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Functional role of *Pseudomonas* rhizobacteria in enhancing plant growth under stress-adaptive agricultural systems

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ABSTRACT

This study explores the functional traits of Pseudomonas spp. isolated from rhizospheric soils and their potential as plant growth-promoting rhizobacteria (PGPR). Four isolates were selected and characterized based on morphological, physiological, and biochemical parameters. The results demonstrated their tolerance to various abiotic stresses, resistance to heavy metals and pesticides, and ability to produce plant-beneficial compounds, including siderophores, hydrogen cyanide, and indole-3-acetic acid. Although certain PGPR traits were absent, the overall profile indicates the potential utility of these isolates in sustainable agriculture and stress-prone environments.

Keywords: *Pseudomonas spp., plant growth-promoting rhizobacteria, stress tolerance, siderophores, sustainable agriculture.*

INTRODUCTION

The dynamic relationship between soil microbiota, crop productivity, and ecological sustainability has become a focal point in modern agricultural research, driven by the global imperative to enhance food production while preserving environmental quality. Soil serves as a physical substrate for plant growth and a critical reservoir of microbial diversity, integral to nutrient cycling, plant health, and resilience to biotic and abiotic stresses.

Conventional agricultural systems, which rely heavily on chemical fertilizers and pesticides, have contributed to soil degradation, loss of microbial biodiversity, and an increased vulnerability of crops to environmental fluctuations (Tilman, 1999; Gomiero, 2016; Nadarajah & Abdul Rahman, 2023). The overuse of agrochemicals has been linked to nutrient imbalances, reduced plant vigor, contamination of water resources, and growing public health concerns (Pešaković et al., 2023; Pešaković, 2024). In response, there is a growing movement toward integrating more sustainable agricultural practices, particularly through the use of beneficial microorganisms that support the ecological intensification of agriculture. One of the most promising strategies within this framework is the application of plant growth-promoting rhizobacteria (PGPR), which has gained significant attention as an eco-friendly approach to enhance crop yield and improve stress tolerance.

PGPR are a diverse group of soil-dwelling bacteria that colonize the rhizosphere, promoting plant development through direct and indirect mechanisms such as phytohormone production, enhanced nutrient availability, and disease suppression (Koskey et al., 2021; Lopes

et al., 2021). PGPR are classified as intracellular (iPGPR) or extracellular (ePGPR) based on their interaction with plant roots (Gray and Smith, 2005). Their growth-promoting activities vary between genera, species, and even strains and include mechanisms such as nitrogen fixation, phosphate solubilization, siderophore production, and induced systemic resistance (Vessey, 2003; Zahir et al., 2004; Singh et al., 2010).

Among the most studied PGPR are species from the *Pseudomonas*, known for their metabolic versatility, strong colonization abilities, and bioactive metabolite production. *Pseudomonas* spp., particularly *P. fluorescens* and *P. putida*, are well-suited for rhizosphere colonization and have been shown to enhance nutrient uptake, suppress phytopathogens through the secretion of antifungal compounds, and improve plant tolerance to abiotic stressors like salinity and heavy metals (Glick et al., 2007; Saharan and Nehra, 2011). Their phosphate-solubilizing ability is particularly notable, linked to producing organic acids such as gluconic acid, which enhances phosphorus availability in nutrient-poor soils (Vyas and Gulati, 2009; Oteino et al., 2015). Furthermore, *Pseudomonas* spp. contributes to iron acquisition in plants via siderophore production and exhibit biocontrol efficacy through enzymes such as chitinase, protease, and ACC-deaminase (Saritha et al., 2015).

Additionally, *Pseudomonas* spp. demonstrate tolerance to a wide range of environmental pollutants, including heavy metals like arsenic, cadmium, and chromium, positioning them as valuable agents in phytoremediation (Parameswari et al., 2009; Singh et al., 2010). Their multifunctionality makes them essential contributors to plant health and environmental sustainability.

The use of *Pseudomonas* species in agricultural systems offers a promising strategy for improving plant health, stress tolerance, and reducing chemical input dependence. Their actions enhance nutrient availability, stimulate plant growth, and suppress a broad range of phytopathogens, fostering a more sustainable and resilient agricultural ecosystem.

Given the increasing interest in sustainable agriculture and the need for adaptable microbial solutions, this study investigates the agronomic potential of *Pseudomonas* isolates obtained from rhizosphere soils. The aim is to assess the physiological, biochemical, and stress tolerance characteristics, as well as their contributions to plant growth promotion through direct and indirect mechanisms of newly isolated strains. By evaluating their functional traits, this research seeks to identify promising bacterial candidates for use as bioinoculants in stress-prone agricultural systems.

MATERIALS AND METHODS

This study employed an integrative methodological framework to isolate, characterize, and evaluate bacterial strains with potential plant growth-promoting traits and environmental resilience.

Morphological and Phenotypic Characterization

The isolates were subjected to detailed phenotypic characterization, including Gram staining, motility testing, and microscopic evaluation to determine cell shape and behavior. Colony morphology—such as shape, pigmentation, margin, and elevation—was recorded following standard microbiological criteria. Identification was guided by the diagnostic keys in Bergey's Manual of Systematic Bacteriology (Holt et al., 1994).

Environmental Stress Tolerance

To evaluate the ecological adaptability of the bacterial isolates, their tolerance to various abiotic stressors was tested. Growth responses were monitored under different

temperature regimes (4°C, 17°C, 25°C, and 35°C), pH levels (4.0, 7.0, and 9.0), and sodium chloride concentrations (0%, 7%, and 15%). Isolates were inoculated on Luria-Bertani medium and incubated for 48 hours under these conditions, after which growth intensity was visually assessed and categorized.

Resistance to selected heavy metals [chromium (Cr), copper (Cu), nickel (Ni), and lead (Pb)] was determined using the agar disc diffusion method. Serial dilutions of each metal salt (10^{-1} to 10^{-3} mol dm^{-3}) were applied, and the presence or absence of inhibition zones was recorded. Pesticide tolerance was similarly evaluated using three commercially available formulations: two fungicides (Luna and Sequence) and one insecticide (Lamdex), tested at recommended application levels as well as at 10-fold and 100-fold concentrations (Randhawa and Kullar, 2010). The capacity of isolates to withstand antibiotics was examined using standard antibiogram methods, with susceptibility assessed against agents such as ampicillin, neomycin, erythromycin, streptomycin, and chloramphenicol. Inhibition zones were measured and interpreted according to established classification criteria (Jarak and Đurić, 2004).

Enzymatic Activities and Metabolic Profiling

The enzymatic potential of the isolates was assessed through a range of biochemical assays relevant to nutrient cycling and plant–microbe interactions. The entire testing are illustrated in Table 1.

Table 1. Enzymatic Tests and Indicators

Enzyme	Medium/Method	Positive reaction indicator	Methods
Catalase	H_2O_2 bubbles	Bubble formation	Gill and Vickers, 1969
Oxidase	Oxidase reagent	Color change (dark purple)	Quinn, P. J., et al. 1994
Lipase	Tween 80 on nutrient agar	Opaque zones	Lanui, 1987
Protease	SIM medium	Black precipitate	MacFaddin, 2000
Cellulase	CMC agar + Congo red	Clear halo	MacFaddin, 2000
Pectinase	Pectin agar + iodine	Clear zone	MacFaddin, 2000
Lecithinase	Egg yolk agar	White precipitate	Galanos et al., 1985
Urease	Christensen's urea agar	Pink color change	Christensen, 1946
Starch hydrolysis	Starch agar + iodine	Clear zone	Edwards and Ewing, 1939
Gelatinase	Nutrient gelatin tubes	Liquefaction	Erdos and Tully, 1986

To further evaluate metabolic flexibility, isolates were tested for their ability to utilize various nitrogen and carbon sources. Nitrate reduction was investigated using nitrate agar, with reagent-based colorimetric confirmation (Christensen, 1946). Citrate utilization was determined on Simons' citrate agar (Simons, 1923), while sugar fermentation profiles for glucose, fructose, sucrose, galactose, lactose, and xylose were assessed using standard media with pH indicators to detect acidification (MacFaddin, 2000).

Plant Growth-Promoting (PGP) Traits

Key biochemical traits associated with direct plant growth promotion were investigated. Ammonia production was determined by incubating isolates in peptone water and applying Nessler's reagent; a yellow to brown color shift was considered a positive result. Indole-3-acetic acid (IAA) production was quantified by culturing isolates in tryptic soy broth supplemented with L-tryptophan, followed by treatment with Salkowski's reagent and spectrophotometric measurement at 540 nm (Patten and Glick, 2002). Siderophore production was assessed using the Chrome Azurol S (CAS) agar assay, where a visible color

change indicated iron-chelating activity (Milagres et al., 1999). Phosphate solubilization was evaluated on Pikovskaya's agar containing tricalcium phosphate; clear halo zones around colonies indicated solubilization capacity (Menkin, 1963). Nitrogen fixation ability was tested by culturing the isolates on nitrogen-free semi-solid malate medium (NFb). The formation of a pellicle near the surface of the medium after incubation was considered indicative of positive nitrogen-fixing activity (Dobereiner, 1995).

In addition, traits associated with indirect plant growth promotion were also examined. Hydrogen cyanide (HCN) production was evaluated using nutrient agar supplemented with glycine and a filter paper saturated with picric acid and sodium carbonate, as described by Bakker and Schippers (1987). A color change from yellow to orange indicated cyanide release. Exopolysaccharide (EPS) production was screened using Congo Red agar, with red-pigmented colonies considered positive (Freeman et al., 1989). Turbidity in liquid cultures was used as a complementary semi-quantitative indicator of EPS production (Zhang et al., 2013).

RESULTS AND DISCUSSION

Bacteria Isolation

Out of 25 rhizospheric soil samples collected from different crops, *Pseudomonas* spp. widely distributed in the rhizosphere and is known for its metabolic versatility and ecological adaptability (Raaijmakers et al., 2002; Silby et al., 2011) were successfully isolated from four samples, designated as PSD1, PSD2, PSD3, and PSD4. Isolates were selected based on colony morphology, repeatedly subcultured to ensure purity, and stored in a culture collection.

Morphological and Phenotypic Characterization

Morphological characterization included both microscopic cell shape and colony appearance (Table 2). All isolates were rod-shaped, motile, and Gram-negative. Colonies were circular, convex, smooth-edged, and beige with a moist texture. These features are consistent with *Pseudomonas* spp. as described by Stanier et al. (1966) and validated by Holt et al. (1994).

Table 2. Morphological characteristics of isolates

Isolates	Cell			Colony				
	Shape	Gram staining	Motility	Shape	Elevation	Margin	Colour	Texture
PSD1	rod-shaped	-	+	circular	convex	smooth	beige	moist
PSD2	rod-shaped	-	+	circular	convex	smooth	beige	moist
PSD3	rod-shaped	-	+	circular	raised	smooth	beige	moist
PSD4	rod-shaped	-	+	circular	convex	smooth	beige	moist

(+) indicates a positive result; (–) indicates a negative result

Stress Tolerance and Resistance Profile of Bacterial Isolates

The resilience of the isolates to temperature, pH, and salinity stress is summarized in Table 3a. All isolates exhibited optimal or abundant growth at 25°C, with PSD3 showing superior adaptability. At 4°C, no growth was observed, confirming the mesophilic nature of these bacteria. Moderate halotolerance was observed up to 7% NaCl, while minimal growth occurred at 15%. These findings align with reports by Alavi et al. (2013), who described the ability of certain *Pseudomonas* strains to tolerate moderate osmotic and pH stress, enhancing their persistence in diverse environments.

Table 3a. Ecological resilience of bacterial isolates

Isolates	Temperature (°C)				pH			NaCl (%)		
	4	17	25	35	4	7	9	0	7	15
PSD1	-	+	++	+	-	++	-	+	+	±
PSD2	-	+	+	+	-	++	-	+	+	±
PSD3	-	++	++	++	±	++	-	+	+	±
PSD4	-	++	++	+	-	++	-	+	+	±

complete absence of growth (-); minimal growth (±); optimal (+); abundant growth (++)

Heavy metal resistance testing (Table 3b) revealed that none of the isolates exhibited inhibition zones in the presence of Cr, Cu, Ni, or Pb at concentrations ranging from 10^{-1} to 10^{-4} mol dm^{-3}, suggesting high intrinsic tolerance. This agrees with previous findings where *Pseudomonas* spp. were recognized for their ability to survive in metal-contaminated environments, aided by metal efflux systems and sequestration mechanisms (Rajkumar et al., 2010; Nies, 2003).

Table 3b. Resistance of isolates to heavy metals

Isolates	Heavy metals (mol dm^{-3})															
	Chromium (Cr)				Copper (Cu)				Nickel (Ni)				Lead (Pb)			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
PSD1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PSD2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PSD3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PSD4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

without zone of inhibition (-); zone of inhibition 1-10 mm (±); zone of inhibition > 10 mm (+)

Resistance to pesticides (Table 3c) showed that *Lamdex* had no inhibitory effect, while *Luna* and *Sequence* were inhibitory at 10× and 100× concentrations. Mild inhibition (±) was observed for *Sequence* at the recommended dose. These results highlight differential sensitivity patterns among isolates, which is consistent with the findings of Bending and Turner (1999), who reported variable microbial responses to pesticide exposure depending on compound, dose, and microbial species.

Table 3c. Resistance of isolates to pesticides

Isolates	Pesticides (mol dm^{-3})								
	Luna			Lamdex			Sekvence		
	MI	(10x>MI)	(100x>MI)	MI	(10x>MI)	(100x>MI)	MI	(10x>MI)	(100x>MI)
PSD1	+	-	-	+	+	+	±	-	-
PSD2	+	-	-	+	+	+	±	-	-
PSD3	+	-	-	+	+	+	±	-	-
PSD4	+	-	-	+	+	+	±	-	-

MI: manufacturer's instructions; without zone of inhibition (-); zone of inhibition 1-10 mm zone (±); zone of inhibition > 10 mm (+)

Antibiotic susceptibility patterns (Table 3d) showed consistent resistance to ampicillin, and moderate sensitivity to erythromycin and neomycin. All isolates were sensitive to streptomycin, with partial sensitivity to chloramphenicol. This profile aligns with earlier studies indicating that *Pseudomonas* spp. are intrinsically resistant to certain

β-lactam antibiotics due to low outer membrane permeability and active efflux systems (Hancock and Speert, 2000).

Table 3d. Resistance of isolates to antibiotics

Isolates	Antibiotics				
	Ampicilin	**Erithromicin**	**Neomicin**	**Streptomycine**	**Chloramphenicol**
	10 µg	**15 µg**	**30 µg**	**300 µg**	**30 µg**
PSD1	-	±	±	+	+
PSD2	-	±	±	+	±
PSD3	-	±	±	+	±
PSD4	-	±	±	+	+

without zone of inhibition (-); zone of inhibition 1-10 mm (±); zone of inhibition > 10 mm (+)

Enzymatic Activities and Metabolic Traits

Biochemical profiling (Table 4) confirmed the production of catalase, oxidase, and lipase in all isolates. Protease activity was detected in three isolates, and lecithinase, urease, and gelatinase were variably expressed. Cellulase and pectinase were produced by selected isolates, consistent with the saprophytic and competitive lifestyle of *Pseudomonas* spp. (Beneduzi et al., 2012). None of the isolates hydrolyzed starch, suggesting the absence of amylase activity. The ability to degrade complex organic substrates enhances nutrient turnover and disease suppression in the rhizosphere (Gomes et al., 2001).

Table 4. Enzymatic, hydrolysis, energy, and carbon source utilization properties of the bacterial isolates

Biochemical Characterization	**Type of test/Isolates**		**PSD1**	**PSD2**	**PSD3**	**PSD4**
Enzymatic Activity	Catalase Production		+	+	+	+
	Oxidase Production		+	+	+	+
	Lipase Production		+	+	+	+
	Protease Production		+	+	+	-
	Cellulase Production		+	-	-	+
	Pectinase Production		-	-	+	+
	Lechitinase Production		+	+	+	+
	Urease Production		+	+	+	+
Complex Organic Substrates Degradation	Starch Hydrolysis		-	-	-	-
	Gelatin Hydrolysis		-	+	-	+
Energy Source Utilization	Nitrogen	Nitrate Reduction	+	+	+	+
	Carbon	Citrate Utilization	+	-	-	+
		Glucose Utilization	+	+	+	+
		Fructose Utilization	+	+	+	-
		Sucrose Utilization	+	+	+	+
		Galactose Utilization	+	+	+	+
		Lactose Utilization	-	-	-	-
		Xylose Utilization	-	-	-	-

(+) positive reaction /produce, hydrolyze, reduce, utilize/; (-) negative reaction /does not produce, hydrolyze, reduce, utilize/

Plant Growth-Promoting Properties

Plant growth-promoting (PGP) traits of the isolates are presented in Table 5.

Indole-3-acetic acid (IAA) production was detected only in PSD1, which may contribute to root elongation and lateral root formation. Although IAA production was limited, all isolates exhibited strong siderophore activity, which facilitates iron acquisition under deficiency conditions and suppresses pathogens (Souza et al., 2015).

Table 5. Plant growth-promoting properties of the isolates

Isolates	IAA	Siderophores	HCN	Solubilize phosphorus	Fixation of Nitrogen	Exopoly saccharide
PSD1	+	+	+	-	+	-
PSD2	-	+	+	-	+	-
PSD3	-	+	+	-	+	-
PSD4	-	+	-	-	+	-

(+) positive reaction / produces/ performs decomposition; (-) negative reaction / does not produce/ does not perform decomposition

Hydrogen cyanide (HCN) production was detected in three isolates. HCN contributes to biocontrol of root pathogens by inhibiting cytochrome c oxidase in target organisms (Datta et al., 2011). All isolates exhibited the capacity for nitrogen fixation, a trait rarely reported for *Pseudomonas* but supported by recent work showing horizontal gene acquisition enabling diazotrophy (Loper et al., 2012). However, none of the isolates solubilized inorganic phosphate, suggesting limited phosphorus-mobilizing capacity.

Exopolysaccharide (EPS) production was not observed in any isolate. While EPS contributes to desiccation resistance and root adhesion, its absence may be compensated by other beneficial traits.

CONCLUSION

This study provides valuable insights into the utilization of *Pseudomonas* species as biofertilizers to enhance soil fertility and promote plant growth in agricultural systems. The isolated strains, obtained from rhizospheric soils, were taxonomically characterized through morphological, physiological, and biochemical analyses. Functionally, they demonstrated notable stress tolerance, resistance to heavy metals, and plant growth-promoting traits. Although certain PGP characteristics, such as phosphate solubilization and exopolysaccharide production, were not expressed, the consistent production of siderophores and hydrogen cyanide (HCN), nitrogen fixation ability, and diverse enzymatic activities suggest their strong potential for use in sustainable agriculture. These functional attributes support the positioning of *Pseudomonas* spp. as eco-friendly alternatives to conventional agrochemicals. Future research should aim to validate these findings through field trials and explore their practical integration into stress-adaptive cropping systems, thereby contributing to long-term agricultural resilience and food security.

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