. The ability to solubilize tricalcium phosphate into plant-available forms is critical in agricultural soils, where phosphorus is often locked in insoluble complexes. Isolates exhibiting PS showed visible zones of clearance around colonies on Pikovskaya agar, indicating their role in enhancing phosphorus availability. These findings align with the work, that demonstrated that phosphate solubilizing PGPR not only increase phosphorus uptake but also improve crop yields in phosphate deficient soils (30). The moderate but significant proportion of phosphate-solubilizing bacteria in this study reinforces their potential use in replacing or reducing synthetic phosphorus fertilizers (31).

Production of nitrogen fixation activity

Approximately 23.8 % of isolates exhibited NF activity, confirmed by growth on nitrogen free minimal medium. NF is one of the most valuable PGP traits, contributing directly to soil fertility by converting atmospheric nitrogen into plant usable forms. In soils of Namakkal, where continuous cereal cultivation may lead to nitrogen depletion, these isolates could serve as natural biofertilizers. Comparable results were reported in early findings, who isolated nitrogen-fixing PGPR such as *Rhizobium*, *Azospirillum* and *Azotobacter* from cultivated soils, noting their contribution to enhanced crop nitrogen nutrition (32).

Production of Siderophore

Siderophore production was noted in 19 % of isolates, which reflects their ability to chelate ferric iron and enhance its availability to plants while simultaneously restricting pathogen growth by depriving them of iron. This dual role of siderophores in nutrient acquisition and pathogen suppression has been reported (33), who demonstrated that siderophore-producing PGPR improved tomato seedling vigor and reduced incidence of phytopathogenic fungi. Though fewer isolates in this study displayed siderophore production, their integration into microbial consortia could strengthen overall biocontrol efficiency.

Production of enzymatic activity

A considerable proportion of isolates exhibited extracellular enzymatic activities. Amylase activity was recorded in 76.1 % of isolates, protease activity in 38 % and cellulase activity in 38 %. Enzymes such as amylase, protease and cellulase contribute to organic matter degradation, nutrient mineralization and pathogen suppression by degrading cell walls of fungi and other pathogens. These findings are comparable to a previous

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report, which documented high enzymatic diversity among PGPR isolates from vegetable cultivated soils in Tamil Nadu (10). The high prevalence of amylase activity in this study suggests that the isolates contribute significantly to soil organic matter turnover, while protease and cellulase producers likely enhance nutrient cycling and disease suppression.

Data analysis

The single factor ANOVA confirmed significant differences among isolates in the expression of key PGP traits. Significant variations were observed for IAA production (F = 129.050, P < 0.0001), amylase (F = 83.251, P < 0.0001), protease (F = 83.251, P < 0.0001), PS (F = 24.667, P < 0.001), NH $_3$ production (F = 8.227, P < 0.05) and cellulase activity (F = 12.774, P < 0.05). However, no significant variation was observed for HCN production (F = 1.422, P = 0.256), NF (F = 3.7294, P = 0.077) and siderophore production (F = 3.0415, P = 0.107). These results indicate that traits such as IAA and amylase are consistently expressed across diverse isolates, while traits like HCN and siderophore production are more sporadic. The identical F-values observed for amylase and protease reflect the dataset structure and confirm that both traits showed comparable levels of inter-isolate variability (Table 1).

Amplification of 16S rRNA of bacterial isolates

One isolate exhibiting all PGP traits was subjected to 16S rRNA sequencing. BLAST analysis revealed 99.5 % similarity to *Bacillus paramycoides* (Accession number: MT373523.1) and phylogenetic analysis confirmed its close relationship with this species. *Bacillus paramycoides* has been increasingly reported as a promising PGPR with diverse beneficial activities, including IAA production, phosphate solubilization and biocontrol potential (34). The identification of this isolate adds to the growing recognition of *Bacillus paramycoides* as a candidate for biofertilizer development.

Antibacterial activity against plant pathogen

The selected *Bacillus paramycoides* isolate demonstrated strong antagonistic activity against *Xanthomonas* sp., a major tomato pathogen. Ethyl acetate extracts at 5 mg concentration produced a 12 mm inhibition zone, surpassing the standard antibiotic streptomycin. This suggests that *Bacillus paramycoides* secretes bioactive compounds with potent antibacterial effects. Similar antagonistic effects were reported, where *Bacillus* isolates inhibited phytopathogenic fungi through the secretion of lipopeptides and polyketides (35). The results here highlight the dual role of *Bacillus paramycoides* as both a growth promoter and a biocontrol agent, making it highly suitable for integrated pest management strategies.

Molecular docking studies

To elucidate the mechanism underlying the observed antagonism, molecular docking was performed using IAA against Xanthomonas sp. protein (PDB ID: 6K62). The docking study revealed a binding affinity of -6.6 kcal/mol (Table 2), with key interactions at amino acid residues VAL 41, GLY 45, VAL 48, ARG 56, VAL 175 and THR 193 (Fig. 1-3). These interactions suggest that IAA interferes with the CHASE domain of the PcrK protein, which is responsible for cytokinin sensing and virulence regulation in Xanthomonas. By targeting this receptor, IAA potentially suppresses the pathogen's ability to recognize host signals, thereby reducing its virulence. Similar docking based insights were reported, demonstrating that plant derived phytohormones can inhibit bacterial signaling proteins and reduce pathogenicity (36). In addition, it was shown through molecular docking that plant hormones naringenin and abscisic acid (ABA) strongly interact with crucial pathogenic enzymes of Phytophthora infestans, such as chitin synthase and calmodulin, suggesting their inhibitory potential at the molecular level (37). The finding that IAA adheres to Lipinski's Rule of Five

Table 1. ANOVA - Single factor for the data on different PGP traits as a function of variation between different PGPB

Source of variation	Sum of squares (SS)	Degrees of freedom (DF)	Mean square (MS)	F-value	P-value
		Indole acetic	acid		
Between groups	23044.571	1	23044.571	129.050	P < 0.0001*
Within groups	2142.857	12	178.571	129.030	P < 0.0001
		Ammonia			
Between groups	7223.143	1	7223.143	0.227	P <0.05*
Within groups	10535.714	12	877.976	8.227	P <0.05
		Hydrogen cya	nide		
Between groups	994.571	1	994.571	1.422 0.	0.250
Within groups	8392.857	12	699.405		0.256
		Phosphate solubi	lization		
Between groups	12480.286	1	12480.286	24.667	P < 0.001*
Within groups	6071.429	12	505.952		
		Nitrogen fixat	tion		
Between groups	4217.786	1	4217.786	3.7294	0.077
Within groups	13571.429	12	1130.952		
		Siderophore prod	luction		
Between groups	3394.571	1	3394.571	3.0415	0.107
Within groups	13392.857	12	1116.071		0.107
		Amylase			
Between groups	21060.643	1	21060.643	83.251	P < 0.0001*
Within groups	3035.714	12	252.976		P < 0.0001
		Protease			
Between groups	21060.643	1	21060.643	02.251	P < 0.0001*
Within groups	3035.714	12	252.976	83.251	P < 0.0001"
		Cellulase			
Between groups	7223.143	1	7223.143	12.774 P < 0.05	D <0.05*
Within groups	6785.714	12	565.476		P <0.05"

Table 2. Results of molecular drug docking using PyRx - Autodock vina 1.5.6 tool and interaction active site region of protein *Xanthomonas* sp. (6K62)

Ligand	Binding affinity	Active site region
Indole-3-acetic acid (IAA)	-6.6 kcal/mol	VAL 41; VAL 175; GLY 45; THR 193; VAL 48; ARG 56

further strengthens its potential as a bioactive compound with drug like properties.

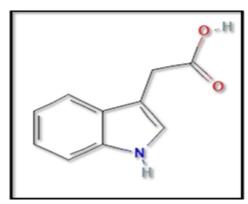


Fig. 1. 2D structure of (A) Indole-3-acetic acid (IAA).

Conclusion

This study underscores the significance of PGPR in promoting sustainable agriculture under the intensive cultivation systems of

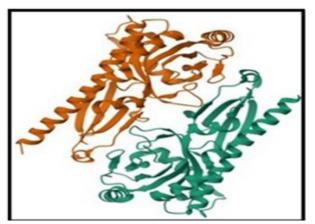


Fig. 2. Protein 3D structure of (6K62) crystal structure of Xanthomonas sp.

Namakkal. From 21 isolates, *Rhizobium* sp. and *Bacillus* sp. demonstrated strong PGP traits, while an unidentified isolate was confirmed as *Bacillus paramycoides* through 16S rRNA sequencing. Notably, *B. paramycoides* exhibited broad spectrum growth promoting attributes and pronounced antagonistic activity against *Xanthomonas* sp. Molecular docking of IAA with the 6K62 protein further revealed a binding affinity of -6.6 kcal/mol, supporting its role in pathogen inhibition. These findings highlight the potential of *B. paramycoides* as a biofertilizer and biocontrol agent, offering an eco-friendly alternative to chemical inputs. Future research should validate its efficacy under greenhouse and field conditions to facilitate large scale application in sustainable crop production systems.

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Authors' contributions

All the authors have made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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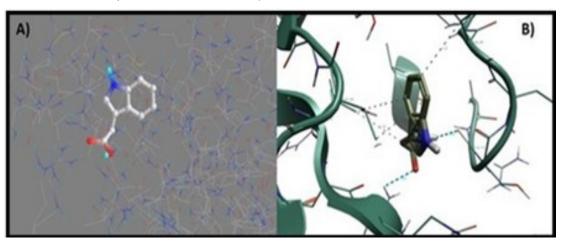


Fig. 3. A) 3D structure of receptor *Xanthomonas* sp (6K62) with Indole-3-acetic acid (IAA) compound docking using (PyRx - Autodock vina 1.5.6 tool) and B) Interaction visualize in (Flare Visualizer 9.0.0).

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