INTERNATIONAL MASTER IN
SUSTAINABLE
DRUG DISCOVERY

STUDIES ON FUNCTIONAL EXPRESSION OF HUMAN CYTOCHROME P450 1A2 AND 2D6 AT THE CELL SURFACE OF *ESCHERICHIA COLI*

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Summary

Enzyme-catalysed reactions provide advantages because of their wide substrate and structural diversity. CYP enzymes are specifically interesting for drug metabolism studies and industrial applications. These applications are challenged by rigorous and costly enzyme purification steps that can be addressed by immobilizing the enzymes to the surface of cells. This work focused on reproducing previously expression of active CYP1A2 at the surface of *Escherichia coli* and adapting this approach to CYP2D6.

The attempt to co-express CYP1A2 and CPR autotransporter fusion proteins (AT-FPs) resulted in inactive cells in the 7-ethoxyresorufin assay. Outer membrane protein isolation and SDS-PAGE showed that both AT-FPs were not present at the cell surface. Single CPR AT-FP but not CYP1A2 or 2D6 AT-FPs could be expressed under the same cultivation conditions. CPR combined with the YeeJ or EhaA translocation unit was active against cytochrome c but activity was significantly higher for YeeJ-CPR (0.575 µmol L^{-1} min^{-1}) than CPR-EhaA (0.150 µmol L^{-1} min^{-1}) at OD_{578nm} 0.25. Surprisingly, lytic polysaccharide monoxygenase (LPMO), which was included as a control showed activity (0.468 µmol L^{-1} min^{-1}) that was comparable to that of CPR. Lower LPMO activity was obtained when copper was available in the growth medium.

The attempt at purifying ATR2, the alternative to CPR as a CYP redox partner, by immobilised metal ion chromatography (IMAC) was not successful. It was shown that ATR2 could not bind to nickel nitrilotriacetic acid (Ni-NTA) agarose. This could be a result of the loss or modification of the His-tag during enzyme expression. Obtained soluble cell lysates containing ATR2 and lower concentrations of other proteins showed cytochrome c activity that was dependent on total protein concentration. However, no activity was observed when single CYP1A2 AT-FP expression samples were combined with the cell lysates containing ATR2.

These results indicate that either the presence of a plasmid encoding CYP enzymes or the selection antibiotic, carbenicillin interfered with both CYP and CPR expression. Future work may investigate the influence of carbenicllin concentration on surface display of CYP and CPR AT-FPs. The mechanism behind cytochrome c activity obtained with surface diaplayed LPMO also requires further investigation.