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"Enhancing Crop Growth through a Lignocellulosic Biofertilizer Formulated with *Paenibacillus* P6: A Sustainable Approach"

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Abstract

The over-reliance on chemical fertilizers has led to significant environmental degradation and a decline in soil health, necessitating the search for sustainable alternatives. This study focuses on the production and evaluation of a lignocellulosic biofertilizer using dry leaves as a carrier material, inoculated with the bacterial strain Paenibacillus P6. The bacterial isolate was characterized for its morphological, cultural, and biochemical properties. A solid-state biofertilizer was prepared by mixing sterilized dry leaves and lignite with a Paenibacillus P6 culture and calcium chloride, allowing it to decompose for 36 days. The efficacy of the prepared biofertilizer was tested in a pot experiment using spinach (Spinacia oleracea). The bacterial strain was identified as Gram-negative, non-motile cocci, testing positive for catalase and oxidase, but negative for zinc solubilization and indole acetic acid (IAA) production under the test conditions. Viability counts over 30 days indicated a sustained microbial population in the biofertilizer compared to the control. Pot trial results demonstrated that the biofertilizer treatment significantly enhanced plant growth, with a maximum shoot length of 8.5 cm and root length of 7.8 cm, compared to the control (shoot: 7.4 cm, root: 3.4 cm). This work suggests that a Paenibacillus P6-based biofertilizer using dry leaf waste is a promising, eco-friendly strategy for improving crop productivity and promoting sustainable agricultural practices.

Keywords: *Biofertilizer, Paenibacillus P6, Dry Leaves, Sustainable Agriculture, Plant Growth Promotion, Spinach.*

1. INTRODUCTION

The Green Revolution, while dramatically increasing agricultural output, was largely fuelled by the indiscriminate use of chemical fertilizers and pesticides. Over the decades, the hazardous effects of this practice have become increasingly apparent, leading to soil degradation, reduced microbial activity, formation of impermeable hardpan layers, and a decline in the nutritional quality of produce (Uzor & Fredrick, 2018). These issues, compounded by the pressures of a growing population and climate change, have necessitated a paradigm shift towards more sustainable agricultural methodologies.

In this context, biofertilizers have emerged as a viable and eco-friendly alternative. Comprising living or latent cells of efficient microbial strains, biofertilizers enhance nutrient availability by fixing atmospheric nitrogen, solubilizing phosphorus, and decomposing organic matter (Mohammadi & Sohrabi, 2012). They are a cornerstone of integrated nutrient management, being low-cost, effective, and renewable (Weselowski *et al.,* 2016; Ahemad & Kibret 2014). These microbial inoculants, often delivered through a carrier material, promote plant growth by converting nutritionally important elements from unavailable to available forms and by producing growth-promoting substances (Rural College of Pharmacy *et al.,* 2009).

Among the diverse group of plant growth-promoting rhizobacteria (PGPR), the genus *Paenibacillus* holds considerable promise. Specifically, *Paenibacillus polymyxa* strains are known for their multiple beneficial traits, including nitrogen fixation, phosphate solubilization, and the production of phytohormones like indole-3-acetic acid (IAA), while also exhibiting antagonistic activity against plant pathogens (Weselowski *et al.,* 2016). The use of agricultural and forest waste, such as fallen dry leaves, as a carrier material for these microbes presents a double advantage: it provides a cost-effective substrate for the inoculant and offers a method for recycling nutrient-rich biomass (Chandran *et al.,* 2014; Kumar *et al.,* 2020).

The primary objective of this study was to develop a solid-state biofertilizer utilizing dry leaves as a carrier for the *Paenibacillus* P6 strain. The work involved characterizing the bacterial isolate, monitoring its viability during the composting process, and evaluating the efficacy of the final product on the growth of spinach (*Spinacia oleracea*) in a controlled pot experiment. This research aims to contribute to the development of sustainable farming practices by providing a practical method to convert organic waste into a valuable agricultural input.

2. MATERIALS AND METHODS

2.1. Collection of Materials

Dry plant leaves were collected from the campus of Modern College, Pune. Soil for the isolation and pot experiment was obtained from the College of Agriculture, Pune. The bacterial strain *Paenibacillus* P6 was procured from a recognized microbial culture collection.

2.2. Characterization of *Paenibacillus* P6

2.2.1. Cultural and Morphological Characterization

The strain was streaked onto sterile Luria Bertani (LB) agar plates using the quadrant method and incubated at 37°C for 24 hours to study colony characteristics (size, shape, margin, elevation, consistency, opacity). Gram staining was performed following the standard protocol. A heat-fixed smear was sequentially stained with crystal violet, Gram's iodine, decolorized with 95% alcohol, and counterstained with safranin. Motility was assessed using the hanging drop technique.

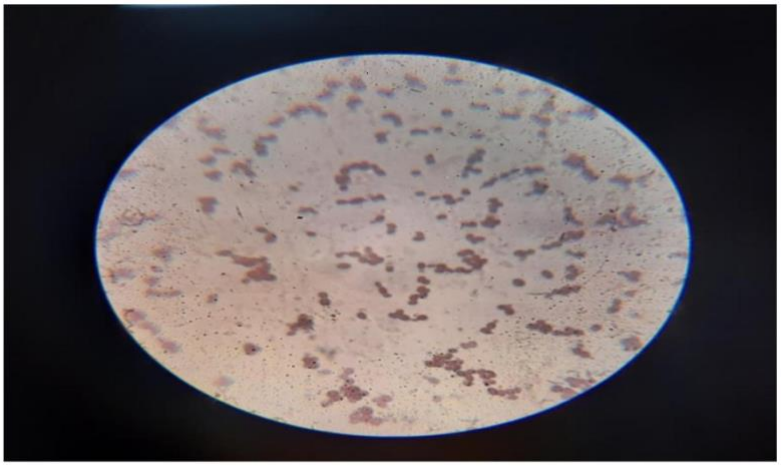

Fig 1. Gram staining

2.2.2. Biochemical Characterization

- **Catalase Test:** A loopful of a 24-hour old culture was placed on a clean slide, and a drop of 3% hydrogen peroxide (H_2O_2) was added. The immediate formation of gas bubbles indicated a positive result.

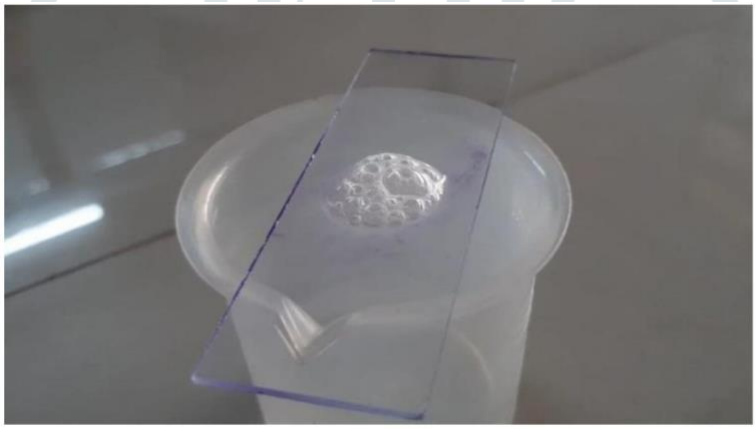

Fig 3. Catalase Test.

- **Oxidase Test:** A drop of oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride) was placed on a filter paper. A colony of the test organism was smeared onto the reagent area. A change to a dark purple colour within 10 seconds was considered a positive test.

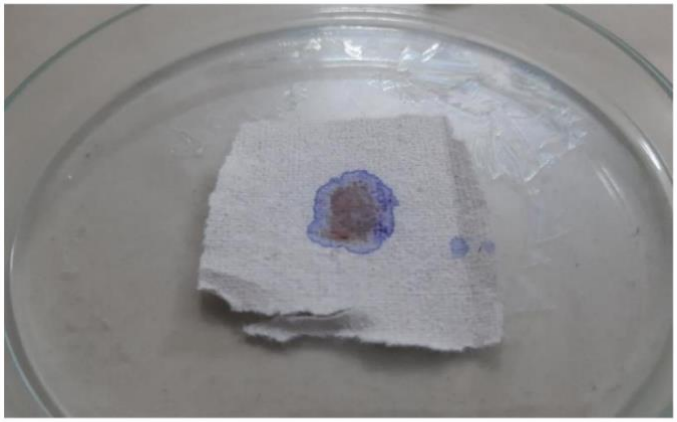

Fig 2. Oxidase Test

2.2.3. Plant Growth-Promoting Traits

- **Zinc Solubilization:** The isolate was spot-inoculated on Tris-minimal medium supplemented separately with 0.1% zinc oxide (ZnO) and zinc phosphate [$Zn_3(PO_4)_2$]. Plates were incubated at 28°C for seven days and observed for the formation of a clear halo zone around the bacterial growth.

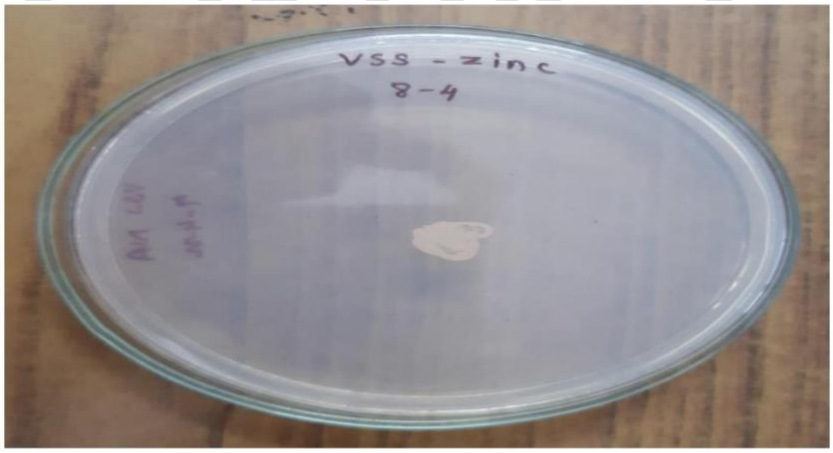

Fig 4. Zinc solubilisation

- **Indole Acetic Acid (IAA) Production:** *Paenibacillus* P6 was grown in LB broth supplemented with 1 mg/ml of L-tryptophan for 48 hours at 28°C. The culture was centrifuged at 10,000 rpm for 15 minutes. One ml of the supernatant was mixed with 4 ml of Salkowski's reagent and incubated in the dark at room temperature for 30 minutes. The development of a pink to red colour was indicative of IAA production. An uninoculated broth with reagent served as the blank, and the optical density was measured at 540 nm.

Fig 5. Indole acetic acid production

2.3. Preparation of Biofertilizer

The collected dry leaves and lignite were sterilized in an autoclave at 121°C for 30 minutes. A 24-hour old culture of *Paenibacillus* P6 was used to inoculate sterile LB broth. The sterilized lignite was then mixed with this bacterial suspension. This mixture was combined with the sterilized dry leaves and calcium chloride ($CaCl_2$) in a sterile container. A control setup was maintained identically but without the microbial inoculation. Both containers were left for 36 days to allow for decomposition. Microbial viability was monitored every 7 days using the ten-fold serial dilution and spread plate technique on LB agar.

2.4. Pot Experiment for Efficacy Evaluation

Soil was sterilized by autoclaving at 121°C for one hour on three alternate days. Six pots were set up, with three serving as the treatment group (biofertilizer) and three as the control group. Each pot was filled three-quarters with sterile soil. In the treatment pots, one-third of the soil was replaced with the prepared biofertilizer and mixed well. The control pots received only sterile soil. Spinach seeds were sown in all pots. The pots were kept in natural sunlight and watered daily. After 18 days, the plants were carefully uprooted, and their shoot and root lengths were measured.

3. OBSERVATIONS AND RESULTS

3.1. Microbial Characterization of *Paenibacillus* P6

The results of the morphological and biochemical tests are summarized in **Table 1** and illustrated in **Figures 1-3**. The strain exhibited faint pink, cocci-shaped cells and was determined to be Gram-negative. It was non-motile and tested positive for both catalase and oxidase enzymes.

Table 1: Morphological and Biochemical Characteristics of Paenibacillus P6

Test	Result
Colony Colour	Faint Pink
Cell Shape	Cocci
Gram Staining	Gram-negative
Motility	Non-motile
Catalase	Positive
Oxidase	Positive

3.2. Assessment of Plant Growth-Promoting Traits

The strain did not show any clear halo zone on the zinc-supplemented media, indicating a negative result for zinc solubilization (**Figure 4**). Similarly, upon adding Salkowski's reagent to the culture supernatant, no colour change was observed, suggesting that *Paenibacillus* P6 did not produce IAA under the given assay conditions (**Figure 5**).

Fig. 6 Bio Fertilizer

3.3. Microbial Viability During Composting

The viability of *Paenibacillus* P6 in the biofertilizer and control (without inoculant) was tracked over 30 days using serial dilution and plating. The colony-forming units (CFU) for various dilutions are presented in **Table 2**.

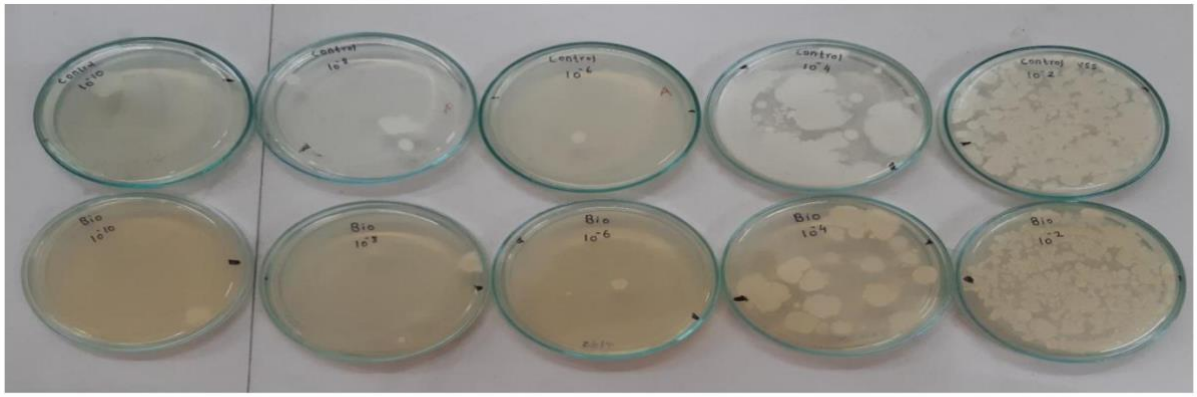

Fig. 7 bacterial colonies of Bio fertilizer

A consistently higher number of colonies were observed in the biofertilizer samples compared to the control across all dilutions and time points, indicating successful colonization and survival of the inoculated strain in the carrier material.

Table 2: Viable Colony Counts (CFU/ml) During 30 Days of Composting

Day	Dilution	Biofertilizer	Control
Day 1	10^{-6}	24	34
	10^{-4}	120	184
	10^{-2}	450	380
Day 15	10^{-6}	102	90
	10^{-4}	211	100
	10^{-2}	537	498
Day 30	10^{-6}	264	237
	10^{-4}	300	321
	10^{-2}	598	440

3.4. Effect of Biofertilizer on Spinach Growth

The pot experiment clearly demonstrated the positive impact of the biofertilizer. After 18 days of growth, spinach plants in the biofertilizer-treated pots exhibited visibly better growth compared to those in the control pots (**Figure 8, 9**). The growth parameters are detailed in **Table 3**.

Fig. 8 Plantation with Bio Fertilizer

Fig. 9 plantation with control

Plants in the biofertilizer treatment achieved an average shoot length of 8.25 cm and an average root length of 6.45 cm, whereas control plants averaged a shoot length of 7.1 cm and a significantly lower root length of 2.65 cm.

Table 3: Effect of Biofertilizer on Shoot and Root Length of Spinach after 18 Days

Parameter	Biofertilizer		Control	
	Plant 1	**Plant 2**	**Plant 1**	**Plant 2**
Root Length (cm)	7.8	5.1	7.4	6.8
Shoot Length (cm)	8.0	8.5	3.4	1.9
Total Length (cm)	15.8	13.6	10.8	8.7

Fig. 10 Measurement of Plant Growth

4. DISCUSSION

The primary goal of this research was to develop a functional biofertilizer from dry leaves using *Paenibacillus* P6 and assess its impact on plant growth. The characterization of the strain provided valuable insights. While *Paenibacillus* species are often reported as Gram-variable or positive, the Gram-negative result here (Table 1, Fig. 1) could be due to the specific strain P6 or the age of the culture. Positive catalase and oxidase tests are common in many soil bacteria and indicate the presence of protective and electron transport chain enzymes, respectively.

Interestingly, the strain tested negative for both zinc solubilization and IAA production, which are classic traits associated with plant growth promotion in many *Paenibacillus* strains (Weselowski *et al.,* 2016; Gupta *et al.,* 2015). This suggests that the growth-promoting effects observed in the pot experiment might be mediated through other mechanisms. These could include the solubilization of other nutrients like phosphate, the production of different volatile organic compounds (VOCs), or the induction of systemic resistance in the plant, which were not assayed in this study (Fasusi *et al.,* 2021; Hayat *et al.,* 2010).

The viability data (Table 2) is a critical success factor for any biofertilizer. The consistently higher colony counts in the inoculated mixture over 30 days confirm that the dry leaves and lignite mixture served as an effective carrier, providing a conducive microenvironment and nutrients for the survival of *Paenibacillus* P6. This aligns with the properties of a good carrier material, which must be non-toxic and have good moisture absorption capacity (Brar *et al.,* 2012; Sharma *et al.,* 2013).

The most significant finding of this study is the clear enhancement of spinach growth by the prepared biofertilizer (Table 3). The increase in both shoot and, more notably, root length in the treated plants suggests a positive plant-microbe interaction. The more robust root system in treated plants likely improved nutrient and water uptake, supporting better overall growth. This result is consistent with previous work demonstrating that *Paenibacillus* biofertilizers can promote plant growth (Xu *et al.,* 2014; Zhou *et al.,* 2020; Sivasakthi *et al.,* 2014). Even without demonstrating IAA production or zinc solubilization in vitro, the *Paenibacillus* P6 strain,

when established in the rhizosphere via the biofertilizer, may have created conditions favourable for plant growth, perhaps through the mechanisms mentioned earlier. The performance of the biofertilizer underscores the potential of using locally available organic waste like dry leaves to create a value-added product for sustainable agriculture (Devi, 2018).

5. CONCLUSION

This study successfully demonstrated the production of a lignocellulosic biofertilizer by inoculating dry leaves with *Paenibacillus* P6. The carrier material effectively supported the viability of the bacterial strain over a one-month period. In a pot trial with spinach, the application of this biofertilizer resulted in superior plant growth, particularly in root development, compared to the uninoculated control. Despite the strain not exhibiting classic PGP traits like IAA production or zinc solubilization in vitro, its positive effect *in vivo* highlights the complexity of plant-microbe interactions and suggests that its growth-promoting ability may stem from alternative mechanisms. This work reinforces the potential of *Paenibacillus*-based biofertilizers formulated from agricultural waste as a cost-effective, environmentally friendly alternative to chemical fertilizers. This approach aligns with the global need for sustainable agricultural intensification and offers a promising avenue for reducing our environmental footprint while maintaining crop productivity.

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