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# Why do some non-ligninolytic fungi possess laccase genes? And why is their expression weak?

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ABSTRACT

While laccase (EC\ 1.10.3.2) is well-established as a paramount copper-dependent oxidase heavily secreted by wood-decaying, ligninolytic fungi (specifically Basidiomycota), high-throughput sequencing (HTS) techniques have unexpectedly revealed laccase-like genetic signatures in non-ligninolytic fungi, like Ascomycota. The true knowledge gap emerges from the stark contrast between the presence of these genes and their minimal or completely absent extracellular enzymatic activity when measured in vitro, especially when compared to the highly efficient basidiomycetes. This discrepancy raises critical questions regarding their structural nature, cellular localization, and alternative physiological roles. In this review, we present a comprehensive and systematic comparison to bridge this knowledge gap. We begin by highlighting the strategic ecological and physiological roles of laccase in basidiomycetes (like white-rot fungi). Conversely, we delve into the specialized roles of laccases in non-ligninolytic fungi, revealing that their functions are entirely decoupled from carbon catabolism. Instead, they drive alternative physiological processes, such as the biosynthesis of DHN-melanin. On a molecular and regulatory level, we discuss the structural and genomic attributes underpinning this divergence. Finally, we shed light on the fundamental differences within the translated proteins. We explain that the low activity measured in non-ligninolytic fungi is largely due to the weakness or absence of secretory signal peptides. This leaves the enzyme intracellular, sequestered within vacuoles or spore walls to perform localized functions, unlike the extracellular laccases of basidiomycetes, which are heavily shielded by glycosylation. We also note the variation in redox potential between the two groups, dictated by the amino acid microenvironment surrounding the copper centers.

## 1. Introduction

The enzyme laccase (EC 1.10.3.2) is classified among the most important fungal enzymes and is one of the most important copper-dependent oxidases (multicopper oxidases) [1]. It plays a crucial role in the oxidation of many compounds, including phenolic and non-phenolic compounds [2,3]. This occurs using oxygen, which is the final electron acceptor [4]. In recent decades, this enzyme has received considerable attention from researchers in both environmental and industrial studies due to its prominent role in the breakdown of lignin compounds, particularly from fungal groups belonging to the Basidiomycota phylum [5].

Furthermore, in fungal groups not classified as ligninolytic, known as non-ligninolytic fungi, such as Ascomycota and some other fungi, a striking paradox has been observed by the detection of these enzymes, or similar enzymes [6,7]. This phenomenon has been confirmed by genomic studies and high-throughput sequencing techniques. This discovery leads to further investigation into the true role of these genes and the reasons for their low gene expression compared to known ligninolytic fungi [8].

This enzyme, composed of a group of proteins, is perfectly structured to break down very complex compounds such as phenolic or non-

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