Fungal oxidoreductases as biocatalysts of fine chemical transformations.

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Due to the increasing industrialisation and the growing sensibility about pollution problems, the identification of energetically and environmentally sustainable processes are currently needed. Nowadays, several industrial sectors are developing bio-based technologies to reduce the high costs and environmental impact of traditional chemical processes. Due to the low energy demand, the reduction of waste, by-product formation and process costs, biocatalysis seems to be an alternative to chemical synthesis. As well this method is a powerful tool acting towards chiral molecules to produce pure enantiomers highly valued in pharmaceutical and flavour fields. Microorganisms and their enzymatic derivatives become interesting for the production of fine chemicals, pharmaceuticals and agrochemical intermediates. In this regard, filamentous fungi may be considered good biocatalysts due to their natural biodiversity and their broad heterogeneous enzymatic pattern. They are currently employed in several applications as the production of citric acid or cyclosporine. Focusing on the reduction of C=C double bonds, this is a widely used process in organic chemistry but currently it is performed by highly polluting and expensive metal catalysts. A viable and sustainable alternative may be ene-reductases which perform the reduction of C=C double bonds conjugated with different electron withdrawing groups (EWGs) such as carbonyl, nitro and ester. These enzymes are well known in yeasts and bacteria but to date are poorly investigated in filamentous fungi.

This PhD project aims to develop fungal whole-cell catalysed processes to provide new sustainable synthetic tools for organic chemistry to produce biotechnologically interesting compounds such as enantiopure chiral compounds. This study is focused on the investigation of ene-reductase activities in different species of filamentous fungi belonging to Basidiomycota, Ascomycota and Zygomycota analysed in the bioconversion of precursors of industrial compounds.

Twenty-eight filamentous fungi were analysed in the bioconversion of cyclohexenone, α-methylnitrostyrene, α-methylcinnamaldehyde and methylcinnamate. Almost all the fungi showed ene-reductase activities and transformed different substrates with high conversion rate producing molecules which can be used for instance in the flavour production. In particular *Gliomastix masseei*, *Mucor circinelloides* and *Mucor plumbeus* resulted versatile and effective reducing all the model substrates quickly and with high yields. Surprisingly *Mucor circinelloides*, *Mucor plumbeus* and *Syncephalastrum racemosum* showed both ene-reductase and carboxylic acid reductase activity. In particular, the latter activity appears to be well sought in several industrial sectors and it is almost unknown in filamentous fungi.

Future perspective will be focused on the optimisation of the biotransformation process and on the molecular analysis of genes coding for ene-reductases in the genome of the fungus *Mucor circinelloides*. Regarding carboxylic acid reductases, intraspecific variability among different strains of *Syncephalastrum racemosum* will be analysed.