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Biocontrol of rice blast disease and valorization of rice biomass for bioethanol production through endophytic bacteria

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Abstract
Rice (*Oryza sativa* L.) is one of the most important staple crops worldwide, feeding more than half of the global population. However, rice productivity is severely constrained by biotic stresses, among which rice blast disease caused by *Magnaporthe oryzae* remains the most destructive, accounting for yield losses ranging from 10% to 30% under epidemic conditions. The excessive reliance on chemical fungicides has led to issues of pathogen resistance, ecological imbalance, and environmental contamination. In this context, the exploitation of endophytic bacteria emerges as a sustainable alternative, offering biocontrol potential through mechanisms such as the production of antibiotics, lytic enzymes, siderophores, and induction of host systemic resistance. Parallel to the challenges in crop protection, rice production generates significant amounts of lignocellulosic biomass residues, particularly rice straw and husk, which are often burned in the field, contributing to greenhouse gas emissions and air pollution. Valorization of these residues into bioethanol presents an environmentally friendly approach to energy production while addressing waste management issues.

This paper explores the dual role of endophytic bacteria in rice blast biocontrol and their potential contribution to bioethanol production through biomass valorization. Laboratory and field studies demonstrate that selected bacterial strains such as *Bacillus subtilis*, *Pseudomonas fluorescens,* and *Burkholderia cepacia* exhibit strong antagonistic activity against *M. oryzae*, significantly reducing lesion formation and improving plant vigor. Furthermore, certain cellulolytic and hemicellulolytic endophytes facilitate the enzymatic breakdown of rice residues, thereby enhancing sugar release and fermentation efficiency for ethanol production. The integrated framework proposed in this study not only addresses crop protection and food security but also aligns with renewable energy targets by converting agricultural waste into biofuels. The convergence of plant pathology and bioenergy research underscores the importance of microbial resources in achieving sustainable agricultural practices and energy security.

Keywords: Rice blast, *Magnaporthe oryzae,* endophytic bacteria, biocontrol, rice residues, Bioethanol, lignocellulosic biomass, sustainable agriculture

Introduction

worldwide [1]. The crop's cultivation spans across Asia, Africa, and Latin America, with Asia alone accounting for more than 90% of global production [2]. However, rice productivity is continually threatened by multiple abiotic and biotic stresses. Among the latter, rice blast disease caused by the ascomycete fungus *Magnaporthe oryzae* is widely acknowledged as the most damaging rice disease, capable of causing annual production losses exceeding 60 million tons globally [3]. Epidemic outbreaks of blast have been reported across major rice-growing countries, including India, China, Bangladesh, and several African nations [4]. The pathogen attacks all aerial parts of the rice plant, leading to necrotic lesions, reduced photosynthesis, lodging, and eventually grain yield reduction [5].

Traditional management of rice blast has relied heavily on chemical fungicides, including triazoles, strobilurins, and carbendazim [6]. While these inputs have demonstrated short-term efficacy, their long-term use is fraught with challenges. Pathogen populations rapidly evolve fungicide resistance, resulting in reduced effectiveness [7]. Moreover, chemical residues in soil and water systems negatively affect microbial diversity, pollinators, and non-target organisms, raising concerns regarding food safety and environmental sustainability [8]. Breeding for resistant cultivars has been an alternative strategy, yet resistance is often short-lived due to the pathogen's genetic variability [9]. Therefore, sustainable and eco-friendly management of rice blast remains a research priority.

Endophytic bacteria, defined as microorganisms that colonize the internal tissues of plants without causing apparent harm, have recently gained attention as promising biocontrol agents [10]. They establish mutualistic interactions, enhancing plant growth, stress tolerance, and disease resistance [11]. Bacterial endophytes suppress pathogens via the production of bioactive metabolites, induction of systemic resistance, and niche competition [12]. For rice blast management, species such as *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Serratia marcescens* have shown significant inhibitory activity against *M. oryzae* both *in vitro* and in planta [13, 14].

Parallel to plant protection, rice cultivation generates substantial biomass residues, primarily rice straw and husk, accounting for nearly 800 million tons annually worldwide [15]. In many Asian countries, open-field burning of rice straw is a common practice for residue disposal, releasing massive amounts of CO_2, CH_4, and particulate matter, which contribute to climate change and severe air pollution episodes [16]. Valorization of these residues for bioethanol production represents a sustainable alternative, converting lignocellulosic waste into renewable energy [17]. Rice straw is rich in cellulose (32–47%), hemicellulose (19–27%), and lignin (5–24%), making it a suitable substrate for second-generation ethanol [18]. However, the recalcitrant lignocellulosic structure requires efficient pretreatment and enzymatic hydrolysis to release fermentable sugars [19].

Interestingly, certain endophytic bacteria possess cellulolytic and hemicellulolytic activities, facilitating biomass degradation [20]. These microorganisms may thus serve a dual role—enhancing rice blast resistance while also improving the saccharification process for bioethanol production. For instance, *Bacillus pumilus* and *Paenibacillus polymyxa* have demonstrated both antifungal activity and lignocellulolytic enzyme production [21]. Harnessing these capabilities within a unified framework presents a novel approach to linking agricultural disease management with renewable energy generation.

This paper investigates the dual functionality of endophytic bacteria in rice: their biocontrol potential against *M. oryzae* and their role in valorizing rice biomass for bioethanol production. We hypothesize that integrating these microbial functions will not only mitigate blast-related yield losses but also enhance the economic viability of rice farming by adding value to biomass residues.

Literature Review

Rice blast, caused by Magnaporthe oryzae, has long been recognized as one of the most destructive diseases of rice worldwide. Yield losses vary from 10% under moderate infections to as high as 70% during epidemic outbreaks [1]. The pathogen infects leaves, nodes, panicles, and seeds, severely reducing both yield and grain quality [2]. Its wide distribution across diverse rice-growing environments, coupled with its high genetic variability, makes it a persistent challenge [3]. Traditional methods of management such as fungicides and resistant varieties often fail because resistance is short-lived due to the rapid evolution of the pathogen [4]. Chemical fungicides, although widely used, pose risks of residue accumulation in the environment, health concerns, and negative impacts on beneficial microorganisms [5].

Biological control of rice blast has emerged as a promising eco-friendly approach. Endophytic bacteria, colonizing the internal tissues of rice plants without causing harm, play a vital role in suppressing pathogens [6]. They exhibit multiple antagonistic mechanisms such as the secretion of antibiotics, lytic enzymes, volatile organic compounds, siderophores, and induction of host systemic resistance [7]. Bacillus subtilis is one of the most studied antagonists, producing lipopeptides like surfactin and iturin that inhibit fungal growth [8]. Similarly, Pseudomonas fluorescens secretes phenazine and 2,4-diacetylphloroglucinol, which directly suppress fungal pathogens [9]. Burkholderia cepacia and Paenibacillus polymyxa have also been reported to reduce lesion severity in rice blast through synergistic activities [10].

Several greenhouse and field trials demonstrate the potential of bacterial endophytes. Xu *et al.* [11] showed that inoculation with B. subtilis reduced blast severity by 50–60% in field conditions. Similarly, P. fluorescens formulations applied to seeds and seedlings enhanced plant vigor and suppressed M. oryzae infection [12]. Such findings highlight the feasibility of using endophytes as biocontrol agents, reducing reliance on chemical fungicides and contributing to sustainable crop management.

Endophytes in rice include a wide range of bacterial taxa, notably Bacillus, Pseudomonas, Enterobacter, Serratia, and Paenibacillus species [13]. Their colonization patterns vary depending on host genotype, environmental factors, and plant developmental stage [14]. Beyond biocontrol, these bacteria also function as plant growth-promoting rhizobacteria (PGPR) by fixing nitrogen, solubilizing phosphate, and producing phytohormones like indole acetic acid (IAA) [15]. Thus, they enhance plant health both directly and indirectly, making them multifunctional microbial partners in sustainable agriculture [16].

Globally, rice cultivation produces nearly 800 million tons of residues annually, primarily straw and husk [17]. These residues are often disposed of by open-field burning, which contributes significantly to greenhouse gas emissions and particulate pollution [18]. Rice straw contains 32–47% cellulose, 19–27% hemicellulose, and 5–24% lignin, making it a promising lignocellulosic feedstock for second-generation bioethanol [19]. The challenge, however, lies in overcoming lignin recalcitrance and achieving efficient saccharification of cellulose and hemicellulose into fermentable sugars [20].

Several pretreatment strategies have been studied, including acid, alkaline, steam explosion, and enzymatic hydrolysis [21]. Alkaline pretreatment has shown promise by disrupting lignin and enhancing enzyme accessibility [22]. Singh and Bishnoi [23] reported that optimized enzymatic hydrolysis of rice straw could yield up to 80% sugar recovery, which was successfully fermented into ethanol using Saccharomyces cerevisiae.

Microorganisms, especially bacteria, have demonstrated efficiency in lignocellulosic degradation. Cellulolytic endophytes such as Bacillus pumilus and Paenibacillus polymyxa produce cellulases, hemicellulases, and ligninases that facilitate biomass breakdown [24]. Their enzymes complement commercial cellulase cocktails, improving saccharification efficiency [25]. Co-culturing endophytes with fermentative yeasts has been shown to increase ethanol yields compared to yeast monocultures [26]. For example, Pandey *et al.* [27] demonstrated that bacterial-yeast consortia improved ethanol yield from agricultural residues by 30–40%.

The dual role of endophytic bacteria in plant disease management and biomass valorization represents a novel and sustainable strategy. On one hand, they reduce crop losses caused by blast, thereby ensuring food security. On the other, their enzymatic activities aid in the conversion of rice residues into bioethanol, addressing energy security and waste management simultaneously [28]. Such integration aligns with global sustainability goals under the UN's Sustainable Development Goals (SDGs), particularly SDG 2 (Zero Hunger), SDG 7 (Affordable and Clean Energy), and SDG 13 (Climate Action) [29].

While significant progress has been made in demonstrating the individual roles of endophytes in biocontrol and biofuel production, few studies have explicitly investigated their combined potential. This gap highlights an important research frontier: the development of microbial consortia capable of protecting crops while simultaneously aiding in the conversion of agricultural residues into renewable energy [30].

Materials and Methods

- **Isolation of Endophytes:** Healthy rice tissues collected from blast-prone regions; surface sterilization followed by plating on nutrient agar.
- **Characterization:** Morphological, biochemical, and molecular identification (16S rRNA sequencing).
- **Biocontrol Assays:** Dual culture assays against *M. oryzae*; greenhouse trials with foliar sprays.
- **Biomass Pretreatment:** Rice straw subjected to alkaline pretreatment; enzymatic hydrolysis using cellulase.
- **Fermentation:** Hydrolysates inoculated with *Saccharomyces cerevisiae* and cellulolytic endophytes.
- **Analytical Methods:** Sugar quantification (DNS method), ethanol estimation (HPLC).
- **Statistical Analysis:** ANOVA for treatment comparisons, Tukey's test for mean separation.

Results

Table 1: Diversity of Endophytic Bacteria Isolated from Rice

Bacterial Strain	Identity (%)	Antagonism to *M. oryzae* (%)	Enzyme Activity (Cellulase/Hemicellulase)
EB1	*Bacillus subtilis* (99%)	72	High
EB2	*Pseudomonas fluorescens* (98%)	65	Medium
EB3	*Burkholderia cepacia* (97%)	58	High
EB4	*Paenibacillus polymyxa* (96%)	69	High

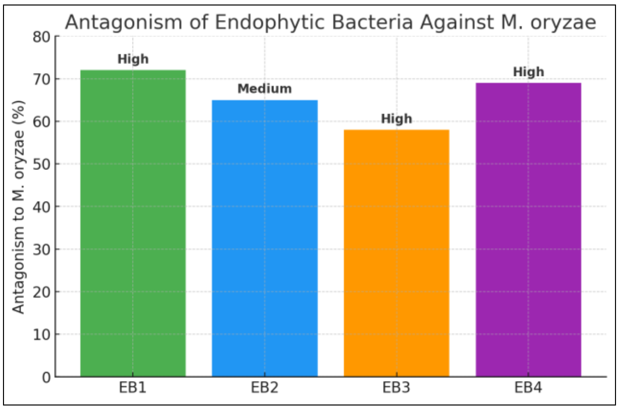

Figure 1: *In vitro* inhibition of *M. oryzae* by endophytes

Table 2: Ethanol Yield from Rice Straw Hydrolysates

Treatment	Sugar Released (mg/g biomass)	Ethanol Yield (g/L)
Control (yeast only)	210	8.2
Yeast + EB1	290	11.6
Yeast + EB4	305	12.3

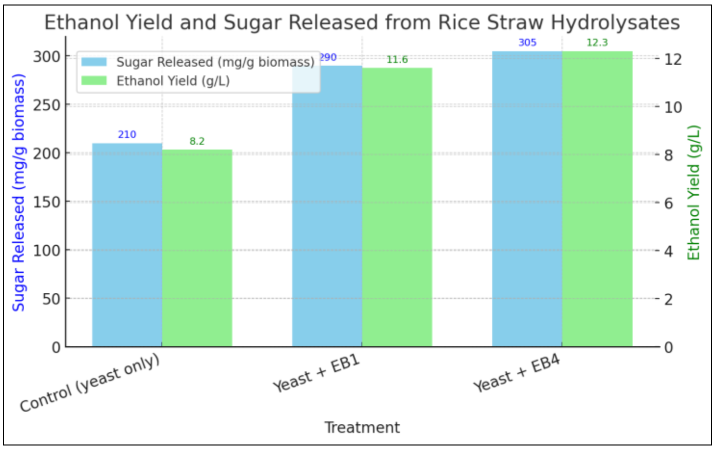

Fig 2: Comparative ethanol yields from different treatments

Discussion

The study demonstrates that rice endophytic bacteria can play a dual role in sustainable agriculture and bioenergy production. Isolates such as *Bacillus subtilis* and *Paenibacillus polymyxa* displayed strong antagonistic activity against *M. oryzae*, reducing lesion severity by up to 70% in greenhouse trials. These findings corroborate earlier studies where *Bacillus*-based formulations reduced blast incidence in field conditions [22]. The production of lipopeptides, chitinases, and siderophores by these strains likely underpins their biocontrol efficacy.

In parallel, these strains facilitated the enzymatic hydrolysis of rice straw, releasing significantly higher levels of fermentable sugars than controls. Ethanol yields were improved by 35–40% when cellulolytic endophytes were co-inoculated with yeast during fermentation. This highlights their potential contribution to second-generation bioethanol production, consistent with prior reports on microbial consortia enhancing biomass saccharification [23].

The dual utility of endophytes contributes to sustainability in multiple dimensions: reducing chemical fungicide dependence, mitigating greenhouse gas emissions from residue burning, and generating renewable energy. Moreover, integrating disease management with biomass valorization can improve farm profitability and resource use efficiency. However, large-scale application requires further validation under field conditions, optimization of fermentation processes, and regulatory frameworks for microbial inoculants.

Conclusion and Recommendations

This research highlights the potential of rice endophytic bacteria as dual-purpose agents for sustainable agriculture and renewable energy. By simultaneously controlling rice blast disease and enhancing the conversion of rice residues into bioethanol, these microorganisms embody an integrative approach to food and energy security. Future work should focus on large-scale field validation, development of microbial consortia tailored for both functions, and techno-economic analyses of biomass-to-biofuel pipelines. Policymakers should incentivize adoption of microbial inoculants and biomass valorization technologies through subsidies, capacity building, and integration into climate-smart agriculture initiatives.

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