Towards the identification of new biocatalysts for the bioreduction of bicyclo[2.2.2]octane-2,6-dione

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INTRODUCTION

The stereoselective reduction of bicyclic diketone **1** (bicyclo[2.2.2]octane-2,6-dione (BCO2,6D), Fig. 1) by baker's yeast generates keto-alcohol **2** (Fig. 1) that is used as starting rigid material for the synthesis of drugs and chemicals[1]. However, previous screening of non-conventional yeasts has identified *Candida* species that produce the exo-alcohol **3** (Fig. 1) as the major product[2] and previous work localized the exo-activity in the membrane fraction of *Candida* sp[3]. The present study aimed at the identification of the *Candida* membrane (-bound) reductase(s) responsible for the production of exo-alcohol **3**.

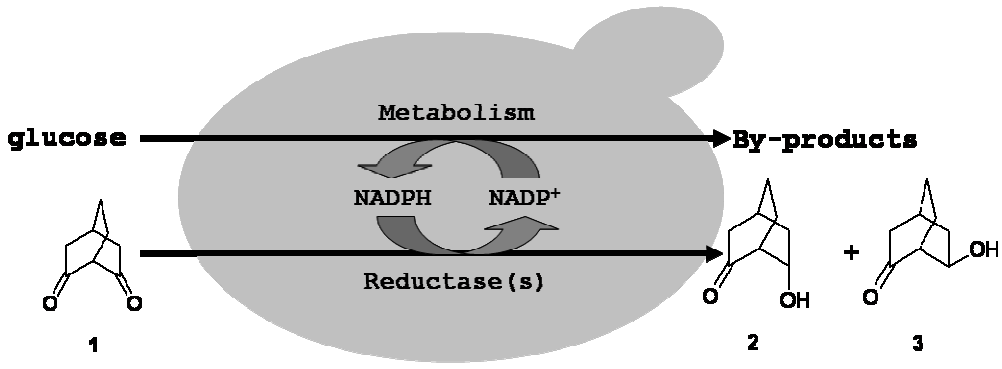

Figure 1. Whole cells bioreduction of bicyclo[2.2.2]octane-2,6-dione, **1**, into *endo*-(1R,4S,6R)-bicyclo[2.2.2]octane-2-one-6-ol, **2**, and *exo*-(2R,4S,6R)-bicyclo[2.2.2] octane-2-one-6-ol **3.**

Screening in *Candida tropicalis*

An *in silico* screening identified seven putative NADPH-dependent membrane reductases in *C. albicans*[3]. Five of the corresponding genes could be expressed in *C. tropicalis.* The corresponding strains were checked for exo/endo ratio after 24 hours bioreduction (Fig. 2).

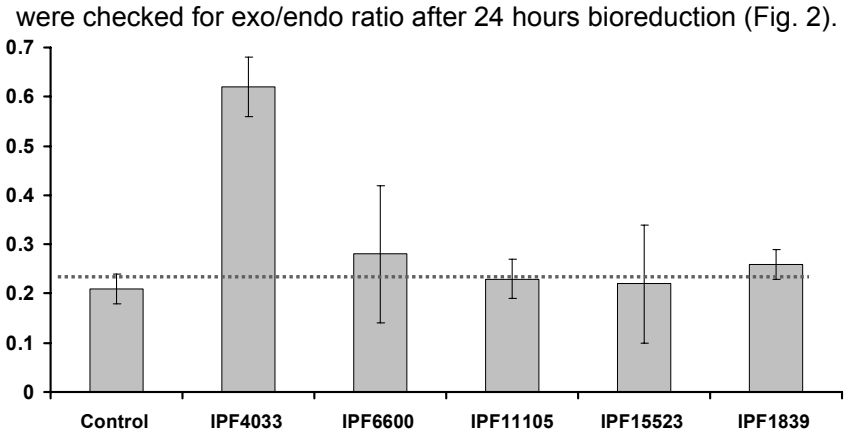

Figure 2. Exo/endo ratios after 24h bioreduction of BCO2,6D with *C. tropicalis* strains over-expressing putative *C. albicans* exo-reductases. Dotted line marks exo/endo ratio 0.23, which was within the 95% confidence limits of all strains, except TMB8001 (IPF4033).

In vitro reductions with IPF4033

In vitro bioreductions of BCO2,6D were performed with the cytosolic (Fig. 3A) and membrane (Fig. 3B) fractions of *C. tropicalis* strain overexpressing IPF4033 (=*AYR1*) gene and the corresponding control strain.

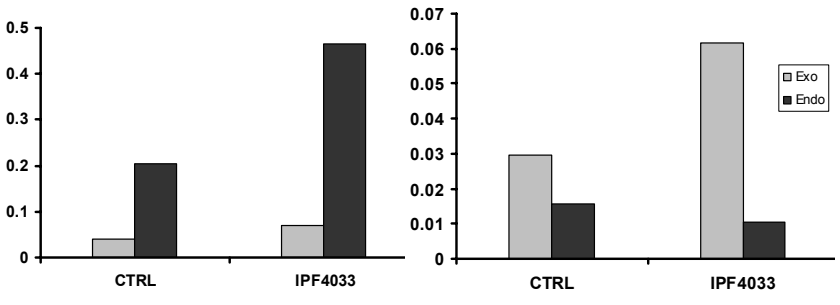

Figure 3. Exo and Endo alcohol generated (g/L) during *in vitro* reductions using cytosolic (A) and membrane (B) fractions of detergent treated *Candida tropicalis* strains overexpressing IPF4033 and Empty plasmid (CTRL).

Expression of *AYR1* in *S. cerevisiae*

C. albicans IPF4033 and its closest *S. cerevisiae* homolog Yil124w were overexpressed in *S. cerevisiae* and functional expression was confirmed by androstanedione reduction (Fig. 4A). The strains were then compared with the control strain for exo/endo ratio after 100 hours bioreduction of BCO2,6D (Fig. 4B).

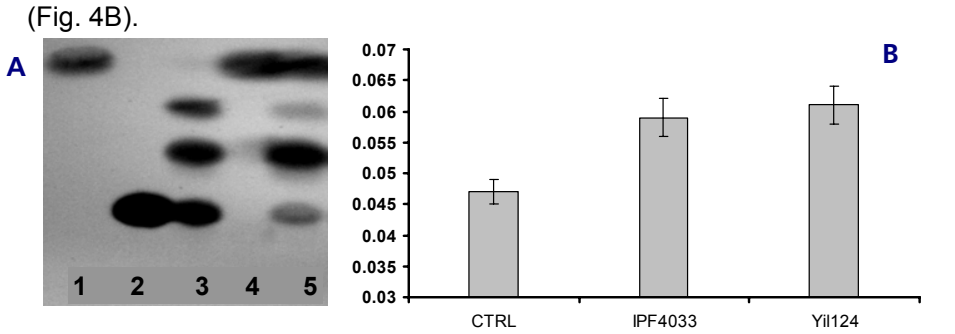

Figure 4. (A) Reduction of androstanedione with whole-cell recombinant *S. cerevisiae* over expressing Yil124w (lane 3), IPF4033 (lane 5), CTRL (lane 4). Lanes 1 and 2 are androstanedione and androstanediol respectively. (B) Exo/endo ratios from whole cell bioreduction (100h) of *S. cerevisiae* overexpressing IPF4033 and Yil124w.

CONCLUSIONS

- ✓ **Overexpression of *C. albicans* IPF4033 in *C. tropicalis* led to significantly higher exo/endo ratio.**
- ✓ **The membrane localization of exo-activity was confirmed.**
- ✓ **Slight differences in exo/endo ratio could be observed in *S.cerevisiae* over expressing *C. albicans* IPF4033 and its closest *S. cerevisiae* homologue Yil124w. However the exo/endo ratio was ~10 fold less than in *C. tropicalis.***

Experimental set-up

In vivo bioreductions were performed in citrate buffer (100mM, pH5.5) using 5 g/l BCO2,6D 1 or 1.0 g/l Androstanedione, 50 g/l glucose and a cell concentration corresponding to OD620nm=5.0.

In vitro bioreductions were performed for 24hrs in phosphate buffer (100 mM, pH 7.0) adding 2 U/ml G6PDH, 12.5 mg/ml G6P, 0.75 mg/ml NADP+ and 5 mg/ml BCO2,6D to the cell extracts. Crude membrane and crude cytosolic fractions were obtained as described previously[3].

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