This project is funded by the European Union (grant agreement 635734) under EU's Horizon 2020 Programme Research and Innovation actions H2020-LEIT BIO-2014-1

Capsaicin Metabolite Synthesis by Fungal Cytochrome P450s

Melissa Willim,[1] Thorsten Bachler,[1] Bettina Schweda,[2] Anna Migglautsch,[2] Anton Glieder,[3,4] Rolf Breinbauer,[1,2] Margit Winkler[1,3]
[1]*Austrian Centre of Industrial Biotechnology (ACIB), Petersgasse 14/4, 8010 Graz, Austria*
[2]*Institute of Organic Chemistry, TU Graz, NAWI Graz, 8010 Graz, Austria*
[3]*Institute of Molecular Biotechnology, TU Graz, NAWI Graz, 8010 Graz, Austria*
[4]*bisy e.U., Wetzawinkel 20, 8200 Hofstätten/Raab, Austria*

Microbial cytochrome P450 enzymes (CYPs) are able to mimic the metabolism of human CYPs. The challenge is to identify authentic human metabolites of **active pharmaceutical ingredients (APIs)**. Here we investigate a class VIII self sufficient CYP505X from *Aspergillus fumigatus* and use it for the synthesis of API metabolites on the preparative scale

Wild-type and mutants of CYP505X were expressed in prokaryotic host *E. coli* [1] or eukaryotic host *P. pastoris,* using IPTG or MeOH as the respective inducers.
Ten mutants showed activity with different substrate specificity in *E. coli*. The most active mutant had five mutations. It was active for the oxidation of eight APIs.

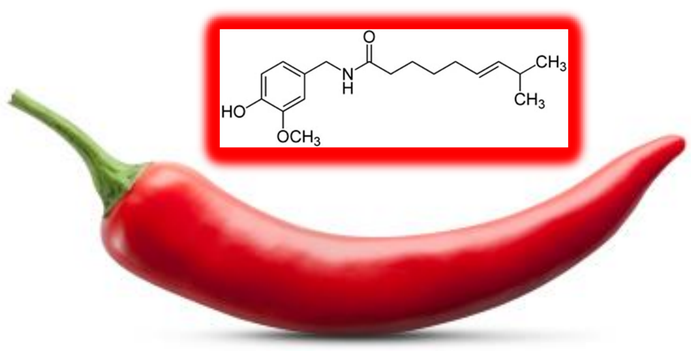


Fig. 1: capsaicin and its natural source

Capsaicin is the active principle of chilli peppers (Fig. 1). This compound is frequently used as an API in heat wraps for pain relief. Among >30 other APIs and heterocyclic compounds, Capsaicin was tested as CYP505 substrate. On the screening level, excellent conversions were observed to at least two oxidized products. The aim was to elucidate the chemical structure of the metabolites.

Workflow

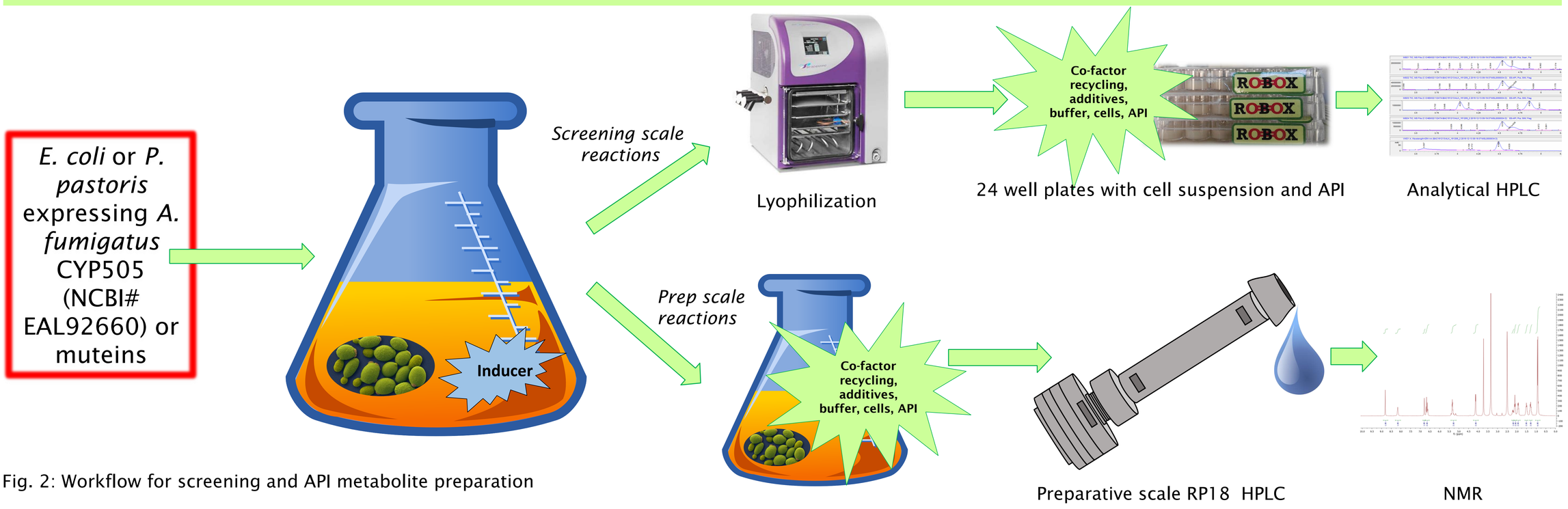


Fig. 2: Workflow for screening and API metabolite preparation

Results and Discussion

Wild-type CYP505X expressed in *E. coli* gave two metabolites on the screening scale (Fig. 2, top). A preparative scale reaction in 160 ml of total volume with 150 mg of capsaicin was carried out and monitored by HPLC/MS (Fig. 3). The products were extracted, purified by reversed phase HPLC and characterized by NMR (Fig. 2, bottom).

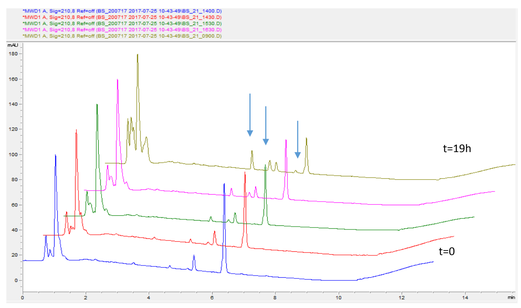
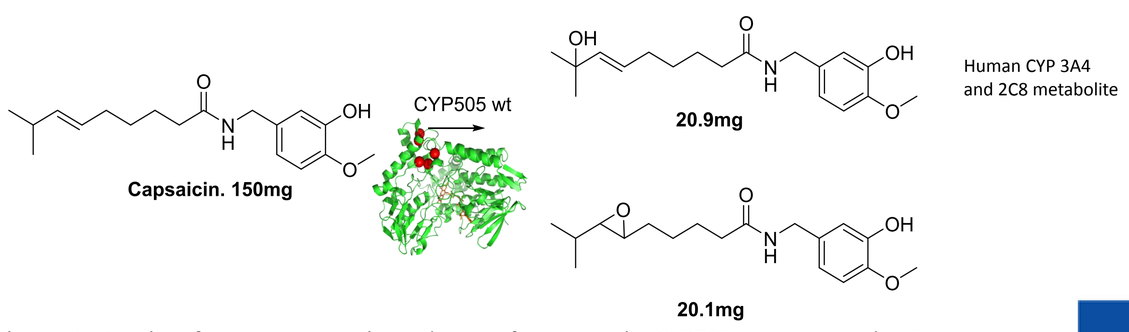


Fig. 3: Time dependent capsaicin oxidation

On the multi-mg scale, two major and one minor metabolites were formed (Fig. 3). The reaction was terminated after 19 h. The two major metabolites could be isolated in 14 and 13% yield, respectively.[2] Unreacted capsaicin was re-isolated in 37%. The tertiary alcohol resembles metabolite M3 as described by Reilley *et al.* that is formed by human CYP3A4 and 2C8).[3]



Scheme 1: Results of preparative scale oxidation of capsaicin by CYP505 wt expressed in *E. coli*. A tertiary alcohol and an epoxide were formed in the aliphatic side chain.

Conclusions

- First multi-mg scale capsaicin conversion
- Human CYP 3A4 and 2C8 metabolite and a epoxide was formed
- WT CYP 505 shows different product range than human CYPs

Outlook

- Optimization of preparative scale reaction conditions
- Kinetic characterization of biocatalyst/substrate pairs
- Scale up and product characterization with other APIs

References

[1]R. Weis, *et al. Adv. Syn. Catal.* **2009**, *351(13),* 2140
[2]A.K. Migglautsch *et al, Tetrahedron*, **2018**, *74*, 6199
[3]C.A. Reilly, *et al. Chem. Res. Toxicol.* **2013**, *26*, 55

email: margit.winkler@acib.at

This work received funding from the EU project ROBOX (grant agreement n° 635734) under EU's H2020 Programme Research and Innovation actions H2020-LEIT BIO-2014-1 and from the Austrian BMWFJ, BMVIT, SFG, Standortagentur Tirol and ZIT through the Austrian FFG-COMET- Funding Program.

This project is funded by the European Union