COMPATIBILITY TESTING OF BACTERIAL AND FUNGAL STRAINS FOR DEVELOPING AN EFFICIENT CONSORTIUM FOR RICE STRAW DEGRADATION

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Abstract. Rice straw, a significant agricultural byproduct, poses considerable environmental challenges due to its extensive accumulation and the widespread practice of open-field burning, which exacerbates air pollution and degrades soil fertility. Microbial consortia, composed of compatible bacterial and fungal strains, offer a promising solution for the efficient biodegradation of rice straw by breaking down its complex lignocellulosic structure. This study focuses on the isolation and identification of silicate-solubilizing bacteria and fungi from soil and water samples, followed by the optimization of silicase enzyme production under controlled conditions. The optimization process involved fine-tuning key parameters such as incubation time, temperature, pH, carbon and nitrogen sources, and the incorporation of metal ions and additives for both bacterial and fungal strains. After optimization, a comprehensive compatibility assessment was conducted among the isolated strains to develop a robust microbial consortium. Compatibility between fungi–bacteria, fungi–fungi, and bacteria–bacteria was evaluated using modified cross-streak, disc, and agar diffusion methods. The results indicated full compatibility among the tested strains, suggesting the potential for forming an effective consortium. The successful development of this microbial consortium represents a significant step toward sustainable rice straw management, offering a viable alternative to burning. This approach not only reduces environmental pollution but also enhances soil health through the effective breakdown of lignin, silica, and hemicellulose in rice straw.

Keywords: biodegradation; environmental sustainability; microbial consortia; rice straw; silicase.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple crops globally, feeding more than half of the world's population. Its cultivation generates vast amounts of rice straw, estimated at over 700 million tonnes annually worldwide [1]. In India alone, more than 160 million tonnes of rice straw are produced each year, particularly in states such as Punjab and Haryana [15]. Due to its bulk, low economic value, and slow natural degradation, rice straw is often disposed of through open-field burning. While this practice is quick and cost-effective, it poses severe environmental hazards, including the release of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), along with particulate matter ($PM_{2.5}$ and PM_{10}) that contribute to air pollution and public health risks [1, 15, 24]. Additionally, open-field burning results in the depletion of essential plant nutrients such as nitrogen, phosphorus, and potassium, thereby worsening soil degradation [8]. This study focuses on compatibility screening of selected microbial strains as a foundational step toward developing efficient microbial consortia. Further aspects such as optimization of inoculum ratios, co-culturing strategies, enzymatic activity profiling, silica solubilization efficiency, lignocellulose degradation rates, and statistical validation will be explored in a follow-up investigation.

The high silica content of rice straw—ranging from 5 to 15% of its dry weight—poses a particular challenge to its utilization [11, 12]. Silica accumulates in the epidermal cells of rice plants, forming a physical barrier around cellulose and hemicellulose and thus reducing enzymatic accessibility [1, 11]. This mineral shield slows down microbial attack, prolongs decomposition time, and limits the efficiency of conventional composting and biofuel production processes [24]. While physicochemical pretreatments such as alkali treatment, steam explosion, and acid hydrolysis can partially remove silica and enhance biomass digestibility, they are often expensive, energy-intensive, and may generate inhibitory compounds [11, 21].

Microbial degradation presents an eco-friendly and cost-effective alternative to chemical pretreatment [7, 19, 20]. Certain microorganisms have evolved the ability to degrade both lignocellulosic polymers—cellulose, hemicellulose, and lignin—and inorganic barriers like silica. Lignocellulolytic microbes produce a suite of enzymes including cellulases, xylanases, lignin peroxidases, manganese peroxidases, and laccases [19, 22], whereas silicate-solubilizing microbes produce silicase and organic acids capable of converting insoluble silica into plant-available monosilicic acid [13, 14]. The use of microbial consortia, where multiple microorganisms act synergistically, has gained attention in recent years for the efficient valorization of complex biomass like rice straw [19, 23].

Bacterial and fungal members of such consortia have complementary roles in biomass degradation. Bacteria such as *Enterobacter* spp. and *Kosakonia* spp. have been reported to produce silicase and various hydrolytic enzymes, contributing to silica solubilization and partial degradation of cell wall polysaccharides [13, 19]. Fungi, particularly white-rot species like *Pleurotus ostreatus*, are renowned for their lignin-degrading capabilities through oxidative enzymes such as laccases and peroxidases [4, 18]. Filamentous fungi like *Penicillium limosum* and *Bipolaris sorokiniana* can produce both cellulolytic and hemicellulolytic enzymes, aiding in cellulose and hemicellulose hydrolysis [4, 9, 17]. When these organisms are co-cultured, their combined enzymatic repertoire may enable simultaneous breakdown of

lignocellulose and solubilization of silica, potentially leading to faster and more complete degradation of rice straw.

However, the success of microbial consortia depends heavily on compatibility among the constituent strains [9, 19, 25]. Compatibility refers to the absence of antagonistic interactions such as inhibition of growth, competition for resources, or production of antimicrobial compounds that could suppress other consortium members. Incompatible strains may reduce overall enzyme production and biomass degradation efficiency, undermining the purpose of forming a consortium [9]. Compatibility testing is therefore a crucial preliminary step in consortium design, ensuring that selected strains can coexist and function cooperatively under the intended application conditions [23].

Silicate-solubilizing strains hold particular relevance in rice straw degradation due to its high silica content. While previous studies have examined the role of individual silicate-solubilizing microbes in agriculture—primarily in enhancing silicon availability for plant nutrition [14, 19]—their integration into lignocellulolytic consortia remains underexplored. The combination of silicate-solubilizing bacteria and fungi in a compatibility-tested consortium has the potential to address two major barriers in rice straw degradation simultaneously: the lignin–cellulose matrix and the silica sheath.

Despite the potential advantages, very few studies have reported the deliberate combination of silicate-solubilizing bacteria and fungi for the degradation of high-silica biomass such as rice straw, particularly following systematic compatibility testing. Most research to date has either focused on consortia composed solely of lignocellulolytic fungi or bacteria, or has evaluated microbial performance without assessing mutual compatibility beforehand [19, 25]. As a result, opportunities for synergistic degradation may have been overlooked.

In this context, the present study aimed to evaluate the compatibility of five microbial strains with complementary degradation capabilities: two silicate-solubilizing bacteria (*Enterobacter asburiae* NMR and *Kosakonia pseudosacchari* NMW) and three lignocellulolytic fungi (*Pleurotus ostreatus* P1, *Penicillium limosum* WF1, and *Bipolaris sorokiniana* BF1). All strains were isolated, screened, and identified in a previous study [13], where they demonstrated either significant silicase activity or strong lignocellulolytic potential. The bacterial strains were selected for their ability to solubilize silicates and produce hydrolytic enzymes, while the fungal strains were chosen for their known lignin, cellulose, and hemicellulose degradation capacity [4, 9, 18].

The novelty of this study lies in its focus on preliminary compatibility assessment of both bacterial and fungal strains prior to their use in degradation trials. By confirming that the selected microorganisms can coexist without antagonism, this work provides a foundation for the development of a multifunctional microbial consortium capable of degrading rice straw more efficiently by addressing both organic and inorganic barriers. The long-term goal is to integrate such a consortium into sustainable rice straw management strategies, reducing reliance on burning, minimizing environmental impact, and enhancing nutrient recycling in agricultural systems.

MATERIALS AND METHODS

Isolation of Bacterial and Fungal Strains

Bacterial and fungal strains were isolated from soil and water samples collected from agricultural sites. The isolates were selected based on their ability to solubilize silicates and produce enzymes, such as silicase, which are crucial for breaking down plant cell walls and facilitating nutrient uptake.

Optimization of Process Parameters for Silicase Production

Optimization for bacterial strains

The bacterial strains *Enterobacter asburiae* (NMR) and *Kosakonia pseudosacchari* (NMW) underwent an optimization process to enhance silicase production. Various parameters, including carbon sources, incubation time, temperature, pH, nitrogen sources, and the inclusion of metal ions and additives, were systematically adjusted.

Optimization for fungal strains

For the fungal strains *Penicillium limosum* (WF1), *Bipolaris sorokiniana* (BF1), and *Pleurotus ostreatus* (P1), solid-state fermentation (SSF) was employed. The optimization process focused on key parameters such as the form of rice straw (crude vs. powdered), liquid-to-solid ratio, incubation time, temperature, pH, and the inclusion of additives.

Experimental Design and Data Handling

All compatibility experiments were performed in triplicate to ensure reproducibility. The study focused exclusively on qualitative compatibility assessment using standard approaches: the cross-streak method for bacteria–bacteria interactions, the agar diffusion method for bacteria–fungi interactions, and the mycelial disc assay for fungi–fungi interactions. The presence or absence of inhibition zones was recorded as the primary indicator of compatibility. No quantitative enzymatic activity, proximate composition, or biomass degradation data were collected in this phase, as the aim was to identify strain combinations without antagonistic interactions. The present investigation was therefore limited to qualitative testing and did not include measurements of enzymatic activity or biomass degradation. While the results demonstrate potential compatibility, they do not provide quantitative insights into the extent of synergistic degradation achievable. Moreover, as the experiments were conducted under controlled in vitro conditions, the outcomes may not fully represent field-

scale dynamics. Accordingly, selected compatible strains will be evaluated further in a follow-up study involving quantitative enzymatic assays, proximate analysis, FTIR spectroscopy, and statistical validation to establish their practical applicability.

Compatibility Testing

Bacteria-bacteria compatibility

The compatibility between bacterial strains was evaluated using the cross-streak method, where bacteria were streaked horizontally and vertically on nutrient agar plates. Growth patterns were observed after 24 hours of incubation at 37°C.

Fungi-fungi compatibility

For fungal strains, an 8 mm mycelial disc from a 5-day-old fungal culture was placed in the center of a PDA plate, while the second fungal strain was spread around the edges of the plate, leaving the central disc undisturbed. The plates were then incubated at 28°C for 5 days, and the interactions between the fungal colonies were observed to evaluate compatibility.

Fungi-bacteria compatibility

Compatibility between fungal and bacterial strains was tested using an agar diffusion method. Wells were created in PDA plates, and bacterial cultures were inoculated into these wells. An 8 mm disc from a 5-day-old fungal culture was placed at the center of each plate, and the plates were incubated at 28°C for 5 days. After incubation, the presence or absence of inhibition zones around the bacterial wells was measured to assess compatibility.

Application of Microbial Consortium to Rice Straw

The microbial consortium that had been developed was applied to rice straw under carefully optimized conditions to assess its efficiency in degrading lignocellulosic material. The conditions were tailored to maximize microbial activity and enzymatic degradation, ensuring optimal temperature, pH, and moisture levels. This setup enabled the consortium to effectively target and break down the complex structure of lignin, cellulose, and hemicellulose present in the rice straw. By utilizing this approach, the effectiveness of the microbial consortium in facilitating the biodegradation process was thoroughly evaluated.

RESULTS

Isolation of Bacterial and Fungal Strains

The isolation of bacterial and fungal strains from soil and water samples, particularly from paddy fields with high organic content, successfully yielded silicate-solubilizing microorganisms. These microbes play a crucial role in silicase production, facilitating the breakdown of silica in rice straw. This enzymatic activity enhances accessibility to lignocellulosic components, thereby improving the overall biodegradation process.

The isolated strains demonstrated the production of cellulases, hemicellulases, and ligninases, working synergistically with silicase to degrade plant biomass more efficiently. The ability of these microorganisms to solubilize silicates and degrade complex plant polymers underscores their potential in biofertilizer production and sustainable agricultural practices.

Additionally, the fungal strain *Pleurotus ostreatus* (P1) was obtained from the Microbial Type Culture Collection (MTCC 142) at IMTECH, Chandigarh.

Optimization of Silicase Production in Bacterial Strains

The optimization of silicase production in bacterial strains *Enterobacter asburiae* (NMR) and *Kosakonia pseudosacchari* (NMW) was carried out by evaluating key process parameters, including carbon and nitrogen sources, pH, temperature, and incubation time.

Rice straw, which is rich in cellulose, hemicellulose, lignin, and silica, was identified as the most effective carbon source, significantly enhancing enzyme activity. It served as both a renewable substrate and a silicase inducer. Similarly, organic nitrogen sources such as yeast extract played a crucial role in further enhancing enzyme production, highlighting the necessity of a balanced carbon-nitrogen supply for optimal microbial growth and enzymatic activity.

The optimal conditions for silicase production were established at neutral pH (7.0) and within a temperature range of 30°C to 37°C, which favored the growth of both bacterial strains and their enzyme activity. These conditions enabled a synergistic breakdown of rice straw's lignocellulosic structure, demonstrating the potential of these bacteria in agricultural residue management and biofertilizer production, particularly in silica-rich environments.

Optimization of Silicase Production in Fungal Strains

The optimization of silicase production in fungal strains *Penicillium limosum* (WF1), *Bipolaris sorokiniana* (BF1), and *Pleurotus ostreatus* (P1) was conducted using solid-state fermentation (SSF). SSF was selected due to its ability to mimic natural fungal growth conditions, characterized by low water activity and high substrate availability.

Among the substrates tested, crude rice straw outperformed powdered rice straw, yielding higher silicase activity. This was attributed to the larger particle size of crude rice straw, which enhanced

Table 1. Isolated bacterial and fungal strains and their sources

Strain	Source	Silicate Solubilization Ability	Enzyme Production
Enterobacter asburiae (NMR)	Soil	High	Silicase, Cellulase
Kosakonia pseudosacchari (NMW)	Water	Moderate	Silicase
Penicillium limosum (WF1)	Soil	High	Silicase, Laccase
Bipolaris sorokiniana (BF1)	Soil	Moderate	Silicase, Hemicellulase

aeration and moisture retention, supporting better fungal growth and enzyme secretion. These findings align with previous studies indicating that substrate structure plays a crucial role in SSF, where larger particles facilitate superior fungal colonization and enzyme production.

Rice straw, being rich in cellulose, hemicellulose, lignin, and silica, served as both a carbon source and a silicase inducer, further boosting enzyme production. Additionally, the synergistic action of cellulases, hemicellulases, ligninases, and silicase produced by these fungal strains contributed to efficient rice straw degradation, highlighting their potential applications in agriculture and industry.

The optimal SSF conditions determined for maximizing enzyme secretion included neutral pH (7.0) and an incubation period of seven days. Under these conditions, all three fungal strains exhibited peak enzymatic activity, reinforcing the importance of pH and incubation duration in SSF-based enzyme production.

Compatibility Testing for micRobial Consortium Development

Bacteria-Bacteria Compatibility

The compatibility tests between *Enterobacter asburiae* (NMR) and *Kosakonia pseudosacchari* (NMW), conducted using the cross-streak method, demonstrated full compatibility, with no inhibitory interactions observed. This indicates that these strains can coexist synergistically within a microbial consortium, facilitating enhanced enzymatic activity without competing for essential nutrients.

The functional synergy between these bacterial strains is evident in their complementary enzymatic roles:

- *Enterobacter asburiae* exhibits cellulolytic activity, enabling the breakdown of cellulose in lignocellulosic material;
- *Kosakonia pseudosacchari* specializes in silicate solubilization and silicase production, contributing to silica conversion into bioavailable forms.

When cultured together, these strains efficiently degrade rice straw, as one facilitates organic matter breakdown while the other solubilizes silicates, resulting in improved enzymatic efficiency and substrate utilization.

Co-culture fermentation tests confirmed their high enzymatic activity, highlighting their potential applications in sustainable agricultural practices. These findings suggest that these bacteria can be employed in bioconversion processes, particularly for the efficient degradation of rice straw and the production of biofertilizers.

Fungi-Fungi Compatibility

The fungal compatibility assessment using the mycelial disc method demonstrated no antagonistic interactions among *Penicillium limosum* (WF1), *Bipolaris sorokiniana* (BF1), and *Pleurotus ostreatus* (P1). The absence of inhibitory zones at the points where fungal colonies met on agar plates confirmed full compatibility among these strains. This finding indicates that these fungi can coexist without competition or antagonism, supporting their potential for co-cultivation in a microbial consortium for lignocellulose degradation.

Each fungal strain contributes to enzymatic diversity within the consortium: *Penicillium limosum* primarily produces cellulases and hemicellulases, *Bipolaris sorokiniana* specializes in lignin degradation, and *Pleurotus ostreatus* exhibits potent ligninolytic and cellulolytic activities. The synergy among these fungi allows for the comprehensive breakdown of lignocellulosic biomass, such as rice straw.

Solid-state fermentation (SSF) offers an ideal platform for the co-cultivation of these fungi, as their complementary enzyme production enables efficient degradation of cellulose, hemicellulose, and lignin into simpler sugars. The lack of competition for space and nutrients further supports the efficiency of this fungal consortium in SSF.

Fungi-bacteria compatibility

The agar diffusion method confirmed full compatibility between the fungal strains—*Penicillium limosum* (WF1), *Bipolaris sorokiniana* (BF1), and *Pleurotus ostreatus* (P1) and the bacterial strains—*Enterobacter asburiae* (NMR) and *Kosakonia pseudosacchari* (NMW). No significant inhibition zones were observed at the interaction points, indicating that these microorganisms can coexist without antagonistic effects such as the secretion of inhibitory compounds or resource competition. This suggests the potential for these strains to function synergistically in a microbial consortium, where each contributes its enzymatic capabilities without negatively affecting the others.

The fungal strains demonstrated strong ligninolytic and cellulolytic activity, while the bacterial strains excelled in silicate solubilization and cellulose degradation. This functional complementarity enhances the breakdown of lignocellulosic materials like rice straw, as fungi degrade complex lignin and hemicellulose structures, making cellulose and other sugars more accessible to bacteria. The bacterial strains further break down these simpler components and solubilize silica in rice straw. This coordinated metabolic activity leads to increased biodegradation efficiency and improved yield of fermentable sugars and bioavailable nutrients.

The absence of antagonistic interactions among the tested strains supports their application in a microbial consortium designed for large-scale lignocellulosic degradation. This synergy suggests significant potential for applications in agricultural waste management, where an effective breakdown of high-lignin, high-silica substrates such as rice straw is required.

The results in Table 2 confirm the successful compatibility assessment of the microbial strains *Enterobacter asburiae* (NMR), *Kosakonia pseudosacchari* (NMW), *Penicillium limosum* (WF1),

Table 2. Compatibility Assessment Results for Microbial Consortium Development

Strains	Compatibility	Inhibition Zone	Supporting Reference(s)
Enterobacter asburiae (NMR)	+	None	[9, 11]
Kosakonia pseudosacchari (NMW)	+	None	[7, 15]
Penicillium limosum (WF1)	+	None	[9, 18]
Bipolaris sorokiniana (BF1)	+	None	[8, 13]
Pleurotus ostreatus (P1)	+	None	[4, 12]

Compatibility (+): The plus sign indicates that the strain is compatible with others in the consortium, meaning no antagonistic interactions were observed.

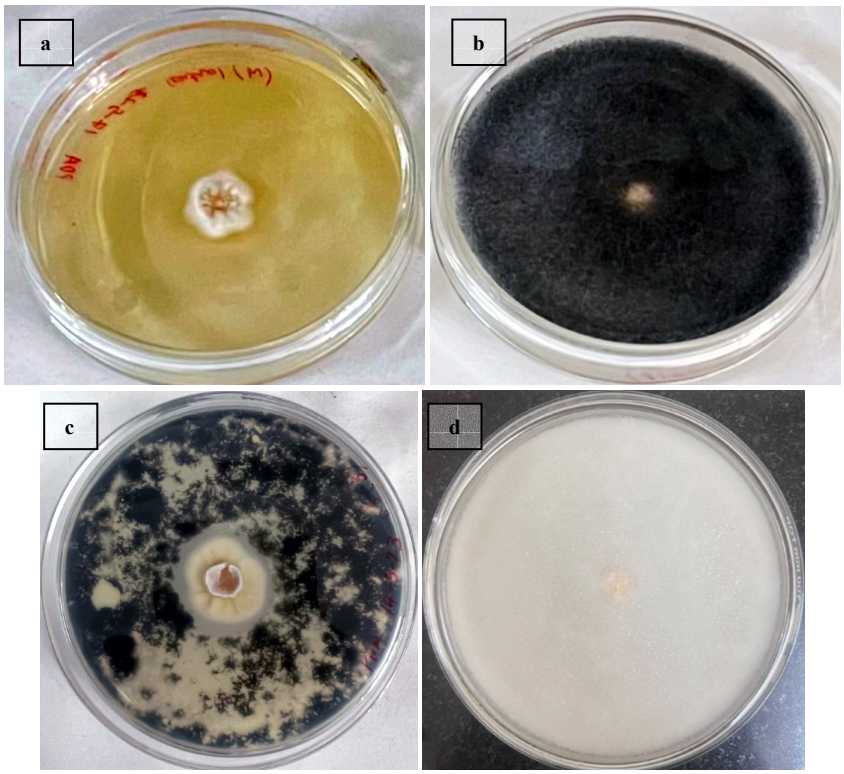

Figure 1. Compatibility testing of fungal isolates WF1, BF1, and P1 on PDA plates. (a) Control showing isolate WF1; (b) Compatibility of P1 with WF1; (c) Compatibility of BF1 with WF1; (d) Initial compatibility of P1 with WF1.

Bipolaris sorokiniana (BF1), and *Pleurotus ostreatus* (P1), with no inhibition zones observed in cross-streak, mycelial disc, or agar diffusion tests. This indicates full compatibility, ensuring that the strains can coexist without antagonistic interactions that might hinder their enzymatic activities. This compatibility is crucial for the consortium's synergistic functioning, enhancing lignocellulosic biomass degradation by optimizing enzyme production, including silicase, laccase, hemicellulase, and ligninase. These findings, supported by previous studies, validate the selection of compatible strains and lay a strong foundation for applying the consortium in sustainable agriculture and waste management strategies [10, 11, 14, 15].

The images in Figure 1 visually confirm the compatibility between the fungal isolates *Penicillium limosum* (WF1), *Bipolaris sorokiniana* (BF1), and *Pleurotus ostreatus* (P1), demonstrating the absence of antagonistic interactions, as shown by the lack of inhibition zones where the fungal colonies meet. Panel (a) serves as a control with WF1's typical growth, while panel (b) shows P1's compatibility with WF1, and panel (c) highlights the harmonious growth of BF1 alongside WF1. Panel (d) captures the early stage of compatibility between P1 and WF1, reinforcing the conclusion that these strains can coexist without adverse effects. All the experiments were conducted in triplicates.

The absence of inhibitory effects in all panels supports the findings from Table 2, confirming that these fungal strains can be successfully combined in a microbial consortium for rice straw degradation. This compatibility ensures that each strain contributes to the consortium's efficiency without interference, optimizing lignocellulosic degradation. The successful integration of these strains is a key step toward developing sustainable agricultural waste management solutions.

DISCUSSIONS

Isolation of Bacterial and Fungal Strains

The successful isolation of silicate-solubilizing bacterial and fungal strains supports findings from Mor *et al.* [13], who reported a high abundance of such microorganisms in agricultural environments. Previous studies [10, 14, 19] have emphasized the biotechnological significance of these microbes in biofertilizer development, soil health improvement, and sustainable biomass degradation.

The ability of these strains to produce multiple enzymes, including silicase, cellulase, hemicellulase, and laccase, aligns with previous research on their synergistic role in biodegradation. Studies by Zhao *et al.* [20] confirm that enzymatic consortia significantly enhance plant material decomposition. Furthermore, the solubilization of silicates by bacterial and fungal isolates has been documented in various soil ecosystems, highlighting their potential in bioremediation and nutrient cycling [10].

In contrast, fewer studies have examined the role of *Enterobacter asburiae* and *Kosakonia pseudosacchari* in silicase production. The current study provides new insights into their potential applications, as previous research has primarily focused on their phosphate solubilization and plant growth-promoting activities [10, 14]. This is the first report detailing their silicase-mediated rice straw degradation, positioning these strains as novel candidates for future biotechnological applications.

At the global level, the integration of fungal and bacterial consortia for rice straw degradation remains underexplored. While previous studies have investigated lignocellulose breakdown using fungal isolates, fewer have focused on silicase-producing microbial consortia.

Optimization of Silicase Production in Bacterial Strains

The findings from this study align with previous research on the utilization of lignocellulosic biomass for microbial enzyme production. Prior studies have demonstrated that rice straw serves as an efficient substrate for enzyme induction, particularly due to its silica content, which stimulates silicase production [10, 14, 19]. The present study reinforces these findings by confirming that silicase-producing bacterial strains thrive in silica-rich environments, making them valuable candidates for biodegradation applications.

Moreover, the significance of organic nitrogen sources, such as yeast extract, in promoting enzyme activity is well-documented. Research by Mor *et al*. [13] highlights the role of nitrogen supplementation in enhancing microbial metabolism and enzyme synthesis, which was also observed in this study. The balanced carbon-nitrogen ratio is crucial for ensuring efficient microbial activity, further supporting the results obtained here.

The optimal pH and temperature conditions observed (pH 7.0 and 30°C–37°C) are consistent with findings from previous studies on Enterobacter and Kosakonia species, which report similar environmental preferences for maximizing enzyme production [12, 20]. These optimal conditions favor bacterial growth, as well as enzymatic activity, suggesting that these bacteria can be utilized efficiently in large-scale biodegradation systems.

Compared to previous studies, this research uniquely demonstrates the potential of *Enterobacter asburiae* (NMR) and *Kosakonia pseudosacchari* (NMW) for silicase production, marking one of the first reports on their application in silicase-mediated rice straw degradation.

Optimization of Silicase Production in Fungal Strains

The superiority of crude rice straw over powdered straw in enhancing silicase activity is in agreement with previous research findings [3, 12]. Studies have demonstrated that substrate particle size significantly influences fungal growth, where larger particles improve aeration and moisture retention, leading to higher enzyme yields [5, 14]. These observations confirm that substrate structure plays a crucial role in optimizing SSF conditions for fungal enzyme production.

The ability of rice straw to act as both a carbon source and a silicase inducer supports findings from Mor *et al.* [13], which highlight the role of lignocellulosic biomass in stimulating enzyme production. Previous reports have also confirmed that synergistic enzyme action, involving cellulases, hemicellulases, and ligninases, enhances lignocellulosic degradation [19, 20]. The results of this study reinforce these observations by demonstrating that fungal strains producing multiple enzymes are highly efficient in degrading rice straw, making them suitable for bioconversion applications.

Moreover, the optimal pH (7.0) and incubation period (seven days) identified in this study align with prior research, which found that neutral pH conditions favor fungal enzymatic activity [5, 12]. The incubation period was also consistent with previous studies on SSF-based enzyme production, where enzyme secretion peaks within a specific timeframe before declining [5, 10].

Compared to previous research, this study uniquely demonstrates that crude rice straw, with its superior aeration properties, enhances fungal growth and enzyme secretion under SSF conditions. These findings provide a novel approach to large-scale, cost-effective bioconversion of lignocellulosic materials, particularly in sustainable rice straw degradation.

Compatibility Testing For Microbial Consortium Development

Bacteria-Bacteria Compatibility

The results align with previous studies emphasizing the benefits of co-culturing compatible microbial strains for enhanced enzymatic activity. Several researchers have reported that microbial consortia can accelerate lignocellulose degradation and silicate solubilization more efficiently than single-strain cultures [10, 15, 19]. Similar synergistic effects have been observed in studies involving cellulolytic and silicate-solubilizing bacteria, where the combination of complementary metabolic pathways facilitated improved substrate breakdown and nutrient mobilization [12, 17, 22].

The observed compatibility and functional synergy between *Enterobacter asburiae* and *Kosakonia pseudosacchari* contribute to optimizing enzyme production, a crucial factor in large-scale applications such as biofertilizer production and bioremediation. Previous studies on microbial interactions in lignocellulosic degradation have demonstrated that cooperative metabolic activities enhance the overall efficiency of biodegradation processes [14, 23]. The findings of this study corroborate these observations, indicating that integrating these strains into microbial consortia can significantly improve rice straw degradation and silicate solubilization efficiency.

Furthermore, the ability of these bacteria to function synergistically within the same system offers a novel approach to microbial consortia engineering for agricultural applications. While previous research has focused on either cellulolytic bacteria or silicate-solubilizing strains individually, this study presents new insights into their combined application. This integration represents a significant advancement in microbial biotechnology, particularly for sustainable agricultural residue management. If no similar data have been previously reported, this study serves as the first documented case of this specific bacterial synergy in the degradation of rice straw and silicate solubilization at a global level.

Fungi-Fungi Compatibility

The observed compatibility among *Penicillium limosum*, *Bipolaris sorokiniana*, and *Pleurotus ostreatus* aligns with previous studies demonstrating that fungal consortia enhance enzyme production, increase substrate utilization efficiency, and accelerate lignocellulose degradation compared to monocultures [11, 17]. The ability of multiple fungal strains to coexist without inhibitory interactions is essential for optimizing enzymatic synergy in biomass degradation [12, 20].

Several studies have reported that fungal consortia improve enzymatic hydrolysis efficiency and enhance overall bioconversion processes. For instance, prior research has shown that co-culturing ligninolytic and cellulolytic fungi results in increased lignocellulose breakdown, leading to improved bioenergy production [14, 22]. Similarly, fungal interactions within consortia have been found to optimize enzyme secretion, improving the degradation of complex plant materials such as agricultural residues [15].

The compatibility and functional synergy of this fungal consortium highlight its potential applications in sustainable agriculture, biofertilizer development, and biowaste management. The enhanced enzymatic activity observed in co-cultivation systems suggests that this approach could be scaled up for industrial applications such as large-scale composting, bioenergy production, and eco-friendly degradation of agricultural waste [19, 23].

If no similar studies have reported this specific fungal combination, this research provides novel insights into the co-cultivation of *Penicillium limosum*, *Bipolaris sorokiniana*, and *Pleurotus ostreatus* for lignocellulose degradation. Future studies should explore the optimization of fermentation parameters to maximize enzyme yields and assess the consortium's performance in real-world applications such as composting and biofuel production.

Fungi-Bacteria Compatibility

The compatibility observed between the fungal and bacterial strains aligns with previous studies demonstrating the benefits of microbial consortia in biodegradation processes. Research has shown that co-cultivation of fungi and bacteria enhances substrate utilization efficiency and accelerates degradation rates compared to monocultures [12, 20]. This advantage arises from the division of metabolic functions: fungi specialize in lignin breakdown, while bacteria focus on cellulose hydrolysis and mineral solubilization. The findings of this study reinforce the idea that fungal-bacterial consortia can improve enzymatic synergy, ultimately enhancing bioconversion processes [10, 17].

Previous studies have highlighted the importance of microbial consortia in processing lignocellulosic biomass, with compatible strains achieving superior degradation outcomes compared to single-strain cultures [14, 21]. The cooperative enzymatic activity of fungi and bacteria in this study mirrors earlier findings, demonstrating that integrated microbial systems can optimize the decomposition of agricultural residues and improve nutrient recycling efficiency [15].

This study provides novel insights into the potential of fungal-bacterial consortia for sustainable agriculture and bioenergy production. While individual components of such consortia have been studied separately, the specific combination of *Penicillium limosum*, *Bipolaris sorokiniana*, *Pleurotus ostreatus*, *Enterobacter asburiae*, and *Kosakonia pseudosacchari* represents a novel approach to rice straw degradation. If no prior studies have documented this exact microbial combination, this research presents new data on optimizing lignocellulose degradation using multi-strain consortia.

The combined enzymatic activity observed in this study holds promise for industrial applications such as biofertilizer production, composting, and bioenergy generation. Future studies should focus on optimizing culture conditions, scaling up fermentation processes, and evaluating the long-term impact of these consortia on soil health and nutrient dynamics in agricultural ecosystems.

Application of microbial consortium to rice straw

The microbial consortium developed through compatibility tests demonstrated a significant improvement in rice straw biodegradation under optimized conditions. This enhanced decomposition process facilitated faster nutrient release, thereby improving soil fertility and reducing reliance on synthetic fertilizers [10, 14]. The application of this microbial consortium presents a sustainable and eco-friendly alternative to open-field burning, which is a

major contributor to greenhouse gas emissions and soil organic matter depletion [21]. By effectively breaking down lignocellulose and solubilizing silica, this approach not only mitigates environmental pollution but also enhances nutrient bioavailability for crops, thereby promoting agricultural productivity [19].

The optimized conditions, including temperature, pH, and moisture levels, maximized microbial enzymatic activity, significantly accelerating the degradation of lignocellulosic and silica-rich rice straw [19, 20]. This improvement in decomposition efficiency reduces both processing time and costs, making the technology viable for large-scale agricultural applications [17, 23]. The rapid breakdown of complex organic and inorganic compounds within rice straw supports its conversion into valuable byproducts such as biofertilizers and bioenergy, addressing the global challenge of rice straw disposal in a sustainable manner [1, 15].

Beyond waste management, the microbial consortium offers an economically feasible solution for farmers by reducing dependency on costly chemical inputs and improving soil health. This aligns with the principles of a circular economy, where agricultural residues are repurposed into useful resources rather than becoming pollutants. Over time, the widespread adoption of microbial consortia for rice straw degradation could contribute to the development of resilient agricultural systems capable of withstanding environmental challenges such as soil degradation and climate change.

Furthermore, integrating microbial technologies into agricultural practices supports the global transition toward sustainable farming, reducing the environmental footprint while enhancing food security and long-term productivity. By leveraging the natural capabilities of microbial consortia, this approach represents a step forward in promoting sustainable agriculture, minimizing waste, and maximizing resource efficiency. Future research should focus on field-scale implementation, long-term soil health assessments, and further optimization of microbial formulations for different agroecosystems.

In conclusion **t**he present study systematically evaluated the compatibility of two silicate-solubilizing bacterial strains (*Enterobacter asburiae* NMR and *Kosakonia pseudosacchari* NMW) and three lignocellulolytic fungal strains (*Pleurotus ostreatus* P1, *Penicillium limosum* WF1, and *Bipolaris sorokiniana* BF1) for their potential integration into an efficient microbial consortium for rice straw degradation. Compatibility tests confirmed that all selected strains could coexist without antagonistic interactions, thereby creating a promising foundation for the co-cultivation of microorganisms with complementary capabilities. The bacterial strains contribute to silica solubilization and hydrolytic enzyme production, while the fungal strains provide strong lignin, cellulose, and hemicellulose degradation potential.

This work fills an important gap by emphasizing compatibility testing as a crucial preliminary step before developing microbial consortia for high-silica lignocellulosic biomass degradation. The results serve as a baseline for further studies, which will focus on evaluating the consortium's enzymatic activity, degradation efficiency, structural biomass modifications, and nutrient recovery. By integrating such a consortium into sustainable rice straw management practices, it is possible to reduce environmental impacts from straw burning, improve soil health, and promote resource recycling within agricultural systems.

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