COMBINATORIAL OPTIMIZATION STRATEGIES TO INCREASE HUMAN P450 CATALYTIC ACTIVITY IN WHOLE CELL BIOTRANFORMATIONS

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INTRODUCTION
Human cytochrome P450 biotransformations are of great potential for drug metabolite synthesis. However, recombinant production yields and product titers are low, which has been the most limiting factor for their application in large scale processes. In this study, we combined protein engineering with expression-, strain-, and process-optimization to obtain high level human cytochrome P450 producing P. pastoris strains for whole-cell biocatalysis.

PROTEIN ENGINEERING
Error prone PCR (epPCR) random mutagenesis and the multiple site saturation mutagenesis method OmniChange [2] were used to generate P450 variants for improved substrate conversion.

We use episomal plasmids, where the library fragment is added to the vector backbone by Gibson assembly. P. pastoris can be directly transformed with a few µl of the mix.

CULTIVATION CONDITIONS
In order to increase the production of human P450s we optimized the cultivation media for deep-well plate and shake flask scale. Several parameters were tested including various cultivation temperatures, additives, pH values and types of media as well as the oxygen supply. The activity was increased up to 5-fold at a higher pH value, when rich media was used.

SUMMARY
By combining these optimization strategies, the enzyme yields for several human P450s were increased from not-detectable to the highest reported in literature.

REFERENCES