The ROBUSTOO Newsletter

With the contribution of:

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OXIDATIVE BIOCATALYST BULLETIN

UPOs: small enzymes, big chemestry

Welcome to the sixth issue of the ROBUSTOO newsletter!

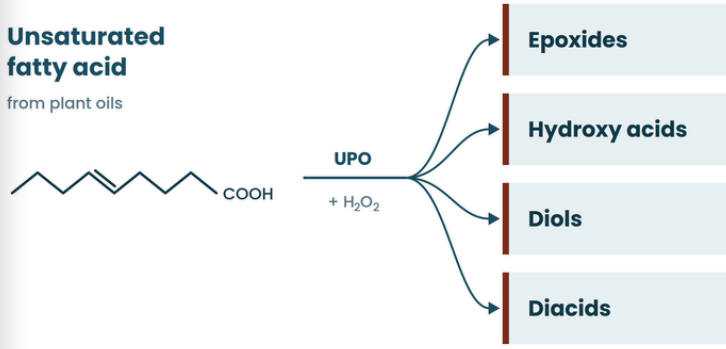
How can complex molecules be modified selectively without relying on harsh chemical processes? One answer lies in a class of enzymes called **unspecific peroxygenases**, or UPOs. These fungal enzymes can introduce oxygen-containing functional groups into a wide range of molecules, enabling the selective production of high-value chemicals and bio-based products.

UPOs are heme-containing enzymes capable of hydroxylation, epoxidation and other selective oxidations of aliphatic and aromatic compounds. Their chemistry resembles that of cytochrome P450 enzymes, but UPOs have an important advantage: they use hydrogen peroxide directly as the oxygen donor without the auxiliary electron-transfer proteins typically needed by P450 systems. This comparatively self-sufficient setup is one reason why UPOs have attracted attention as potential “dream biocatalysts”.

From plant oils to valuable building blocks

One promising application is the oxyfunctionalisation of unsaturated fatty acids, major constituents of plant oils and a renewable feedstock for the chemical industry.

Through epoxidation or hydroxylation, UPOs can generate epoxides, hydroxy acids, diols and diacids – valuable intermediates for crosslinkers, binder components, polymer precursors or additives.



Achieving this control is a central challenge. UPOs are highly versatile, but the regio- and stereoselectivity needed for a particular product varies between enzymes. ROBUSTOO therefore combines the discovery of new UPOs with enzyme engineering to identify and develop variants with the activity and selectivity needed for specific industrial transformations.

Funded by the European Union

More than lipids

The possibilities extend beyond plant oils. Within **ROBUSTOO**, UPOs are being explored for selective oxygenation reactions that can lead to high-value intermediates and fine chemicals. The project specifically targets oxygenated lipophilic compounds that are difficult to obtain selectively by conventional chemical synthesis.

A striking example is the selective conversion of vitamin D2 and D3 into their 25-hydroxy derivatives, which are valuable metabolites used in clinical and nutritional applications. Since conventional synthesis is multistep, uses strong oxidants and offers limited control over the hydroxylation site, an enzymatic route is highly attractive. In a recent ROBUSTOO-supported study, screening a broad panel of UPOs identified promising biocatalysts, while subsequent reaction optimisation and scale-up achieved high conversion and regioselectivity.

And why is selectivity so important here? In a complex molecule, introducing oxygen at a different position or with a different stereochemistry can result in a product with very different properties. Finding or engineering the right UPO for a particular transformation is therefore key to unlocking these applications.

From promising enzymes to industrial biocatalysts

But how can promising UPO chemistry be translated into industrial applications? One major challenge is producing enough enzyme. Although fungal genomes contain many UPO sequences, only a relatively small number have so far been produced and studied in the laboratory, limiting their characterisation and wider application.
ROBUSTOO is addressing this bottleneck by combining different microbial production hosts with computational tools, host engineering, protein engineering, and optimisation of cultivation conditions. Particular attention has been given to *Komagataella phaffii*, a yeast widely used for recombinant protein production. By testing UPO sequences, secretion signals and production conditions, the consortium has considerably expanded the number of UPOs available for further investigation.

The next challenge is to bring these two sides together: producing the most suitable UPO efficiently for a given reaction. From renewable lipids to complex molecules such as vitamin D, ROBUSTOO aims to develop UPOs into practical biocatalysts for high-value products that are difficult to obtain selectively by conventional chemistry.

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