Master Research Project

Turning hyperthermostable metallo-oxidases into laccases by directed evolution

Multicopper oxidases are multifunctional enzymes that can be broadly divided into two functional classes: metallo-oxidases (with a higher activity towards metals, such as Cu⁺ or Fe²⁺) and laccases (with a superior efficiency for organic compounds). Laccases are green catalysts with an outstanding redox capability over a wide range of aromatic substrates using O₂ as an electron acceptor and releasing water as reduced product [1].

In this study, a laboratory evolution approach will be conducted to improve the efficiency of the metallo-oxidase McoP from the hyperthermophilic archaea *Pyrobaculum aerophilum* for aromatic compounds, in particular for phenolic compounds [2]. Directed laboratory evolution is a powerful protein engineering tool to tailor biocatalysts with improved features or new functions [3, 4]. By mimicking the principles of natural selection through iterative rounds of random mutagenesis and/or DNA recombination and screening, the epical time scale of evolution can be shortened to an experiment which can be conducted in the laboratory. One of the major technologies underlying synthetic biology is the use of directed evolution for creating novel biocatalysts aiming at the synthesis of chemicals or degradation of toxic pollutants able to replace current polluting processes based on petrochemical feedstocks, organic solvents and heavy metal catalysts.

These studies will allow (i) obtaining hyperthermostable laccases that are highly in demand for their robustness in biotechnological applications and (ii) a better insight on the structure-function relationships within the multicopper oxidase family including the mechanisms of multifunctionality. This has several general implications in the understanding of molecular recognition to ligands, e.g. for drug discovery programs and in the engineering of proteins in the realm of biotechnology, on the synthetic biology field.

**Areas:** Microbiology/Biochemistry/Enzymology/Biotechnology/Molecular Biology

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**References:**