Expression of fungal laccases in *Pichia pastoris* and characterization using lignin depolymerization

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Project Goals: This work demonstrates that we can heterologously express lignin-degrading enzymes (laccases in this case) that are active on lignin-like compounds and catalyze the cleavage of specific bond types critical to depolymerizing lignin.

Abstract
Enzymatic cocktails from white fungi secretomes are potentially an efficient and cost-effective approach to developing mixtures of ligninolytic enzymes for biorefinery implementation, but complex interactions among the different agents highly can lead to difficulty in condition optimization and mechanism elucidation. Instead of mixed secretion, heterologous expression of individual genes is a valuable approach to understand the structure-function relationships of these important enzymes. Herein, we report structure-based selection of three novel laccases, designated as Cer_Lc1, Cer_Lc2, and For_lac from white-rot fungi, expression in *Pichia pastoris* and characterization of their catalytic performance, thermal stability, and solvent stability. This work aimed to improve our understanding of the mechanism underlying the enzymatic degradation of lignin by monitoring and quantifying β-O-4 ether, C_α-C_β, and C_α-C_{aryl} bond cleavage in a model lignin-like dimer synthesized for use in a nanostructure-initiator mass spectrometry (NIMS) assay. We present detailed studies of the effect of a natural mediator, syringaldehyde, on bond cleavage occurrence. This study also provides a comprehensive understanding of the structure-function and the structure-stability relationship of these novel fungal laccases, which will facilitate developing this important class of enzymes for applications in the conversion of lignin to valuable products and use in biorefinery implementations.

References

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