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Research review paper

# *Candida* spp. redox machineries: An ample biocatalytic platform for practical applications and academic insights

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ABSTRACT

The use of oxidoreductases as biocatalysts for the production of a wide number of chiral building blocks is presently a mature (bio-)technology. In this context some industrial applications are currently performed by means of those enzymatic approaches, and new examples are expected to be realized. Moreover, oxidoreductases provide an interesting academic platform to undertake fundamental research in enzymology, to acquire a better understanding on catalytic mechanisms, and to facilitate the development of new biocatalytic applications. Within this area, a wide number of oxidoreductases from genus *Candida* spp. have been characterized and used as biocatalysts. These enzymes are rather diverse, and are able to carry out many useful reactions, like highly (enantio)selective keto-reductions, (de)racemizations and stereo-inversions, and promiscuous catalytic imine reductions. In addition, some *Candida* spp. dehydrogenases are very useful for regenerating the cofactors, with the aid of sacrificial substrates. Addressing those features, the present paper aims to give an overview of these enzymes, by focusing on practical applications that these biocatalysts can provide. Furthermore, when possible, academic insights on the enzymatic performances will be discussed as well.



**Contents**



## 1. Background

Biotransformations discipline is currently gaining an increasingly important position in the field of (asymmetric) organic synthesis. By means of enzymes and/or whole cells as selective biocatalysts, environmental issues and economic aspects can be tackled effectively. In this respect, there are currently an ample number of examples of biotransformations at industrial scale (Faber, 2004; Hilterhaus et al., 2007; Patel, 2008; Ran et al., 2008; Tao et al., 2007). Key to this rapid growth are the tremendous developments achieved in the molecular biology field, which have opened new avenues in practical industrial microbiology (Beloqui et al., 2008), directed evolution (Reetz, 2004), and metagenomics (Gabor et al., 2007; Lorentz et al., 2005). Overall, these outstanding technologies are fueling biotransformations, thus triggering the set up of (more sustainable) practical applications.

Within the field of biotransformations, the redox platform represents a key-area of research, development, and innovation (Faber, 2004; Goldberg et al., 2007a,b; Hilterhaus et al., 2007; Nakamura et al., 2003). Actually, in just some decades the general perception on these bio-based-redox performances has impressively moved from being considered as a simple *lab curiosity*, to become (almost) the *first choice* in catalyst search in specific industries, when a certain redox processes is envisaged (De Wildeman et al., 2007).

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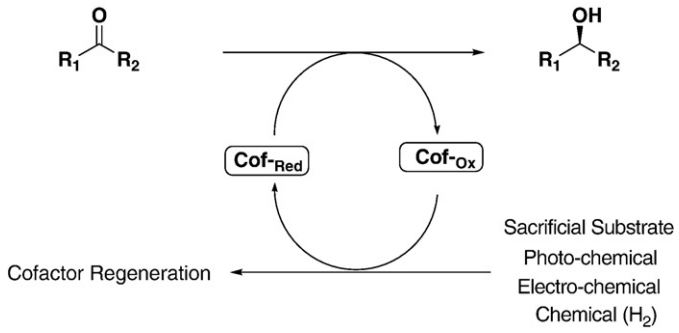



**Scheme 1.** General model of classic bio-based redox process, involving the different alternatives for cofactor regeneration (De Wildeman et al., 2007; Faber, 2004; Goldberg et al., 2007a,b; Liese et al., 2006; Nakamura et al., 2003; Ruinatscha et al., 2006).

Again, the successful cloning and overexpression of some of those oxidoreductases – to build the so-called *designer cells* (Gröger et al., 2006) – , has led to powerful applications, with high productivities and enantioselectivities.

It is common knowledge that redox enzymes make use of different cofactors to transfer the electrons from donor to acceptors, when catalyzing their natural processes. Therefore, the achievement of an efficient *in situ* regeneration of these (very expensive) cofactors, represents a key-issue in the implementation of a successful bio-based redox application (Scheme 1).

As depicted in Scheme 1 several strategies have been (more or less) successfully developed to overcome the problem of cofactor regeneration. These strategies comprise different biological, or photo-, or electro-chemical concepts (De Wildeman et al., 2007). Although this challenging cofactor regeneration is nowadays largely solved, there is still room for improvements, and therefore it is matter of an intense research. As an example, very recently the known use of phosphite dehydrogenase (PTDH) as coupled enzyme for a monooxygenase-based Baeyer–Villiger redox process was improved. This PTDH accepts phosphite as substrate, thus providing an inexpensive and an efficient way of regenerating cofactors (Torres Pazmiño et al., 2008). Surely many other innovative ways to close this loop of cofactor regeneration will be developed in the forthcoming decades.

In this particular area of bio-based redox reactions, enzymes from genus *Candida* spp. play a relevant role. Involving useful reductions, oxidations, and/or (de)racemizations or stereoinversions, to mention some fields, these *Candida* spp. redox enzymes have been reported in a wide number of interesting processes. Moreover, many of these enzymes can be taken as model for academic studies on enzymology and/or to validate new concepts.

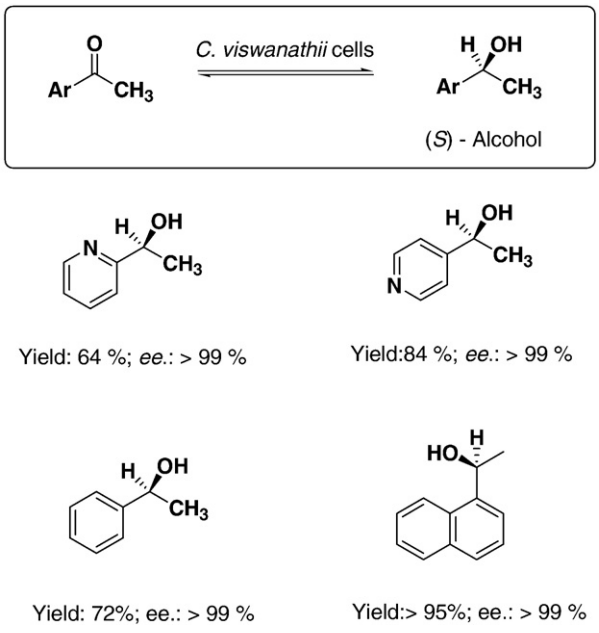
A wide number of these biocatalysts have been already cloned and overexpressed. In other cases, however, performances are carried out on a "black-box" whole cell catalytic basis. Aiming to draw the attention to those promising biocatalysts, in this article we aim to give an overview on the possibilities that these enzymes can have for a diverse number of practical applications. Aspects on substrate acceptance, (enantio)selectivities, type of biotransformation, and/or other relevant issues, will be outlined. In addition, whenever possible, academic aspects on the (natural) performance of these enzymes will be briefly discussed as well.

## 2. The *Candida* spp. oxidoreductase platform

Several *Candida* spp. oxidoreductases have been reported as powerful biocatalysts to produce a wide number of chiral compounds. In this area, a promising and recently described biocatalyst, is the *Candida viswanathii* carbonyl reductase. This yeast was initially isolated from a soil sample, and was subsequently grown with acetophenone as the sole carbon source. Optimization for production of this carbonyl reductase was conducted (Soni et al., 2006b), and at a later stage, the enzyme was purified and characterized (Soni et al., 2007). Recently, it was also shown that resting cells of *C. viswanathii*, immobilized in calcium alginate, were able to perform the enantioselective reduction of acetophenone efficiently. The immobilization allowed the re-use of the biocatalyst up to 12 cycles (Fatima et al., 2007). Moreover, preliminary studies on substrate acceptance show that this carbonyl reductase may be a promising biocatalyst. In all cases (*S*) selectivity was observed (Fatima et al., 2007; Soni et al., 2007) (Scheme 2).

In the same area, *Candida boidinii* was also described as an efficient biocatalyst in redox processes (Chen et al., 2008; Ferré et al., 2002). Thus, Ferré et al. described the resolution of racemic phenylamines with *C. boidinii* in oxidation reactions, with the production of either alcohols, aldehydes, or ketones, but with only moderate enantioselectivities for (*R*)-enantiomer (*ee* 52–70%) (Ferré et al., 2002). Yet, more recently, the enantioselective reduction of 2-oxophenylbutyrate to ethyl (*R*)-2-hydroxy-4-phenylbutyrate in pure aqueous medium, with high yield (92%) and enantioselectivity (>99%), was reported. This latter compound is a key intermediate in the production of angiotensin-converting enzyme (ACE) inhibitors (Chen et al., 2008) (Scheme 3).

In this section, another relevant representative of oxidoreductases useful for biocatalysis is that of *Candida parapsilosis*. Some decades ago, a novel ketopantoyl lactone reductase was described for the first time in that yeast (Shimizu et al., 1984). *C. parapsilosis* enabled the reduction of ketopantoyl lactone to D-(−)-Pantoyl lactone in a stereoselective manner with moderate-to-high yields (60–80%). The reduction process was carried out using a wide range of carbon and nitrogen sources, and the desired product was achieved with moderate *ee*'*s* (66–85%). At a later stage a crude extract containing a novel NADH-dependent carbonyl reductase from *C. parapsilosis* (CPCR) was tested for the first time regarding its ability to reduce keto acids or keto esters with different chain length and position of the keto group (Peters et al., 1992). Higher activities were observed especially when ketoesters were employed as substrates, as compared to ketoacids. CPCR are thus able to reduce β, γ, and δ ketoesters. To induce the enzyme biosynthesis, a ketoester as substrate was added when the microorganism was grown in glycerol as the sole carbon source. CPCR was subsequently purified and characterized, and




**Scheme 2.** Selected examples of the substrate acceptance reported for carbonyl reductase from *Candida viswanathii*. More examples can be found in literature (Fatima et al., 2007; Soni et al., 2007).