Microbial and Enzyme Technology Lab

Master Projects 2012

Enzyme Engineering for Biotechnological Applications

Project 1 – Unravel the structural bases for azoreductases promiscuity

The goal is to probe structure–function relationships that are determinants of substrate specificity and mechanisms of promiscuity among the family of flavoproteins. The azoreductase PpAzoR from *Pseudomonas putida* MET94 shows a high non-specificity towards the degradation of synthetic dyes. Flavin-dependent azoreductases, such as PpAzoR, share strong similarities with regard to sequence, structure, and reaction mechanism with the large family of quinone reductases that includes Lot6p from *S. cerevisiae* and the mammalian NQO1. These enzymes are involved in the reduction of quinones, quinoneimines, azo dyes, and nitro groups and are assumed to take part in the organism's enzymatic detoxification systems. Mutagenic and kinetic studies with PpAzoR, for which a crystal structure is already available, will be used to study the molecular determinants of the enzyme promiscuity. Understanding the relative contributions of substrate binding vs. chemistry will promote our understanding of enzyme evolution and of ligand-protein interaction in general. These issues also have biotechnological implications, because substrate ambiguity and catalytic promiscuity provide the basis of biocatalytic applications.

Project 2 – Improve the stability of peroxidases towards hydrogen peroxide inactivation

The goal is the development of efficient and robust bacterial DyP type peroxidases that can act as biocatalysts in the depolymerization of lignin. Lignin is by far the most abundant polymer based on aromatic moieties in nature, excluding petrochemical raw materials. Biocatalysis is regarded as the key enabling technology for lignin valorization but biocatalytics development is essential for selective and efficient lignin valorization. Peroxidases are important lignolytic enzymes. However all heme-containing peroxidases have a low operational stability mostly due to their rapid inactivation by H_2O_2 that limits their utilization. Laboratory evolution strategies will be followed to generate optimized peroxidases with increased stability towards peroxide. The robustness of evolved catalysts will be validated from laboratory experiments to industrial scale trials.

Project 3 - High-yields expression of heterologous enzymes through directed evolution approaches

The goal is the development of an improved *Escherichia coli*-based expression system capable of producing 0.1-1 g of purified recombinant enzymes per liter of culture, volumetric yields at least 10-fold higher than presently achieved. Indeed, the utilization of biocatalysts for biotechnological applications is presently limited by the need for scalable and high-yielding methods to supply active enzymes. Engineering strategies including directed evolution approaches will be used to generate highly expressed heterologous proteins. These will be assessed at lab and pilot scales and developed as prototypes for commercial scale-up. The production system developed will facilitate basic enzyme investigations as well as the development of new technologies that utilize the producing enzymes.

Project 4 – Enzymatic bioremediation of petroleum hydrocarbon contaminants

The goal is to test the oxidation efficiency of bacterial laccases and DyP type peroxidases in the degradation of polyaromatic hydrocarbons (PAHs). PAHs are pollutants of great concern due to their potential toxicity, mutagenecity and carcinogenicity. They are the most important components of crude oil, creosote, asphalt, and coal tar. One of the major environmental problems today is hydrocarbon contamination resulting from the activities related to the petrochemical industry. Biodegradation is generally regarded as the safest, least disruptive and most cost-effective treatment method and enzymes such as fungal laccases and peroxidases are potential biocatalysts. As representatives of mono- and poly-aromatic pollutants toluene and naphtalene will be used and the conversions will be optimized in microstructure systems such as micellar and microemulsioned solutions to enhance the bioavailability of the hydrophobic substrates. The integration of these results will hopefully allow and the set-up of an effective bioremediation process.

Areas:
Biochemistry/Enzyme Engineering/Biotechnology/Molecular Biology/Microbiology/

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