Protein and DNA technologies for functional expression of membrane-associated cytochromes P450 in bacterial cell factories

The heavy dependence and massive consumption of fossil fuels by humans is changing our environment very rapidly. Some of the side effects of industrial activity include the pollution of the natural resources we rely on, and the reduction of biodiversity. Some chemicals found in nature exhibit great potential as medicines, fuels or food for humans. Plants conquered different environments thereby developing adaptation strategies based on the biosynthesis of a myriad of compounds. Unfortunately they are present in small amounts in plants and are too complex and to produce by organic chemical synthesis. In most of biosynthetic pathways leading to these chemicals the cytochrome P450 enzyme family (P450s) is responsible for their final functionalization. However, the membrane-bound nature of P450s, makes their expression in microbial hosts a challenge. In order to meet the global demand for these natural compounds without compromising sustainability, biological production needs to substitute the traditional manufacturing methods. Thus, new methodologies for expression and characterization of P450 enzymes are in great need. This thesis explores state-of-the-art techniques at the core of membrane protein, metabolic engineering and protein engineering to provide new solutions to the P450 expression bottleneck in bacteria. The work primarily focuses on developing a fluorescence high-throughput platform to easily assess proper folding and expression levels of plant cytochromes P450. The platform has been designed to fit in metabolic engineering and structural biology applications. Furthermore in this thesis a systematic engineering rationale is proposed to improve P450 expression. For this, anew set of N-terminal tags has been developed in order to provide a streamlined optimization scheme for P450 expression. The application of these N-terminal tags has been also tested to elucidate the structure of the plant cytochrome P450 CYP79A1. The present work demonstrates the usefulness of the above mentioned technologies to optimize P450 expression for biotechnological applications. The thesis provides new P450 engineering guidelines and serves as platform to improve performance of microbial cells, thereby boosting recombinant production of complex plant P450-derived biochemicals. The knowledge generated, could guide future reconstruction of functional plant metabolic pathways leading to high valuable chemicals. This work is in the foundations of sustainability, as it contributes to find alternatives that limits or relief exploitation of scarce natural resources vital for the survival of our future generations.

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