Isolation and Functional Characterization of Plant Growth-Promoting Paenibacillus polymyxa from Wheat Rhizosphere

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ABSTRACT

Background: Plant growth-promoting rhizobacteria (PGPR) are increasingly recognized as sustainable alternatives to chemical fertilizers in cereal production. Among these, Paenibacillus polymyxa is notable for its multifunctional plant growth-promoting (PGP) traits and broad-spectrum antagonism against phytopathogens.

Objective: This study aimed to isolate and functionally characterize a P. polymyxa strain from wheat rhizosphere soil and evaluate its growth-promoting potential under greenhouse conditions.

Methods: Rhizosphere soil was sampled from healthy wheat fields, and bacteria were isolated on nutrient agar, purified, and identified by morphological, biochemical, and 16S rRNA gene sequencing analyses. PGP traits, including IAA production, phosphate and potassium solubilization, nitrogen fixation, siderophore and ammonia production, HCN production, ACC deaminase activity, and biofilm formation, were quantified. Antagonism against wheat phytopathogens and a pot-based plant growth assay were also performed, with data analyzed by one-way ANOVA and Tukey's test.

Results: The isolate, designated WR-7, was confirmed as P. polymyxa with 99.1% 16S rRNA sequence similarity to reference strains. It produced substantial IAA (28.4 µg/mL), solubilized phosphate (312 µg/mL) and potassium, fixed atmospheric nitrogen, and generated siderophores, ammonia, and biofilm. Inoculation significantly ($p<0.05$) improved germination percentage, root and shoot length, biomass, and chlorophyll content relative to untreated controls, and inhibited three major phytopathogens in vitro.

Conclusion: P. polymyxa WR-7 exhibits robust, multifaceted plant growth-promoting activity and represents a promising candidate biofertilizer for sustainable wheat cultivation, warranting further field-scale validation.

Keywords: *Paenibacillus polymyxa*; Plant growth-promoting rhizobacteria (PGPR); Wheat rhizosphere; Biofertilizer

1. Introduction

Wheat (*Triticum aestivum L.*) is considered to be one of the most cultivated cereal crops in the world and is very vital for the global food supply. Wheat yields are negatively affected by low soil fertility, nonbiological stress, and excessive usage of fertilizers which degrades the soil quality and increases input costs over time. The rhizosphere is a narrow part of the soil which is affected by the roots and is home to a dynamic group of microbes that are responsible for nutrient cycling and stress resilience.

In the rhizosphere, PGP agents (plant growth-promoting agents) thrive and help boost host productivity through direct and indirect ways including the production of phytohormones, nutrient dissolving bacteria, fixing nitrogen biologically, and combating diseases. One of the examples of PGPRs is Penicilliums polymyxin which is an endospore-forming anaerobic bacterium and is known for its diversity and metabolic capabilities.

Chemical fertilizers which rely on PGPRs are an efficient and eco-friendly substitute for fertilizers.

There is an interest in these chemicals, yet there are few local strains that have functional characteristics, thus the current study was undertaken to isolate P. polymyxin.

Materials and Methods

Sample Collection

Rhizosphere soil adhering to wheat roots was collected from an experimental wheat field at a depth of 0–15 cm during the tillering stage. Composite samples from five randomly selected plants were pooled, transported in sterile polybags at 4°C, and processed within 24 h.

Isolation of Bacteria

Soil suspensions were serially diluted and spread on nutrient agar and Pikovskaya's agar, followed by incubation at 28±2°C for 48 h. Distinct colonies were repeatedly sub-cultured on nutrient agar to obtain pure isolates, which were preserved in 20% glycerol at −80°C.

Identification

Colony morphology, Gram reaction, and endospore staining were recorded. Biochemical tests (catalase, oxidase, citrate utilization, starch hydrolysis, Voges-Proskauer) were conducted using standard protocols. Genomic DNA was extracted, and the 16S rRNA gene was amplified using universal primers 27F/1492R, sequenced, and compared against the NCBI database using BLASTn; a phylogenetic tree was constructed in MEGA11 using the neighbor-joining method with 1000 bootstrap replicates.

Functional Characterization

IAA production (with and without L-tryptophan) was quantified colorimetrically using Salkowski's reagent. Phosphate and potassium solubilization were assessed on Pikovskaya's and Aleksandrov's media, respectively, and quantified spectrophotometrically. Nitrogen-fixing ability was tested on nitrogen-free Jensen's medium. Siderophore production was detected using Chrome Azurol S assay, ammonia production via Nessler's reagent, and hydrogen cyanide production on HCN-amended agar. ACC deaminase activity was measured using α-ketobutyrate quantification, and biofilm formation was evaluated by the crystal violet microtiter assay.

Antagonistic Activity

Antifungal activity was screened against Fusarium graminearum, Bipolaris sorokiniana, and Rhizoctonia solani using the dual culture plate assay, and percentage inhibition of radial growth was calculated after 7 days.

Plant Growth Assay

Surface-sterilized wheat seeds (cv. HD-2967) were treated with bacterial suspension (10^8 CFU/mL) or sterile broth (control) and evaluated for germination percentage after 7 days. A pot experiment in sterilized soil under greenhouse conditions (25±2°C, 60–65% relative humidity) assessed root length, shoot length, dry biomass, and chlorophyll content (SPAD meter) at 30 days post-sowing.

Statistical Analysis

All experiments were performed in triplicate (n=3) with three biological replicates per treatment. Data were analyzed by one-way ANOVA followed by Tukey's honestly significant difference (HSD) test using SPSS v26, with $p<0.05$ considered statistically significant.

Results

Isolation and Identification

The isolate WR-7 formed opaque, cream-colored, irregular colonies with a wrinkled surface on nutrient agar. Microscopy revealed Gram-variable, rod-shaped, motile cells with central to sub-terminal endospores. Biochemical and molecular characteristics are summarized in Table 1. BLASTn analysis confirmed 99.1% similarity to P. polymyxa reference sequences, and phylogenetic clustering placed WR-7 within the P. polymyxa clade (Figure 1).

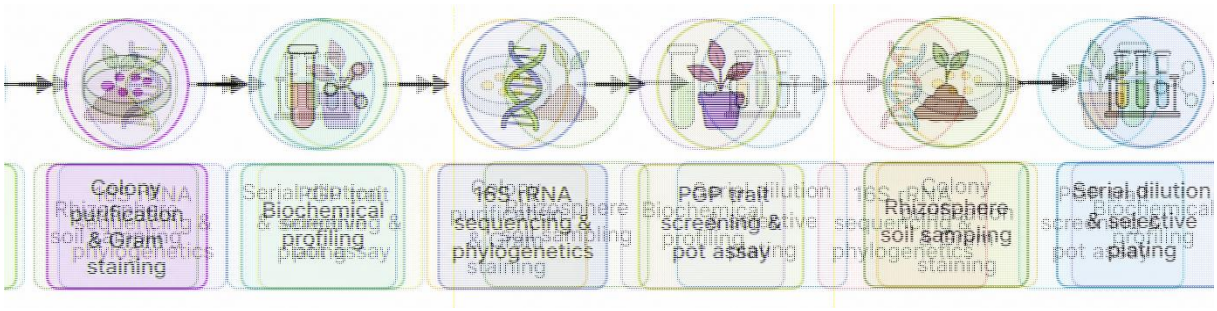

Fig 1: Workflow for isolation and characterization of Paenibacillus polymyxa from wheat rhizosphere soil.

Functional Characterization

WR-7 exhibited strong multifunctional PGP activity (Table 2), including substantial IAA production, phosphate and potassium solubilization, positive nitrogen fixation, siderophore, ammonia, and HCN production, ACC deaminase activity, and dense biofilm formation. The relationship among these mechanisms and their contribution to wheat growth promotion is depicted schematically in Figure 2.

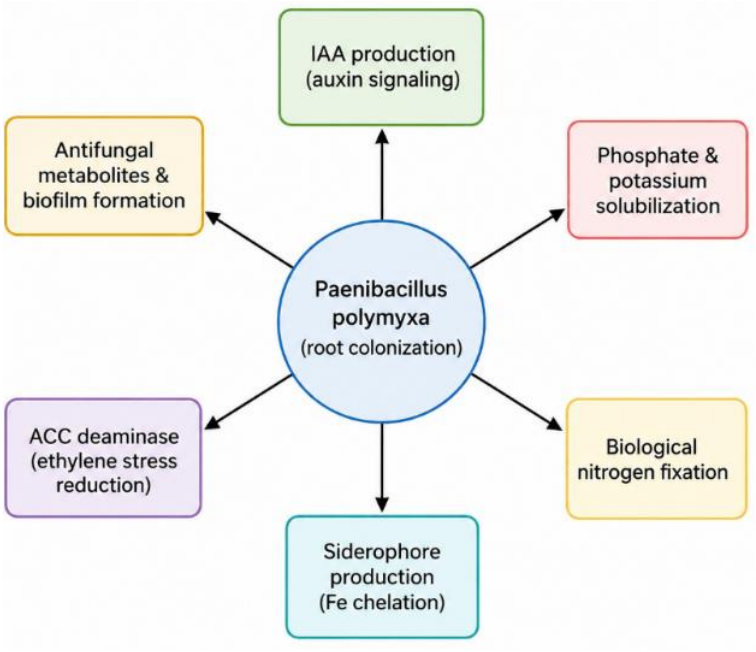

Fig 2: Plant growth-promoting mechanisms of Paenibacillus polymyxa in wheat rhizosphere.

Plant Growth Promotion and Antifungal Activity

Seed inoculation with WR-7 significantly enhanced germination percentage, root and shoot length, dry biomass, and chlorophyll content relative to uninoculated controls (Table 3, Figure 3; $p<0.05$, Tukey's HSD). In dual culture assays, WR-7 inhibited F. graminearum (61.2%), B. sorokiniana (54.8%), and R. solani (48.3%) radial growth.

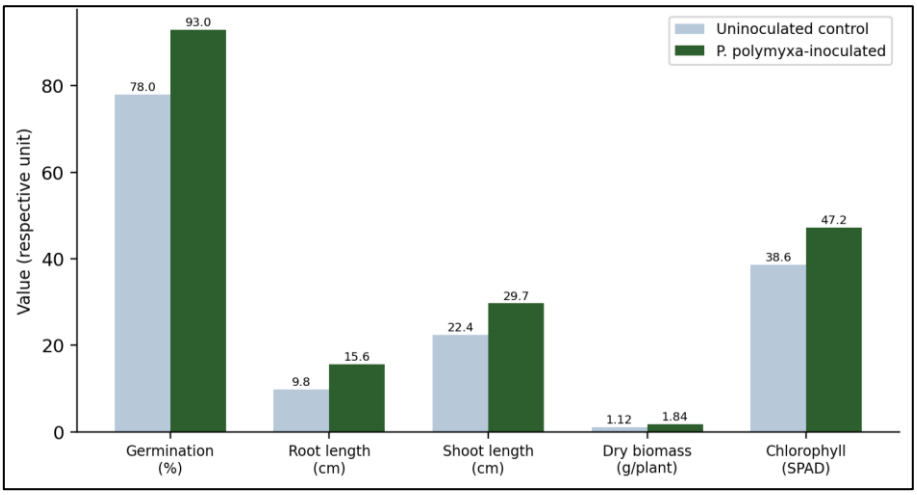

Fig 3: Comparative growth performance of treated and untreated wheat plants

Discussion

The polyphasic identification of WR-7 as P. polymyxa aligns with prior reports describing this species as a common, functionally versatile inhabitant of cereal rhizospheres. The isolate's IAA and siderophore production levels are comparable to, or exceed, those reported for other wheat-associated Paenibacillus strains, suggesting strong phytohormonal and iron-acquisition capacity that likely contributed to the observed improvements in root architecture and biomass.

Concurrent phosphate and potassium solubilization indicate that WR-7 can enhance nutrient bioavailability in the rhizosphere, complementing nitrogen fixation to support balanced nutrition. ACC deaminase activity further suggests a role in mitigating ethylene-induced stress during seedling establishment, while biofilm formation likely promotes persistent root colonization, a prerequisite for consistent field performance. The antifungal activity against major wheat pathogens highlights additional disease-suppression potential, mechanistically linked to antimicrobial lipopeptide and volatile compound production characteristic of this species.

Compared with many single-trait PGPR, P. polymyxa's combination of hormonal, nutritional, and biocontrol functions offers a broader agronomic advantage, though greenhouse gains do not always translate proportionally to field conditions due to variable soil, climatic, and microbial competition factors. This study is limited by its single-season, single-cultivar greenhouse design and the absence of genome-level functional validation. Future work should incorporate comparative genomics,

metabolomic profiling of active compounds, multi-location field trials, and formulation studies to support commercialization of WR-7 as a biofertilizer.

Conclusion

The research identified and isolated a multi-functional P. polymyxa strain WR-7 in wheat rhizosphere soil, exhibiting potent plant growth-promoting and antifungal properties that resulted in observable increases in wheat seedling growth. Overall, WR-7 can be a potential biofertilizer candidate for enhancing sustainable wheat production, and further studies may require field and formulation studies to arrive at practical application.

Table 1: Morphological, biochemical, and molecular characteristics of isolated Paenibacillus polymyxa WR-7

Characteristic	Observation	Method/Remarks
Colony morphology	Cream, irregular, opaque, wrinkled surface	—
Gram reaction	Gram-variable, rod-shaped	—
Endospore staining	Central to sub-terminal endospores present	—
Catalase / Oxidase	Positive / Negative	—
Citrate utilization	Positive	—
Starch hydrolysis	Positive	—
16S rRNA identity	99.1% similarity to P. polymyxa	GenBank BLASTn
Phylogenetic placement	Clustered within P. polymyxa clade	Neighbor-joining, 1000 bootstraps

Table 2: Functional plant growth-promoting characteristics of isolate WR-7

Trait	Result	Assay/Method
IAA production (with tryptophan)	28.4 µg/mL	Salkowski colorimetric assay
Phosphate solubilization	312 µg/mL	Pikovskaya's medium
Potassium solubilization	Positive (halo zone)	Aleksandrov's medium
Nitrogen fixation	Positive (growth on N-free medium)	Jensen's medium
Siderophore production	Positive (orange halo)	CAS assay
Ammonia production	Positive	Nessler's reagent
HCN production	Weakly positive	Picric acid method
ACC deaminase activity	0.42 µmol α-ketobutyrate/mg protein/h	Spectrophotometric assay
Biofilm formation	Strong (OD570 = 1.86)	Crystal violet microtiter assay

Table 3: Plant growth performance of wheat under different treatments (mean ± SD, n=3)

Parameter	Uninoculated control	WR-7 inoculated	Significance (Tukey's HSD)
Germination (%)	78.0 ± 2.6	93.0 ± 2.1	$p<0.05$
Root length (cm)	9.8 ± 0.7	15.6 ± 1.0	$p<0.05$
Shoot length (cm)	22.4 ± 1.3	29.7 ± 1.5	$p<0.05$
Dry biomass (g/plant)	1.12 ± 0.09	1.84 ± 0.12	$p<0.05$
Chlorophyll content (SPAD)	38.6 ± 1.8	47.2 ± 2.0	$p<0.05$

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