**STRUCTURAL CHARACTERIZATION OF A NOVEL LACCASE-LIKE MULTICOPPER OXIDASE FROM *THERMOTHELOMYCES THERMOPHILUS***

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**ABSTRACT**

In this study, we report complete structure analysis of a novel laccase-like multicopper oxidase from the thermophilic fungus *Thermothelomyces thermophila* (*Tt*LMCO1).

Laccases (EC 1.10.3.2) are multicopper oxidases that oxidize phenolic substrates using O2 as electron acceptor. The wide substrate spectrum of laccases makes them ideal candidates for a variety of applications such as bioremediation, biocatalytic synthesis and biosensors [1]. However, the low selectivity against potential substrates hampers their use for the biocatalytic synthesis of valuable chemicals. Intensive research led to the discovery of laccase-like multicopper oxidases (LMCOs), that in contrast to known laccases, have low redox potentials (E0), leading to a more narrow substrate range [2].

*Tt*LMCO1 was previously biochemically characterized and found to be a thermostable, acidophilic enzyme, with satisfactory stability against organic solvents, capable of oxidizing phenolic compounds and amines. Also, the redox potential of the enzyme was determined [4], classifying it to low-redox potential oxidases. *Tt*LMCO1 performed the cyclization reaction of 2′,3,4-trihydroxychalcone to the respective aurone with satisfactory yield (48%) [3], displaying improved selectivity to traditional laccases. *Tt*LMCO1 can thus be exploited in biocatalytic applications for the production of novel compounds with bioactive properties.

In this work we report the crystal structure of *Tt*LMCO1, which was determined in order to identify the structural characteristics that append the aforementioned properties to the enzyme. The recombinant enzyme was purified, deglycosylated and and crystallized using the vapor diffusion method. X-ray data to 1.9 Å resolution were collected at EMBL beamline P13. The groundbreaking algorithm Alphafold 2.0 [5] was used to generate a starting model for molecular replacement (MR) and structure determination. The resulting initial model led to a successful structure solution, while refinement led to a final model with Rwork/Rfree 0.17/0.20. The final structure is under deposition in the Protein Data Bank.

**KEYWORDS:** Biocatalysts, LMCO, Protein X-ray Crystallography, Structure

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