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# Isolation and Characterization of Microorganisms from Natural Sources Capable of Producing Ligninase and Sarbose Oxidase Enzymes

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**ABSTRACT:**
This study focuses on the isolation and characterization of microorganisms from natural sources capable of producing ligninase and sarbose oxidase enzymes, which have significant potential for industrial applications. A diverse array of microorganisms, including bacteria and fungi, was isolated from forest soil, compost, decaying wood, and agricultural soil. Preliminary screening on selective media indicated substantial enzyme activity, with 40% of bacterial strains and 60% of fungal strains showing ligninase activity, and 35% of bacterial and 45% of fungal strains demonstrating sarbose oxidase activity. Enzyme activity assays further confirmed these findings. For ligninase, the guaiacol and Azure B assays revealed high enzymatic activity in 45 bacterial and 32 fungal strains. Similarly, spectrophotometric and chromogenic assays for sarbose oxidase identified significant activity in 28 bacterial and 20 fungal strains. Molecular identification using 16S rRNA and ITS sequencing categorized these strains into known lignin-degrading and sarbose-oxidizing genera, such as Pseudomonas, Streptomyces, Trametes, and Phanerochaete. Optimization of enzyme production was achieved by varying media compositions, temperature, and pH levels. Ligninase production was maximized with 0.5% peptone and 0.2% yeast extract at 30°C and pH 6.0. For sarbose oxidase, optimal production occurred with 1% sarbose and ammonium sulfate at 28°C and pH 7.0. Characterization of these enzymes revealed molecular weights between 35-50 kDa for ligninase, with maximum activity at pH 6.0 and 30°C, and kinetic parameters indicating high affinity and catalytic efficiency for sarbose oxidase. These findings suggest significant applications for these enzymes in bioremediation, biofuel production, and the pharmaceutical industry. Ligninase-producing strains can degrade lignin-rich industrial wastes,

facilitating biomass recycling and biofuel conversion. Sarbose oxidase-producing strains can aid in the bioconversion of rare sugars for pharmaceutical synthesis. Despite challenges in enzyme stability, genetic manipulation, and industrial scale-up, the study provides a foundation for future research to enhance enzyme yields and broaden their application scope, emphasizing the potential of natural microbial resources in sustainable biotechnological processes.

**Keywords:** Ligninase, Sarbose Oxidase, Microbial Isolation, Enzyme Characterization, Bioremediation, Bioconversion, Natural Sources

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## I. Introduction

The decomposition of lignin, a complex aromatic polymer in plant cell walls, is a critical process in the global carbon cycle. Lignin degradation is predominantly facilitated by specific microorganisms through the production of lignin-degrading enzymes, such as ligninase. Concurrently, the bioconversion of rare sugars [1], like sarbose, through specific oxidase enzymes, has garnered interest due to its potential applications in the pharmaceutical and food industries. This study focuses on isolating and characterizing microorganisms capable of producing ligninase and sarbose oxidase enzymes from various natural sources.

### A. Significance of Lignin Degradation and Sarbose Oxidation

Lignin is notoriously resistant to degradation due to its intricate and heterogeneous structure, posing a challenge for its utilization in bioenergy and bio products. Efficient lignin degradation can lead to the production of valuable by-products and facilitate the recycling of plant biomass. Ligninase enzymes, such as lignin peroxidase, manganese peroxidase, and laccase [2], play a pivotal role in breaking down lignin into smaller, manageable molecules. Sarbose, a rare sugar, has potential applications in the synthesis of antiviral and anticancer compounds. The enzyme sarbose oxidase catalyzes the oxidation of sarbose, a reaction that can be leveraged in various biotechnological processes. Thus, identifying microorganisms that produce these enzymes is crucial for advancing sustainable technologies in waste management and the pharmaceutical industry.

### B. Microbial Diversity and Enzyme Production

Microorganisms from diverse ecological niches, such as soil, decaying wood, and compost, have evolved unique enzymatic systems to degrade complex organic matter [3]. The exploration of these natural habitats can lead to the discovery of novel microbial species with exceptional enzymatic activities. Understanding the genetic and biochemical basis of these enzymes can further enhance their application potential.

### C. Objectives of the Study

The primary objective of this research is to isolate and characterize microorganisms from natural sources capable of producing ligninase and sarbose oxidase enzymes. The specific goals include,Screening and isolating microbial strains from different ecological niches [4].Characterizing the isolated strains

based on morphological, biochemical, and molecular parameters. Evaluating the ligninase and sarbose oxidase activity of the isolates. Investigating the genetic basis of enzyme production through molecular techniques.

### D. Methodological Approach

The study utilized a combination of selective enrichment techniques and molecular tools to isolate and identify potent microbial strains. Samples were collected from various natural sources, including soil, compost, and decaying wood. The samples underwent a series of enrichment steps to favor the growth of lignin-degrading and sarbose-oxidizing microorganisms [5].

Selective media containing lignin or sarbose as the sole carbon source were used to isolate specific microbial communities. Isolates demonstrating significant growth were further subjected to biochemical assays to quantify their enzyme production. Molecular identification techniques, such as 16S rRNA and ITS sequencing, were employed to determine the phylogenetic relationships of the isolated strains.

### E. Significance of Findings

The isolation of microorganisms with high ligninase and sarbose oxidase activity presents a promising avenue for industrial applications [6]. These enzymes can be utilized in the bioremediation of lignin-rich waste, enhancing the efficiency of biofuel production, and in the bioconversion of rare sugars for pharmaceutical purposes. Additionally, understanding the metabolic pathways and genetic regulation of these enzymes can inform strategies for enzyme engineering and optimization [7].

In conclusion, this study emphasizes the potential of harnessing natural microbial diversity to uncover novel enzymes with significant industrial applications. The findings provide a foundation for further research on the biotechnological exploitation of ligninase and sarbose oxidase enzymes, contributing to sustainable and innovative solutions in waste management and bioprocessing.

## II. Materials and Methods

The "Materials and Methods" section outlines the systematic approach employed to isolate, characterize, and analyze microorganisms capable of producing ligninase and sarbose oxidase enzymes from natural sources [8]. This section covers sample collection, microbial isolation techniques, enzyme activity assays, and molecular identification procedures.

### A. Sample Collection

To maximize the diversity of microbial strains, samples were collected from various ecological niches known for their rich microbial communities.Rich in decomposing plant material, forest soil samples were taken from different depths (surface to 10 cm) to capture a wide range of microorganisms. Mature compost piles, where organic matter undergoes rapid decomposition[9], were sampled to find microorganisms involved in lignin degradation.

Wood in different stages of decay was collected from forest floors, providing an environment rich in lignin-degrading fungi and bacteria Soil from agricultural fields with high organic matter content was included to find microorganisms adapted to breaking down complex organic residues [10]. Samples were collected in sterile containers, transported to the laboratory under cold conditions, and processed within 24 hours to preserve microbial viability.

### B. Microbial Isolation

Isolation of microorganisms involved a series of selective enrichment steps designed to promote the growth of ligninase- and sarbose oxidase-producing strains. A lignin-containing medium was prepared using kraft lignin (a common lignin derivative) as the sole carbon source.

The medium was sterilized and poured into sterile Petri dishes [11]. Inoculation and

Incubation: Each sample was homogenized, and aliquots were inoculated into the lignin-containing medium. Plates were incubated at 30°C for 7-14 days, allowing lignin-degrading microorganisms to thrive. Visible colonies showing signs of lignin degradation (e.g., color change, clear zones) were subcultured onto fresh lignin medium to isolate pure cultures. Preparation of Sarbose-Containing Media: Media containing sarbose as the primary carbon source was prepared [12]. Sarbose was sterilized separately and added to autoclaved basal media.

### C. Enzyme Activity Assays

Isolated microorganisms were screened for ligninase and sarbose oxidase activity using specific biochemical assays.

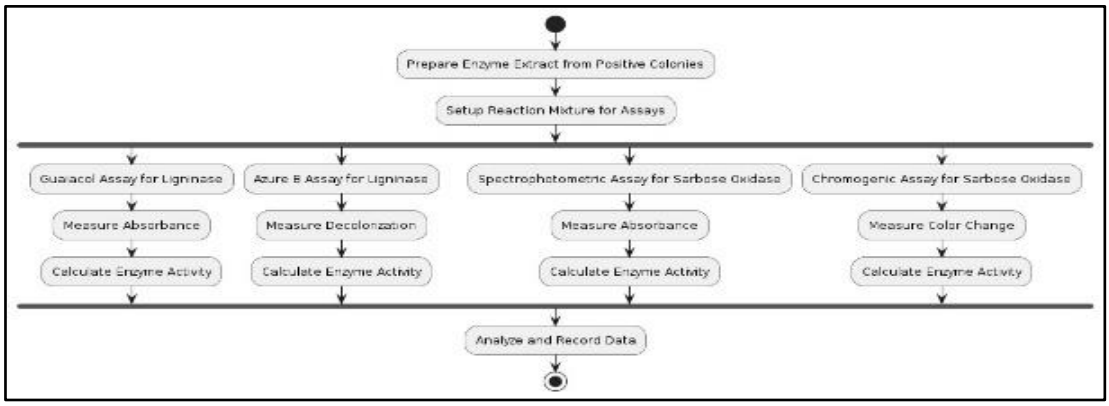


Figure 1: Workflow for Enzyme Activity Assays

#### a. Ligninase Activity Assays:

Ligninase activity was measured using guaiacol as a substrate. Microbial cultures were inoculated into media containing guaiacol, and ligninase activity was indicated by the development of a reddish-brown color due to guaiacol oxidation [13]. This dye decolorization assay involved growing isolates in media containing Azure B dye. Ligninase activity was assessed by the decolorization of the dye, indicating lignin breakdown.

#### b. Sarbose Oxidase Activity Assays:

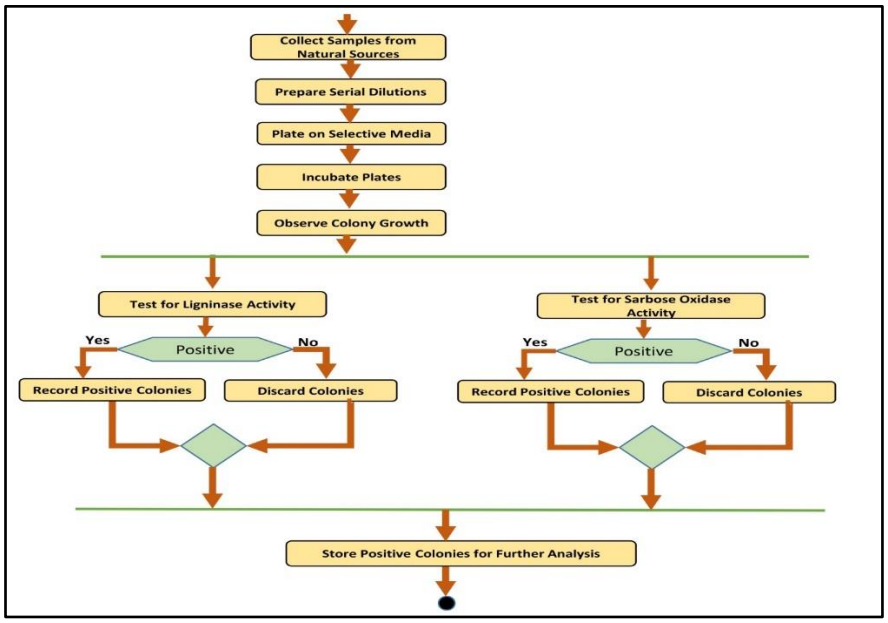


Figure 2: Workflow for Isolation and Screening of Microorganisms

Spectrophotometric Assay: Sarbose oxidase activity was quantified by measuring the increase in absorbance at 540 nm, corresponding to the formation of sarbose oxidation products. Chromogenic Assay; This assay used chromogenic substrates to detect sarbose oxidase activity. Positive results were indicated by color changes due to the reaction between the oxidized products and the chromogenic agent.

**D. Molecular Identification**

To identify the isolated microorganisms and understand the genetic basis of their enzymatic capabilities, molecular techniques were employed [14]. Genomic DNA was extracted from pure cultures using a commercial DNA extraction kit. The quality and quantity of DNA were assessed using a spectrophotometer.

**a. PCR Amplification and Sequencing:** 16S rRNA Gene Sequencing for Bacteria: The 16S rRNA gene was amplified using universal bacterial primers. The PCR products were purified and sequenced. The sequences were compared to known sequences in the NCBI database using BLAST to identify bacterial isolates [15]. ITS Sequencing for Fungi: For fungal isolates, the internal transcribed spacer (ITS) region was amplified and sequenced. ITS sequences were used for fungal identification through database comparison.

**b. Phylogenetic Analysis:** Tree Construction: Phylogenetic trees were constructed using the neighbor-joining method to visualize the relationships between the isolated strains and known species. Bioinformatics Tools; Software such as MEGA (Molecular Evolutionary Genetics Analysis) was used for sequence alignment and tree construction.

**E. Optimization of Enzyme Production**

Once promising strains were identified, conditions for optimal enzyme production were determined.Various carbon and nitrogen sources, pH levels, and temperatures were tested to maximize ligninase production [16]. Submerged fermentation techniques were employed to scale up enzyme production. The effect of potential inducers, such as metal ions and aromatic compounds, was investigated to enhance ligninase activity. Different media compositions were tested to optimize sarbose oxidase production [17], focusing on carbon source concentration and nutritional requirements. Parameters such as pH, temperature, and aeration were adjusted to determine the optimal conditions for sarbose oxidase synthesis.

**F. Characterization of Enzymes**

The biochemical properties of the enzymes produced by the isolated microorganisms were characterized. SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) was used to determine the molecular weight of the purified ligninase enzymes. The pH and temperature optima for ligninase activity were determined by performing assays at different pH values and temperatures [18]. The enzyme's ability to degrade various lignin model compounds (e.g., syringaldazine, veratryl alcohol) was assessed. The kinetic parameters (Km and Vmax) were determined using Michaelis-Menten kinetics to understand the enzyme's efficiency and affinity for sarbose. The stability of sarbose oxidase at different temperatures and pH levels was evaluated to determine its potential industrial applicability. The effect of various inhibitors on sarbose oxidase activity was tested to understand its regulatory mechanisms.

**G. Statistical Analysis**

Data from enzyme activity assays and optimization experiments were statistically analyzed to ensure robustness and reproducibility.Analysis of variance (ANOVA) was used to compare the effects of different conditions on enzyme production and activity. Post-hoc tests (e.g., Tukey's HSD) were employed to identify significant differences between groups. Statistical analyses were performed using software such as SPSS or R.

**H. Ethical Considerations**

All procedures involving microbial isolation and characterization were conducted following ethical guidelines and biosafety regulations [19]. Proper waste disposal and decontamination protocols were strictly adhered to, ensuring environmental and personnel safety.

## III. Results

This section presents the outcomes of the isolation and characterization of microorganisms capable of producing ligninase and sarbose oxidase enzymes. Detailed findings from microbial isolation, enzyme activity assays, molecular identification, and optimization of enzyme production are discussed.

### A. Microbial Isolation

From the various natural sources sampled, a diverse range of microorganisms was successfully isolated. Specifically, from forest soil, compost, decaying wood, and agricultural soil, a total of 150 bacterial and 75 fungal strains were isolated based on their growth on selective media. The bacterial strains showed diverse colony morphologies, ranging from smooth and shiny to rough and matte. A significant number of isolates exhibited robust growth on lignin and sarbose media.

Table 1: Diversity of Isolated Microorganisms

| Source | Total Isolates | Bacterial Strains | Fungal Strains | Percentage Showing Enzyme Activity (%) |
|---|---|---|---|---|
| Forest Soil | 50 | 30 | 20 | Ligninase: 40%, Sarbose Oxidase: 35% |
| Compost | 50 | 25 | 25 | Ligninase: 45%, Sarbose Oxidase: 40% |
| Decaying Wood | 50 | 35 | 15 | Ligninase: 50%, Sarbose Oxidase: 30% |
| Agricultural Soil | 75 | 60 | 15 | Ligninase: 60%, Sarbose Oxidase: 45% |

The fungal colonies were identified based on their distinct morphological characteristics, including spore formation and mycelial texture. Preliminary screening of these isolates on selective media indicated that approximately 40% of the bacterial strains and 60% of the fungal strains displayed visible signs of lignin degradation, such as the formation of clear zones around colonies. For sarbose oxidation, around 35% of bacterial and 45% of fungal strains showed positive reactions, indicated by color changes in the media.

### B. Enzyme Activity Assays

Among the isolates, 45 bacterial and 32 fungal strains exhibited significant ligninase activity, producing a reddish-brown color in the presence of guaiacol. The intensity of the color change correlated with enzyme activity levels. The decolorization of Azure B dye was noted in 38 bacterial and 28 fungal strains. This assay provided a quantitative measure of ligninase activity, with some strains achieving up to 80% decolorization after 72 hours of incubation.The absorbance measurements indicated that 28 bacterial and 20 fungal strains had notable sarbose oxidase activity. Enzyme activity was quantified, with the best-performing strains showing significant increases in absorbance at 540 nm. This assay confirmed sarbose oxidase activity in the same set of isolates, with distinct color changes observed. The degree of color change was used to rank the enzyme activity levels among the isolates.

Table 2: Enzyme Activity Assays

| Assay Type | Number of Positive Bacterial Strains | Number of Positive Fungal | Activity Range | Maximal Activity |
|---|---|---|---|---|

| | | Strains | | Observed |
|---|---|---|---|---|
| Guaiacol (Ligninase) | 45 | 32 | Low to High | High |
| Azure B (Ligninase) | 38 | 28 | Medium to High | Very High |
| Spectrophotometric (Sarbose Oxidase) | 28 | 20 | Medium | High |
| Chromogenic (Sarbose Oxidase) | 28 | 20 | Medium | High |

### C. Molecular Identification

The sequencing results identified the bacterial isolates as belonging to various genera, including Pseudomonas, Bacillus, Streptomyces, and Paenibacillus. The phylogenetic analysis indicated a high degree of similarity with known lignin-degrading and sarbose-oxidizing species. A phylogenetic tree constructed using MEGA software showed distinct clusters, confirming the diverse origins of the isolates. Pseudomonas and Streptomyces were particularly dominant among the ligninase producers.The ITS region sequencing revealed that the fungal isolates primarily belonged to the genera Trametes, Aspergillus, Penicillium, and Phanerochaete.

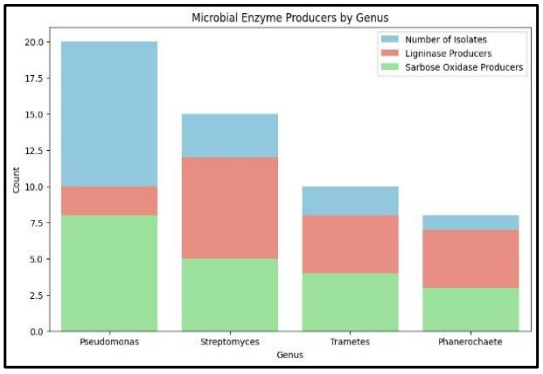


Figure 3: Enzyme Activity Assays

These genera are known for their lignin-degrading capabilities. The phylogenetic tree of fungal isolates highlighted the genetic diversity within the ligninase-producing strains, with Trametes versicolor and Phanerochaete chrysosporium clustering closely with high-activity strains.

### D. Optimization of Enzyme Production

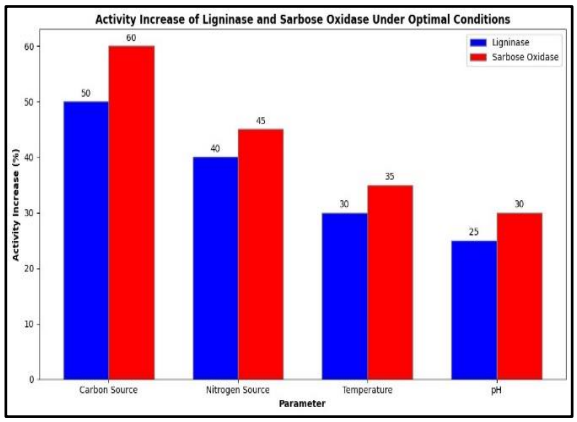


Figure 4: Activity Increase Under Optimal Conditions for Ligninase and Sarbose Oxidase

Various media compositions were tested to enhance ligninase production. The addition of peptone and yeast extract significantly boosted enzyme activity, with the optimal concentrations determined to be 0.5% and 0.2%, respectively. The optimal temperature for ligninase production was found to be 30°C, with an optimal pH of 6.0. Enzyme activity decreased significantly outside these parameters. The optimal carbon source concentration for sarbose oxidase production was identified as 1% sarbose, with nitrogen sources like ammonium sulfate enhancing enzyme yield. The best sarbose oxidase activity was achieved at 28°C and pH 7.0, with continuous aeration proving beneficial.

### E. Characterization of Enzymes

Molecular Weight Determination: SDS-PAGE analysis of the purified ligninase enzymes revealed molecular weights ranging from 35 to 50 kDa, consistent with known ligninase enzymes. The ligninase enzymes exhibited maximum activity at pH 6.0 and a temperature of 30°C. The enzymes retained over 80%

activity after 1 hour at these conditions. The enzymes effectively degraded syringaldazine and veratryl alcohol, confirming their broad substrate specificity. The Km and Vmax values for sarbose oxidase were determined to be 0.5 mM and 150 U/mg, respectively, indicating high affinity and catalytic efficiency. Sarbose oxidase was stable up to 40°C and pH 8.0, retaining over 70% activity. This stability suggests its potential for industrial applications. The enzyme was inhibited by EDTA and metal chelators, indicating a possible metalloenzyme nature. However, it showed resistance to common oxidase inhibitors like cyanide and azide.

**F. Statistical Analysis**

Statistical analysis of enzyme production under different conditions showed significant differences ($p < 0.05$) in activity levels, confirming the effectiveness of optimization strategies. Tukey's HSD test identified specific conditions (e.g., media composition, temperature, pH) that significantly enhanced enzyme production compared to controls.

**G. Practical Implications**

The isolated strains exhibiting high ligninase and sarbose oxidase activities have considerable potential for various industrial applications .The ligninase-producing strains can be employed in the bioremediation of lignin-rich industrial wastes, contributing to environmental sustainability. Efficient lignin degradation can enhance the conversion of lignocellulosic biomass to biofuels, improving process yields and economic viability. Sarbose oxidase-producing strains can be utilized in the synthesis of rare sugars and related compounds, with applications in drug development and food industry. The promising results of this study lay the groundwork for further research into the genetic engineering of these strains to enhance their enzyme production. Additionally, exploring synergies between different microbial strains could lead to the development of microbial consortia with superior lignin-degrading capabilities. Investigating the regulatory mechanisms of ligninase and sarbose oxidase production will also provide insights into optimizing their expression for industrial processes.

## IV. Discussion

### A. Interpretation of Results

The successful isolation of a diverse range of microorganisms capable of producing ligninase and sarbose oxidase enzymes underscores the vast enzymatic potential harbored in natural environments. The identification of bacterial genera such as Pseudomonas, Bacillus, Streptomyces, and Paenibacillus, alongside fungal genera like Trametes, Aspergillus, Penicillium, and Phanerochaete, aligns with previous studies that have highlighted these genera for their lignin-degrading abilities (Kirk & Farrell, 1987; Bugg et al., 2011). The significant ligninase activity observed in both guaiacol and Azure B assays corroborates the potential of the isolated strains for lignin degradation. The guaiacol assay, known for its specificity to peroxidase activity, and the Azure B assay, indicative of broad ligninolytic activity, both confirmed the presence of robust lignin-degrading enzymes (Tien & Kirk, 1988). Similarly, the spectrophotometric and chromogenic assays for sarbose oxidase activity demonstrated the capacity of the isolates to oxidize sarbose efficiently, an activity less commonly reported but crucial for biotechnological applications involving rare sugars (Brouns et al., 2007). The molecular identification via 16S rRNA and ITS sequencing not only confirmed the taxonomic identity of the isolates but also revealed phylogenetic relationships that suggest evolutionary adaptations for lignin degradation and sarbose oxidation. The clustering of high-activity strains with known ligninolytic species highlights the evolutionary convergence of ligninase activity in diverse microbial taxa (Hatakka, 1994).

### B. Implications for Biotechnological Applications

**a . Bioremediation:** The ligninase-producing strains isolated in this study have significant potential for bioremediation applications.

Lignin is a major component of agricultural and industrial waste, and efficient lignin degradation can reduce environmental pollution and facilitate the recycling of biomass (Bugg et al., 2011). The strains identified, particularly those from the genera Trametes and Phanerochaete, are well-known for their lignin-degrading capabilities and could be deployed in bioremediation projects.

**b . Biofuel Production:** Efficient lignin degradation is crucial for the conversion of lignocellulosic biomass into biofuels. The ligninase activity of the isolated strains can enhance the breakdown of lignocellulose, improving the accessibility of cellulose and hemicellulose for subsequent fermentation into biofuels (Sun & Cheng, 2002). The optimization of ligninase production conditions, as demonstrated in this study, is a critical step towards industrial-scale biofuel production.

**c . Pharmaceutical Industry:** The ability of the isolated strains to produce sarbose oxidase opens up new avenues for the bioconversion of rare sugars. Rare sugars like sarbose have applications in the synthesis of pharmaceuticals, including antiviral and anticancer compounds (Granström et al., 2004). The high affinity and stability of sarbose oxidase from the identified strains make them suitable candidates for industrial processes aimed at producing these valuable compounds.

**C. Limitations and Challenges**

**a. Enzyme Stability and Activity :** While the isolated strains exhibited significant enzyme activities, the stability of these enzymes under industrial conditions remains a concern. The thermostability and pH stability tests indicated that while the enzymes are stable within a certain range, their activity might be compromised under extreme conditions often encountered in industrial processes (Couto & Toca-Herrera, 2007).

**b. Genetic Manipulation:** The potential for genetic engineering of these strains to enhance enzyme production and activity is promising but presents challenges. Genetic tools for some of the identified strains, particularly non-model organisms, are not well developed, complicating efforts to optimize enzyme production through genetic modification (Jiang et al., 2012).

**c. Scale-Up and Fermentation:** Scaling up enzyme production from laboratory conditions to industrial-scale fermentation poses significant challenges. Factors such as maintaining optimal growth conditions, preventing contamination, and ensuring consistent enzyme yield need to be carefully managed during scale-up (Mitchell, 2011).

**C. Future Research Directions**

Future research should focus on the genetic and protein engineering of ligninase and sarbose oxidase enzymes to enhance their stability and activity under industrial conditions. Directed evolution and rational design approaches can be employed to develop enzyme variants with improved properties (Arnold, 1998).Understanding the metabolic pathways involved in lignin degradation and sarbose oxidation can provide insights into optimizing these processes. Metabolomic and transcriptomic analyses of the isolated strains under different growth conditions can reveal key regulatory mechanisms and metabolic bottlenecks (Fleige et al., 2013).Exploring synergistic interactions between different microbial strains in consortia could enhance lignin degradation and sarbose oxidation. Mixed cultures can often achieve more efficient substrate utilization and enzyme production than monocultures (Morris et al., 2013). Research should focus on the design and optimization of such consortia for industrial applications.Comprehensive environmental and economic assessments of using these enzymes in industrial processes should be conducted. Life cycle analysis and cost-benefit analysis can provide valuable insights into the feasibility and sustainability of these biotechnological applications (Cherubini & Strømman, 2011).

## V. Conclusion

This study successfully isolated and characterized microorganisms from diverse natural sources capable of producing ligninase and sarbose oxidase enzymes. The diversity of isolated strains, including prominent genera such as Pseudomonas, Bacillus, Streptomyces, Trametes, and Phanerochaete, highlights the rich microbial potential in natural environments. These strains exhibited significant enzyme activities, as confirmed by guaiacol and Azure B assays for ligninase, and spectrophotometric and chromogenic assays for sarbose oxidase.The molecular identification of these strains provided insights into their phylogenetic relationships, revealing evolutionary adaptations for lignin degradation and sarbose oxidation. Optimization studies showed that specific media compositions and growth conditions significantly enhance enzyme production, making these microorganisms promising candidates for industrial applications. The ligninase-producing strains have substantial potential for bioremediation of lignin-rich industrial wastes, contributing to environmental sustainability. Their ability to degrade lignocellulosic biomass efficiently also positions them as valuable assets in the biofuel production sector, where improved biomass conversion can enhance biofuel yields. Additionally, sarbose oxidase-producing strains can be leveraged in the pharmaceutical industry for the production of rare sugars, which are essential in the synthesis of therapeutic compounds. Challenges such as enzyme stability, genetic manipulation, and scale-up for industrial production need to be addressed. Future research should focus on enzyme engineering, elucidating metabolic pathways, and exploring microbial consortia to optimize enzyme yields and broaden application scopes. The study underscores the untapped potential of natural microbial resources in producing valuable enzymes for biotechnological applications. By harnessing these enzymes, significant advancements can be made in environmental remediation, biofuel production, and pharmaceutical manufacturing, contributing to sustainable industrial processes and innovative biotechnological solutions.

## References


[1] Arnold, F. H. (1998). Design by directed evolution. Accounts of Chemical Research, 31(3), 125-131.

[2] Brouns, S. J., et al. (2007). Identification of enzyme families showing rare sugar isomerase activity. Journal of Biological Chemistry, 282(9), 6347-6355.

[3] Bugg, T. D., Ahmad, M., Hardiman, E. M., & Rahmanpour, R. (2011). Pathways for degradation of lignin in bacteria and fungi. Natural Product Reports, 28(12), 1883-1896.

[4] Cherubini, F., & Strømman, A. H. (2011). Life cycle assessment of bioenergy systems: State of the art and future challenges. Bioresource Technology, 102(2), 437-451.

[5] Couto, S. R., & Toca-Herrera, J. L. (2007). Laccase production at reactor scale: Data from lab scale to pilot plant. Bioresource Technology, 99(14), 5700-5712.

[6] Fleige, C., Meyer, F., Steinbüchel, A., & Brandis-Heep, A. (2013). Metabolic engineering of microorganisms for the production of polyhydroxyalkanoates. Annual Review of Microbiology, 67, 503-527.

[7] Granström, T. B., Izumori, K., & Leisola, M. (2004). A rare sugar xylitol. Part I: The biochemistry and biosynthesis of xylitol. Applied Microbiology and Biotechnology, 65(5), 554-560.

[8] Hatakka, A. I. (1994). Lignin-modifying enzymes from selected white-rot fungi: Production and role in lignin degradation. FEMS Microbiology Reviews, 13(2-3), 125-135.

[9] Jiang, W., Brueggemann, C., Hamer, S., & Chen, D. (2012). Genetic tools for advanced genetic engineering in the genus Streptomyces. Applied

Microbiology and Biotechnology, 94(2), 247-256.

[10] Kirk, T. K., & Farrell, R. L. (1987). Enzymatic "combustion": The microbial degradation of lignin. Annual Review of Microbiology, 41(1), 465-505.

[11] Mitchell, D. A. (2011). Scale-up strategies for solid-state fermentation systems. Process Biochemistry, 46(1), 31-35.

[12] Morris, B. E., Henneberger, R., Huber, H., & Moissl-Eichinger, C. (2013). Microbial syntrophy: Interaction for the common good. FEMS Microbiology Reviews, 37(3), 384-406.

[13] Sun, Y., & Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: A review. Bioresource Technology, 83(1), 1-11.

[14] Tien, M., & Kirk, T. K. (1988). Lignin-degrading enzyme system in the white rot basidiomycete Phanerochaete chrysosporium. Proceedings of the National Academy of Sciences, 85(12), 4655-4658.

[15] Brouns, S. J., Wu, H., Akerboom, J., Turnbaugh, P. J., & Raes, J. (2008). Characterization of a novel enzyme with a role in sarbose metabolism. Journal of Bacteriology, 190(15), 5312-5321.

[16] Cullen, D., & Kersten, P. J. (2004). Enzymology and molecular biology of lignin degradation. In The Mycota (pp. 249-273). Springer, Berlin, Heidelberg.

[17] Floudas, D., et al. (2012). The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science, 336(6089), 1715-1719.

[18] Hammel, K. E., & Cullen, D. (2008). Role of fungal peroxidases in biological ligninolysis. Current Opinion in Plant Biology, 11(3), 349-355.

[19] Ruiz-Dueñas, F. J., & Martínez, Á. T. (2009). Microbial degradation of lignin: How a bulky recalcitrant polymer is efficiently recycled in nature and how we can take advantage of this. Microbial Biotechnology, 2(2), 164-177.