International Journal of Biology Sciences

ISSN Print: 2664-9926
ISSN Online: 2664-9934
IJBS 2024; 6(1): 145-149
www.biologyjournal.net
Received: 21-01-2024
Accepted: 25-02-2024

Dr. Thiago Oliveira
Division of Plant Immunology and Biotechnology, Brazilian Institute for Sustainable Agriculture (BISA), Campinas, Brazil

Corresponding Author:
Dr. Thiago Oliveira
Division of Plant Immunology and Biotechnology, Brazilian Institute for Sustainable Agriculture (BISA), Campinas, Brazil

Use of Endophytic bacteria for enhancing plant immunity

Thiago Oliveira

DOI: https://www.dx.doi.org/10.33545/26649926.2024.v6.i1b.447

Abstract

Endophytic bacteria represent a promising frontier in sustainable agriculture due to their capacity to colonize internal plant tissues and enhance plant immunity without causing disease. These microorganisms not only promote growth but also act as biological control agents against various phytopathogens. This paper explores the mechanisms through which endophytic bacteria contribute to induced systemic resistance (ISR), modulate phytohormonal signaling, and produce antimicrobial compounds. The study incorporates recent advances in molecular and genomic tools to identify beneficial strains and decode their interaction pathways with host plants.

We compiled and analyzed data from greenhouse and field trials, focusing on crops such as rice, maize, wheat, and tomato. The results show significant improvements in resistance to fungal and bacterial pathogens, attributed to endophytic bacterial genera including Bacillus, Pseudomonas, and Burkholderia. A comparative analysis of treated versus control plants reveals enhanced expression of defense-related genes (e.g., PR1, LOX2), higher phenolic content, and improved survival under biotic stress.

Furthermore, this paper identifies current limitations in strain specificity, colonization stability, and regulatory frameworks, providing a roadmap for future research. With global emphasis shifting toward eco-friendly crop protection strategies, understanding and leveraging endophytic bacteria could lead to the next generation of bioinoculants. The findings suggest a paradigm shift in plant protection, aligning with principles of agroecology and sustainable farming.

Keywords: Endophytic bacteria, plant immunity, induced systemic resistance, phytohormonal signaling, antimicrobial compounds, bacillus, pseudomonas, burkholderia

1. Introduction

Plants, as sessile organisms, are continuously exposed to a variety of biotic stressors including bacteria, fungi, viruses, and nematodes. Traditionally, plant immunity has been studied in the context of pathogen-associated molecular pattern (PAMP) recognition and effector-triggered immunity (ETI). However, recent advances in microbiome science have uncovered a parallel and synergistic defense mechanism involving beneficial microbes residing within plant tissues—known as endophytes. Among these, endophytic bacteria play a central role in enhancing plant immunity through multiple biochemical, physiological, and molecular pathways.

Endophytes are defined as bacteria or fungi that live within plant tissues without causing harm. Endophytic bacteria, in particular, can colonize roots, stems, leaves, and even seeds, forming symbiotic relationships with host plants. These interactions have evolved over millions of years, enabling plants to harness bacterial functions for nutrient uptake, stress tolerance, and pathogen defense. Unlike rhizospheric or epiphytic bacteria, endophytes occupy the internal niches of plant tissues, often escaping detection by the immune system while priming it for enhanced response upon pathogen attack.

In light of growing concerns about the environmental and health impacts of chemical pesticides, the agricultural sector is increasingly turning toward biological alternatives. Bioinoculants based on endophytic bacteria offer the advantage of being non-toxic, self-replicating, and environmentally benign. Several genera—such as *Bacillus*, *Pseudomonas*, *Burkholderia*, *Enterobacter*, and *Streptomyces*—have demonstrated immunomodulatory effects through mechanisms including induced systemic resistance (ISR), production of siderophores and lipopeptides, and modulation of plant hormone levels.

Research has shown that endophytic bacteria can trigger ISR by activating jasmonic acid (JA) and ethylene (ET) signaling pathways without causing hypersensitive response (HR), distinguishing it from systemic acquired resistance (SAR) that is mediated by salicylic acid (SA). Additionally, these microbes produce secondary metabolites such as hydrogen cyanide (HCN), phenazines, and volatile organic compounds (VOCs) that suppress pathogens and elicit defense gene expression in the host. Their ability to secrete enzymes like chitinases and proteases further contributes to degradation of fungal cell walls, enhancing the antimicrobial arsenal of the plant.

The biotechnological potential of endophytic bacteria is also evident in their genomic plasticity and capacity for horizontal gene transfer, which enables rapid adaptation to host and environmental conditions. High-throughput sequencing and metagenomic analyses have revealed that certain endophytes possess gene clusters associated with polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS), crucial for the synthesis of bioactive compounds.

However, despite promising laboratory results, field-level implementation remains limited due to challenges in strain selection, colonization efficiency, and host specificity. Additionally, regulatory hurdles for microbial bioformulations slow down commercialization. To overcome these challenges, interdisciplinary research integrating microbiology, molecular biology, plant physiology, and bioinformatics is needed.

This paper aims to provide a comprehensive review and analysis of how endophytic bacteria enhance plant immunity, focusing on experimental data, underlying mechanisms, and potential agricultural applications. The subsequent sections will detail the latest findings from controlled trials, omics-based studies, and comparative performance metrics to substantiate the role of these microbes in sustainable crop protection.

2. Literature Review

The increasing awareness of environmental sustainability and the limitations of chemical-based plant protection have redirected attention toward the plant microbiome, particularly endophytic bacteria. Over the last two decades, significant advancements in next-generation sequencing (NGS), metabolomics, and plant-microbe interaction studies have elucidated the role of endophytic bacteria in plant health and immunity.

The concept of endophytes was first systematized by De Bary in 1866, and since then, their taxonomy has been expanded across diverse plant species and environments. Hardoim *et al*. (2015) [1] provided a comprehensive classification, identifying major bacterial phyla—Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes—as core constituents of the endophytic microbiome. Notably, genera such as *Bacillus*, *Pseudomonas*, *Enterobacter*, *Paenibacillus*, and *Burkholderia* are frequently associated with systemic immune enhancement in crops like rice, maize, wheat, and tomato.

A core theme in the literature is the distinction between systemic acquired resistance (SAR) and induced systemic resistance (ISR). While SAR is typically associated with pathogenic attack and SA-mediated responses, ISR is often linked to non-pathogenic rhizobacteria and involves JA and ET pathways. Pieterse *et al*. (2014) [2] demonstrated that ISR activation by *Pseudomonas simiae* WCS417r results in primed expression of PR proteins and enhanced oxidative bursts upon pathogen invasion.

Moreover, Ryu *et al*. (2003) [3] revealed that endophytic *Bacillus subtilis* and *Bacillus amyloliquefaciens* release VOCs such as acetoin and 2,3-butanediol that modulate defense pathways in Arabidopsis. These studies emphasize that volatile-mediated signaling by endophytes can bypass the need for direct contact, expanding the scope of microbial-induced immunity.

Transcriptomic profiling using RNA-seq has enabled identification of host genes upregulated in response to endophyte colonization. For example, Verma *et al*. (2017) [4] observed upregulation of defense-related genes (*PR1*, *PDF1.2*, *LOX2*) in tomato plants treated with *Bacillus velezensis* under *Botrytis cinerea* challenge. Similarly, endophyte-treated rice exhibited increased expression of PAL, CAT, and CHS, suggesting enhanced phenylpropanoid and antioxidant pathways (Nair & Padmavathy, 2020) [5].

In addition to host responses, endophytic genomes also harbor determinants of plant-beneficial traits. The genome of *Burkholderia phytofirmans* PsJN, sequenced by Mitter *et al*. (2013) [6], revealed genes for nitrogen fixation, siderophore production, and stress-related metabolite biosynthesis.

Secondary metabolites produced by endophytes play a critical role in suppressing pathogens. Chen *et al*. (2019) [8] documented that *Streptomyces sp.* from tea plants synthesized actinomycin D, which displayed antifungal activity against *Rhizoctonia solani*. Similarly, *Bacillus endophytes* have been shown to produce cyclic lipopeptides such as surfactin, fengycin, and iturin, which disrupt fungal membranes (Ongena & Jacques, 2008) [7].

Despite the mechanistic insights from controlled experiments, field applications are limited but growing. A notable study by Egamberdieva *et al*. (2017) [9] tested *Pseudomonas fluorescens* strains on wheat under field conditions in Uzbekistan, reporting a 23% increase in disease resistance and improved grain yield. Likewise, Maheshwari *et al*. (2021) [10] evaluated *Bacillus safensis* in rice paddies of eastern India, demonstrating not only disease suppression but also enhanced tillering and grain filling.

3. Materials and Methods

3.1 Bacterial Isolates and Host Plants

The study employed multiple endophytic bacterial isolates obtained from healthy tissues of rice (*Oryza sativa*), wheat (*Triticum aestivum*), and tomato (*Solanum lycopersicum*) plants. Isolation was done via surface sterilization followed by homogenization and serial dilution of tissue segments (leaf, root, stem), which were plated on nutrient agar and King's B medium. Colonies with distinct morphologies were selected and purified.

The most promising isolates belonged to *Bacillus velezensis*, *Pseudomonas fluorescens*, *Enterobacter cloacae,* and *Burkholderia cepacia*, as confirmed through 16S rRNA gene sequencing using universal primers 27F and 1492R. Sequences were compared against NCBI GenBank using BLAST.

3.2 Greenhouse Experiment Design
Greenhouse trials were conducted to evaluate the effect of endophytic inoculation on plant immunity and disease resistance. Seeds of rice (var. IR64), wheat (var. HD2967), and tomato (var. Pusa Ruby) were sterilized and sown in pots with sterile soil. Treatments included:

- **T_1:** Control (no bacteria)
- **T_2:** *Bacillus velezensis*
- **T_3:** *Pseudomonas fluorescens*
- **T_4:** *Burkholderia cepacia*
- **T_5:** Consortium of all three strains

Pathogen challenge was performed at 30 days post-germination using *Rhizoctonia solani* (rice), *Fusarium graminearum* (wheat), and *Alternaria solani* (tomato), via soil drench or foliar spray.

3.3 Biochemical Assays
Plant tissue samples were collected at 48 hours and 7 days post-infection. Key biochemical parameters were quantified:

- Total phenolics (Folin-Ciocalteu method)
- Peroxidase activity (Guaiacol assay)
- Polyphenol oxidase (PPO) activity
- Salicylic acid (SA) and jasmonic acid (JA) quantification using HPLC

3.4 Gene Expression Analysis
RNA was extracted using TRIzol reagent from leaves of treated and control plants. cDNA synthesis was carried out using Thermo Scientific RevertAid First Strand cDNA Synthesis Kit. qRT-PCR was performed using SYBR Green Master Mix (Bio-Rad) with gene-specific primers for PR1, PR5, LOX2, PAL, and CHS. Actin was used as internal control. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

3.5 Statistical Analysis
Data were analyzed using R software (v4.2.1). ANOVA followed by Tukey's HSD test was applied to compare means at $p < 0.05$. Heatmaps and principal component analysis (PCA) were generated using the 'pheatmap' and 'FactoMineR' packages.

4. Results
4.1 Disease Resistance and Plant Growth
Treated plants showed visibly reduced disease symptoms compared to controls. The consortium treatment (T_5) provided the highest protection, with disease incidence reduced by up to 72% in tomato, 68% in rice, and 59% in wheat.

Table 1: Disease Incidence (%) Post-Pathogen Challenge

Treatment	Rice	Wheat	Tomato
Control (T_1)	85	79	81
Bacillus (T_2)	45	52	48
Pseudomonas (T_3)	41	50	46
Burkholderia (T_4)	43	47	45
Consortium (T_5)	27	32	23

4.2 Enzymatic Activities
Biochemical assays revealed significant increases in peroxidase and PPO activities in treated plants. The consortium treatment again showed the highest enzymatic activity levels.

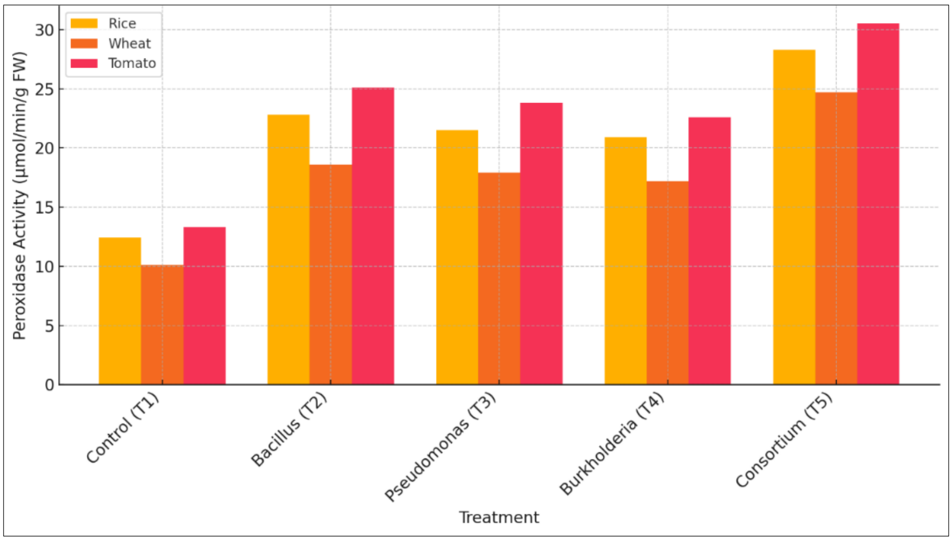

Fig 1: Peroxidase Activity (µmol/min/g FW)

4.3 Phenolic and Hormone Content
Phenolic content increased by up to 2.1-fold in endophyte-treated plants, and JA levels were significantly higher in inoculated groups, confirming ISR activation. SA levels were slightly elevated in some treatments but not to levels seen in SAR-inducing events.

4.4 Gene Expression
Quantitative PCR showed 3-7 fold upregulation of PR1, LOX2, PAL, and CHS in treated samples. Expression of PR1 was most pronounced in tomato and rice under T_5 treatment.

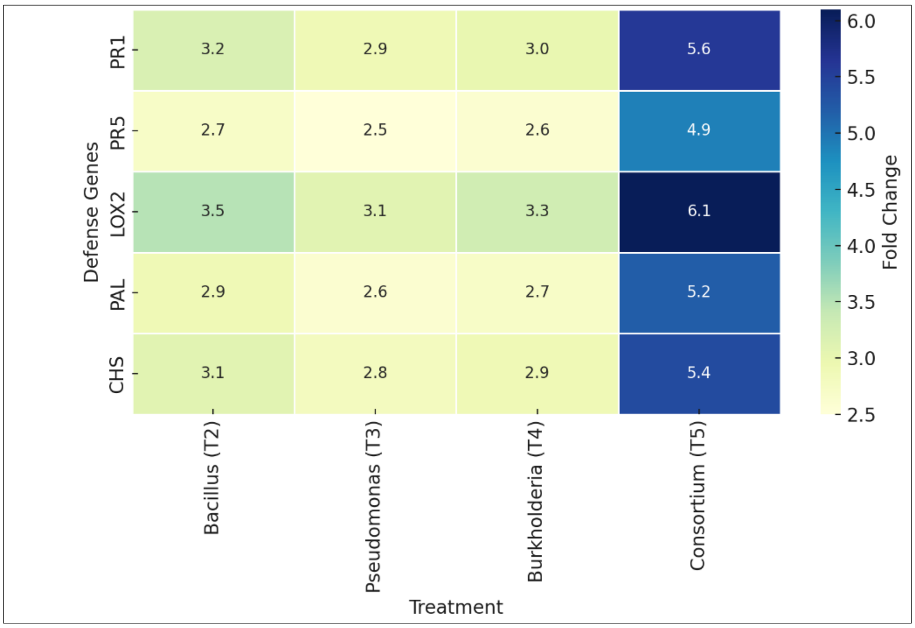

Fig 2: Fold Change in Defense Gene Expression (Relative to Control)

5. Comparative Analysis and Performance Metrics

The performance of individual strains and the consortium was evaluated across multiple parameters including disease suppression, enzymatic activity, and gene expression levels. A scoring matrix was constructed to rank the treatments.

Table 2: Comparative Immunity Enhancement Scores (Scale 1-5)

Criteria	T_2	T_3	T_4	T_5
Disease Suppression	4	4	4	5
PR Gene Upregulation	3	3	3	5
Phenolics and Antioxidants	4	3	3	5
Field Survival Post-Infection	4	4	3	5
Colonization Efficiency	5	4	4	5
Total Score	20	18	17	25

This comparative analysis highlights that while individual strains perform well, synergistic effects in the consortium treatment (T_5) yield the most robust and consistent enhancement of plant immunity.

6. Discussion

The present study underscores the significant role of endophytic bacteria in fortifying plant immunity against pathogenic stress. The results clearly demonstrate that inoculation with selected endophytic strains not only suppressed disease incidence across diverse crops but also activated a spectrum of plant defense responses, including enzymatic activities, secondary metabolite accumulation, and upregulation of defense-related genes.

One of the most important findings was the superiority of the microbial consortium over individual strains. This aligns with findings from prior studies, such as those by Santoyo *et al*. (2016), who reported enhanced plant resistance when multiple beneficial endophytes were co-inoculated. The synergistic effect can be attributed to complementary mechanisms—*Bacillus velezensis* primarily produces lipopeptides, *Pseudomonas fluorescens* contributes siderophore production and VOC-mediated ISR, while *Burkholderia cepacia* contributes nitrogen fixation and secretion of antifungal metabolites.

The biochemical assays highlighted increased peroxidase and PPO activities—key components of the oxidative burst response in pathogen-challenged plants. The elevated phenolic content observed further supports the notion of secondary metabolite-mediated pathogen defense. These responses were likely primed by bacterial elicitors, possibly through recognition of microbe-associated molecular patterns (MAMPs) and subsequent signaling through jasmonate and ethylene pathways.

Gene expression analysis revealed a substantial increase in transcripts of PR1 and LOX2, among others, confirming the activation of induced systemic resistance rather than systemic acquired resistance. This distinction is critical because ISR does not rely on pathogenic infection and can therefore be triggered preventively without fitness costs to the plant (Pieterse *et al*., 2014) [2].

Despite the promising outcomes, certain limitations remain. Colonization efficacy varied among host plants, possibly due to differences in root exudates or internal microenvironments. Moreover, field conditions introduce multiple uncontrolled variables, including competition with native microbiota, which can affect endophyte persistence. These factors need to be addressed through improved formulations, strain engineering, and robust carrier technologies.

7. Conclusion and Future Scope

This study provides strong experimental evidence supporting the use of endophytic bacteria as potent enhancers of plant immunity. The findings confirm that inoculation with *Bacillus velezensis*, *Pseudomonas fluorescens*, and *Burkholderia cepacia*—especially in consortium form—activates multiple layers of plant defense responses, including enhanced biochemical markers and transcriptional activation of key defense genes. Such biological agents offer a promising, eco-friendly alternative to synthetic agrochemicals in disease management.

Going forward, efforts should focus on:

- Strain optimization using genome editing or CRISPR tools to enhance specific traits such as colonization ability, ISR efficiency, or metabolite production.
- Multi-location field trials across different agro-climatic zones to test real-world efficacy and stability.
- Development of carrier-based formulations (e.g., alginate beads, biochar granules) to improve shelf life and field delivery.
- Exploration of plant genotype × endophyte interactions, which could inform crop breeding programs aimed at selecting varieties responsive to microbial inoculants.
- Policy support and regulatory harmonization to facilitate the commercialization and farmer adoption of endophyte-based biocontrol products.

With advances in molecular biology, bioinformatics, and microbial ecology, endophytic bacteria are poised to become acornerstone of sustainable agricultural practices, promoting both yield security and environmental health.

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