Localization of wood-degrading enzymes on natural samples

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The transition to renewable resources for energy and raw materials is a key realization in the ongoing struggle towards a sustainable and environmentally friendly way of life. Lignocellulosic biomass holds the unique role of being the only renewable carbon source and will therefore become the major source for organic bulk and fine chemicals [1]. However, the recalcitrant structure of lignocellulose makes its degradation and decomposition a challenging process [2]. In nature, wood-degrading fungi have evolved a versatile set of specialized enzymes for the efficient separation and depolymerization of (hemi)cellulose and lignin. The classical concept of wood breakdown by polysaccharide-degrading hydrolases is well studied but only presents an incomplete picture of the whole process. It is well accepted that also many redox-active enzymes are essential for the complete decomposition of lignocellulose and that they all act in a concerted manner [3].

For a better understanding of their mode of action, investigations to reveal their interactions are required. In our ERC Consolidator Grant-funded project "OXIDISE" we aim to shed light on the localization and distribution of fungal enzymes on natural wood samples. We identified key enzymes from the white-rot fungus *Phanerochaete chrysosporium* and expressed them heterologously. In the course of that, a cysteine residue was introduced on the surface of each protein to enable fluorescence labeling through a maleimide coupling reaction [4]. To allow for simultaneous imaging of several enzymes on one sample, different fluorescent dyes of the Thermo Scientific DyLight series were selected for labeling. Dependent on the enzyme and fluorophore used, degrees of labeling between 20 and almost 50% were achieved. Protein preparations were applied to poplar wood samples of different orientations and cell structures, and the localization of the enzymes was imaged and analyzed using a laser scanning confocal fluorescence microscope.

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