Discovering fungal ene-reductases: transcripts and in silico analysis

Discovering fungal ene-reductases: transcripts and *in-silico* analysis

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The reduction of C=C double bonds and carboxylic acids and esters is often a crucial step in organic chemistry, currently performed by highly polluting and expensive metal catalysts. A viable alternative is given by ene-reductase (ERs) activity which is able to reduce C=C conjugated with different electron-withdrawing groups as carbonyl, nitro and ester and carboxylic acid reductases. To date, most of the information about this enzymatic class comes from bacteria and yeasts. Even though filamentous fungi are good biocatalysts due to their natural biodiversity and their broad heterogeneous enzymatic pattern, they have been poorly investigated.

This research aimed to develop fungal whole-cell processes to provide new sustainable synthetic tools for organic chemistry, producing enantiopure chiral compounds. Filamentous fungi belonging to Ascomycota, Basidiomycota and Zygomycota were investigated and most of them were capable of expressing ERs activity. Among them, *Mucor circinelloides*, *M. plumbeus* and *Syncephalastrum racemosum* were the most versatile and effective reducing all the substrates already within the first 1-3 days.

M. circinelloides was used to assess the dynamics of the bioconversion of three target analytes through a time-course assay. The biotransformation correlated with the expression profile of the putative ERs genes found in *M. circinelloides* genome: the maximal peak of expression always occurred just before the beginning of C=C reduction, and sharply decreased as soon as the reaction started. Eight out of 10 genes have been expressed, among which ER1 and ER2 reached the highest expression levels.

A homology modeling approach was adopted to study the 3-D features of the putative ERs and identify peculiarities between them. The overall structure of these enzymes, the FMN-binding site and the catalytic residues were mostly conserved. The models displayed peculiar features, mainly regarding a specific loop, the size of the active site and the surface charge. The substrate-enzyme interaction was studied by a molecular docking approach, revealing high variability in the binding mode, partially justifying the differences observed in the biotransformation assay.

Further study will be aimed at the production of ERs of *Mucor circinelloides* by heterologous expression in order to purify and catalytically characterize these isoenzymes.