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Enzyme biomass combustion by shared oxidation and hydrolysis mechanisms

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Biomass is abundant and renewable, useful in biochemical commodity production systems if polymers may perhaps be extracted. *Myceliophthora thermophila* secretes in addition to the classic hydrolytic enzymes an array of oxidoreductases, some of whose functions remain unknown. The objective of this work was to determine the nature of polysaccharide decomposition mechanisms used by *M. thermophila*. Liquid chromatography-tandem mass spectrometry was employed in quantitative evaluation of secretome compositions. A molecular genetics manipulated protein overproduction and secretion system combined with high-resolution chromatography purification was employed to obtain pure proteins. Advanced spectroscopic electron-transfer enzymology (dye-coupled reduction/oxidation of heme and flavin domains) was used to determine how electrons flow in the system. To determine enzymatic products and intermediates (oxidized sugars, H_2O_2, OH^-, etc.) HPLC, HPAEC, LC-MS/MS, MALDI-TOF and liquid chromatography was used. Three-dimensional protein structure and protein intermediate folding states were deduced from X-ray crystallography and SAXs experiments. The most abundant enzyme in *M. thermophila* secretomes grown on natural cellulosic substrates was cellobiose dehydrogenase followed by three cellobiohydrolases, a beta glucosidase, an aryl-alcohol oxidase, an aldose epimerase and a glyoxal oxidase. Cellobiose dehydrogenase, a hemoflavoenzyme oxidized cellobiose to cellobiolactone in the flavin domain, which interacted with high redox potential aryl-alcohol and glyoxal oxidase as well as at least three low abundant polysaccharide mono oxygenases producing excessive amounts of hydrogen peroxide and oxygen radicals that resulted in non-selective Fenton like oxidation of glycosidic bonds. Our findings show that *M. thermophila* preferably oxidizes biomass glycosidic linkages rather than hydrolyzing them.

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Rapid and accurate identification of clinically significant fungi by molecular methods

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Rapid and accurate identification (ID) of clinically significant fungi is essential and DNA sequencing of specific gene has become an important tool for fungal molecular ID. The molecular methods are used to solve varies diagnostic dilemma related to the fungi infection such as: Sensitivity and accuracy of testing: Although the internal transcribed spacer (ITS) and domains 1 and 2 of the large ribosomal subunit (D1/D2) are recommended for fungi identification by the CLSI guideline MM18-A, these targets may not be sufficient to discriminate between certain species. For example, the identification of *Aspergillus,* a clinically important species in immunocompromised patients may be enhanced by sequencing of the beta tubulin and calmodulin targeting regions. Similarly *Fusarium* species and potential human pathogen, causing local or disseminated infections in immunocompromised patients can be identify by Sanger sequencing of ITS, translation elongation factor and RNA polymerase II second largest subunit. Distinction pathogenic from non-pathogenic organisms *R. arrhizus* causes a local infection of the lungs, brain, etc., and severe systemic mycosis. Closely related and morphologically indistinguishable *R. Delemar* may have a different pathogenesis and antifungal susceptibility. They can be separated by ITS sequence. Drug susceptibility: *Azole antifungals* (*itraconazole, voriconazole, posaconazole* and *isavuconazole*) are effective treatments for invasive aspergillosis. However, drug resistance in *A. fumigatus* has been reported and can be identified by a testing for *cyp51A* missense mutations. Sanger sequencing is an effective supportive diagnostic method for fungi identification, differentiation between pathogenic and non-pathogenic organisms and for assessment of susceptibility to the anti-fungus treatment.

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