FUNGAL OXIDOREDUCTASES AS BIOCATALYSTS FOR THE REDUCTION OF CHEMICALS.

<u>Alice Romagnolo¹</u>, Federica Spina¹, Michele Crotti², Fabio Parmeggiani², Elisabetta Brenna², Giovanna Cristina Varese¹.

¹ Department of Life Science and Systems Biology, University of Turin, viale Pier Andrea Mattioli 25, 10125 Turin, Italy;

² Department of Chemistry, Materials and Chemical Engineering, Politecnico di Milano, via Luigi Mancinelli 7, 20131 Milan, Italy.

e-mail: alice.romagnolo@unito.it

Presentation

The Mycotheca Universitatis Taurinensis (MUT) is the fungal collection of the Department of Life Sciences and Systems Biology of the University of Turin (Italy). The MUT is one of the most important banks of fungal biodiversity in Italy, and it has a great value from the systematic, ecological and applications point of views.

The aims of the MUT are the acquisition, identification, characterization, preservation and distribution of both macromycetes and micromycetes.

The MUT preserves about 5000 strains belonging to almost all classes of filamentous fungi of about 1100 species. Moreover, some yeast and bacterial strains are conserved basically for didactic aims.

The collection is particularly interesting from an ecological and applicative point of view. Actually, many fungal isolates preserved by MUT have been characterized for their ecological and physiological properties and includes mycorrhizal strains, biocontrol and bioindicator agents as well as antibiotic and enzyme producers to be used in industrial and bioremediation applications. The collection includes also several potentially harmful fungi isolated from both human, animal, and vegetal pathological specimens and environmental sites.

Since 2008, the MUT has been affiliated to the World Federation Culture Collections (WFCC), which represents a global network aimed to the ex-situ conservation of microbiological biodiversity. Moreover, since 2008, the MUT has become member of the European Culture Collections' Organisation (ECCO). Thus, the MUT collection is embedded in a scientific-academic environment, which guarantees the quality of the fungal isolates and their correct conservation. Moreover, the MUT promotes the development of scientific programmes to enlarge its collection and to improve the quality of the material entrusted to them.

Web site: http://www.mut.unito.it/it

Abstract

The development of biocatalysts for the reduction of C=C double bonds and carboxylic acids and esters are of great relevance due to the need of several industrial sectors of developing biobased technologies to reduce the high costs and environmental impact of traditional chemistry. In recent years, a growing interest has been devoted to biocatalysis due to the low energy demand, the reduction of waste, by-product formation and process costs and the chemo- regioand stereo-selectivity. This last feature is highly valued in pharmaceutical and flavour fields. Microorganisms and their enzymes become interesting for the production of fine chemicals, pharmaceuticals and agrochemical intermediates. Particularly, filamentous fungi may be considered good biocatalysts due to their natural biodiversity and their broad heterogeneous enzymatic pattern. They are currently employed in several important applications as the production of citric acid or cyclosporine.

The reductions of C=C double bonds and carboxylic acids and esters are currently performed by highly polluting and expensive metal catalysts. A viable alternative may be the use of enzymes; particularly ene-reductases (Stuermer et al. 2007) reduce C=C double bonds conjugated with different electron-withdrawing groups such as carbonyl, nitro and ester and carboxylic acid reductases (Venkitasubramanian et al. 2006) which reduce carboxylic acids and esters to primary alcohols. To date, these enzymes are poorly investigated in filamentous fungi and no information is available relating to the structure of these proteins. To date no ene-reductases or carboxylic acid reductases have been purified and characterised from this microorganisms.

This research aims to develop fungal whole-cell catalysed processes to provide new sustainable synthetic tools for organic chemistry, producing useful molecules such as enantiopure chiral compounds. This study focused on the investigation of ene-reductases and carboxylic acid reductases activities in different species of filamentous fungi belonging to Ascomycota, Basidiomycota and Zygomycota analyzed in the bioconversion of industrial compounds. Twenty-eight fungi were evaluated in the conversion of cyclohexenone, α -methylnitrostyrene, α -methylcinnamaldehyde and methyl cinnamate. Almost all the fungi showed ene-reductase activities and transformed the substrates producing molecules which can be used for instance in the flavours production (i.e., methyldihydrocinnamyl alcohol from α -methylcinnamaldehyde). In particular *Mucor circinelloides, Mucor plumbeus* and *Syncephalastrum racemosum* resulted versatile and effective reducing all the substrates quickly and with high yields. These three fungi showed both ene-reductase and carboxylic acid reductase activity. The latter activity appears to be well sought in several industrial sectors and it is almost unknown in filamentous fungi.

As regard ene-reductases, future perspective will be focused on the optimisation of the biotransformation processes and in particular on the molecular and bioinformatics analysis of genes coding for these enzymes in the genome of *Mucor circinelloides*. Regarding carboxylic acid reductases, intraspecific variability among different strains of *Syncephalastrum racemosum* will be analysed.

References

1. Stuermer, R.; Hauer, B.; Hall, M.; Faber, K., 2007, 11, 203-213.

2. Venkitasubramanian, P.; Daniels, L.; Rosazza J.P.N., 2006, 1, 478-485.