Carboxylate reductase – a novel route for 3-hydroxytyrosol production

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Introduction

3-Hydroxytyrosol (3-HT) is a phenolic antioxidant that exhibits a number of beneficial effects on human health [1]. It is also a valuable building block in the synthesis of various pharmaceutically active ingredients [2]. So far, it was obtained from natural sources as well as by chemical synthesis, in very laborious and expensive procedures.

Herein we report a new method for 3-HT production through reduction of cheap, commercially available 3,4-dihydroxy-phenylacetic acid (DOPAC). The reduction to 3,4-dihydroxy-phenylacetaldehyde (DOPAL) was carried out in whole *E. coli* BL21 Gold (DE3) cells heterologously overexpressing carboxylic acid reductase (CAR) from *Nocardia* and phosphopantetheinyl-transferase (PPT-ase) from *E. coli.* Further reduction to 3-hydroxytyrosol was catalyzed by an endogenous oxidoreductase [3] (Figure 1).

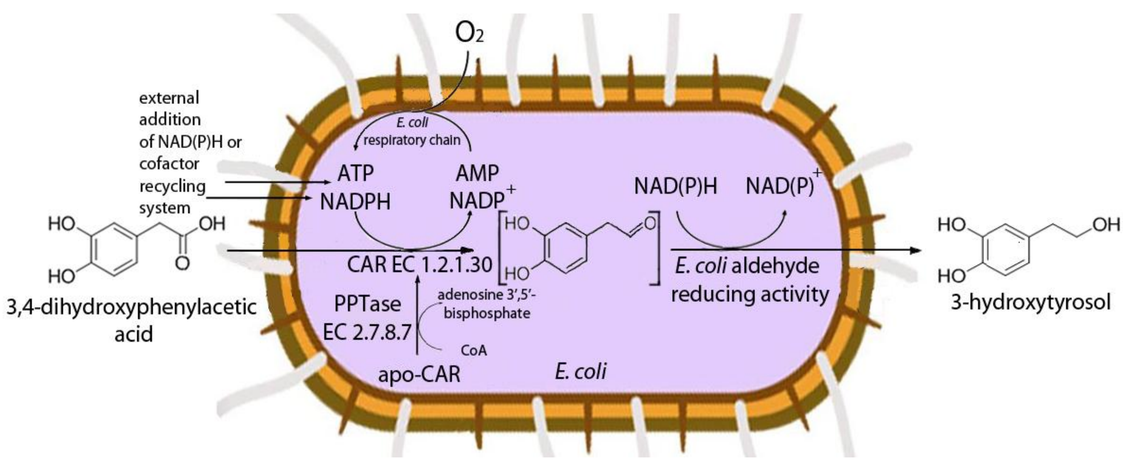

Figure 1: Whole cell bioconversion of 3,4-dihydroxyphenylacetic acid to 3-hydroxytyrosol

Results

On the way to the best conditions

Catechols tend to autooxidize at elevated pH [4] and this results in orange-brown color development and the loss of substrate and products. To avoid this, pH and buffer composition were studied in detail (Figure 2). The optimum pH and temperature (enzyme still active; no substrate or product loss) appeared to be pH 6.0 (Table 1) and 28° C (Figure 3) respectively. The substrate was fully converted to the product after 8 h of reaction time (Figure 4).

Table 1: Typical procedure for reaction condition optimization

Material used for reduction direction	Volume [µl]	End concentration
Whole cell suspension (OD approx. 150)	100	
50mM MES, 10 mM $MgCl_2$, 1 mM DTT, 1 mM EDTA, 10% v/v glycerol, pH 6.0	330	33 mM
0.5 mM NADPH	20	20 mM
100 mM DOPAC in water	50	10 mM
Total	**500**	
The reactions proceeded in 1.5 ml reaction tube with open lid, overnight at1000 rpm and 28°C		

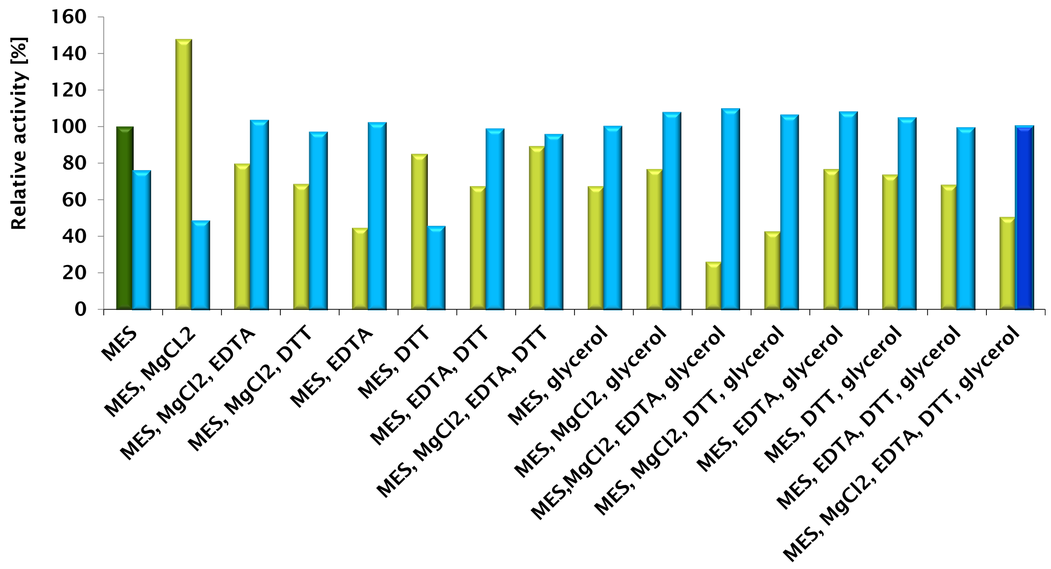

Figure 2: Effect of different buffer components on DOPAC reduction and recovery of substrate and product in 50 mM MES buffer at pH 6.0; **green bars**: relative conversion; **blue bars**: relative recovery (sum of DOPAC and 3-HT); the darkest bars were used as 100% benchmarks

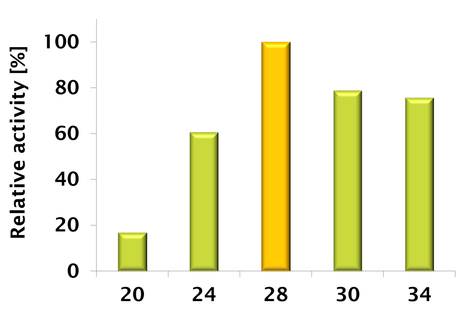

Figure 3: Temperature optimum of the reaction

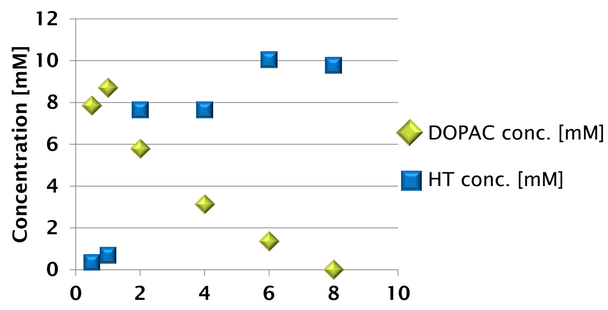

Figure 4: Reaction time course

Cofactor recycling methods

The next aim was the exploration and minimization of the need for additional nicotinamide cofactor; therefore, several combinations of nicotinamide cofactors were added to the biotransformation reactions in different concentration (Figure 5).

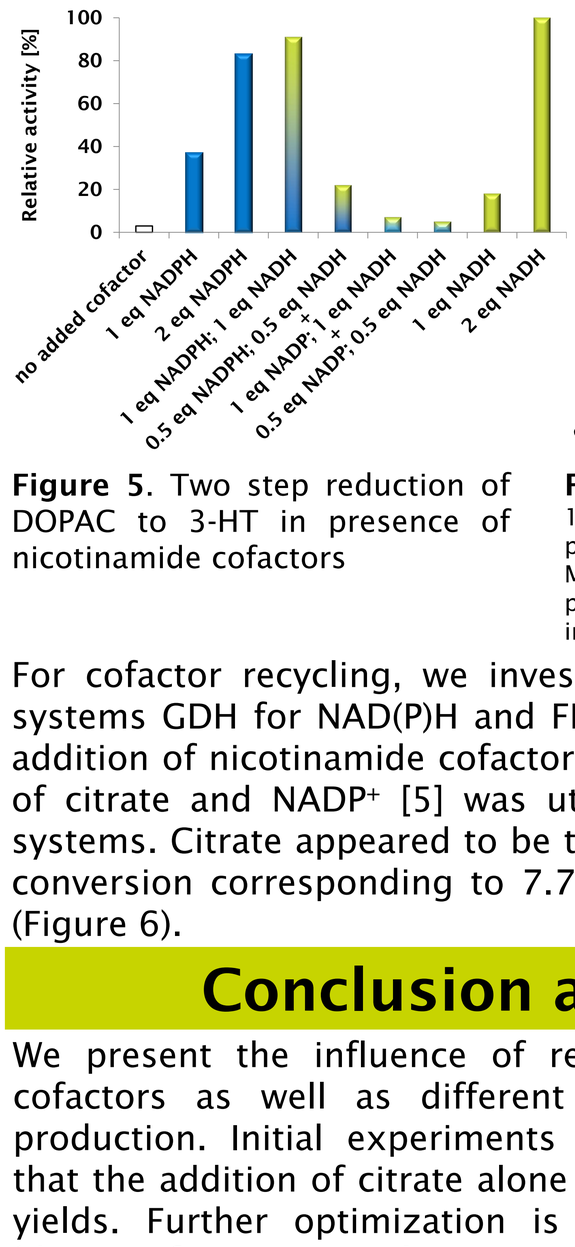

Figure 5. Two step reduction of DOPAC to 3-HT in presence of nicotinamide cofactors

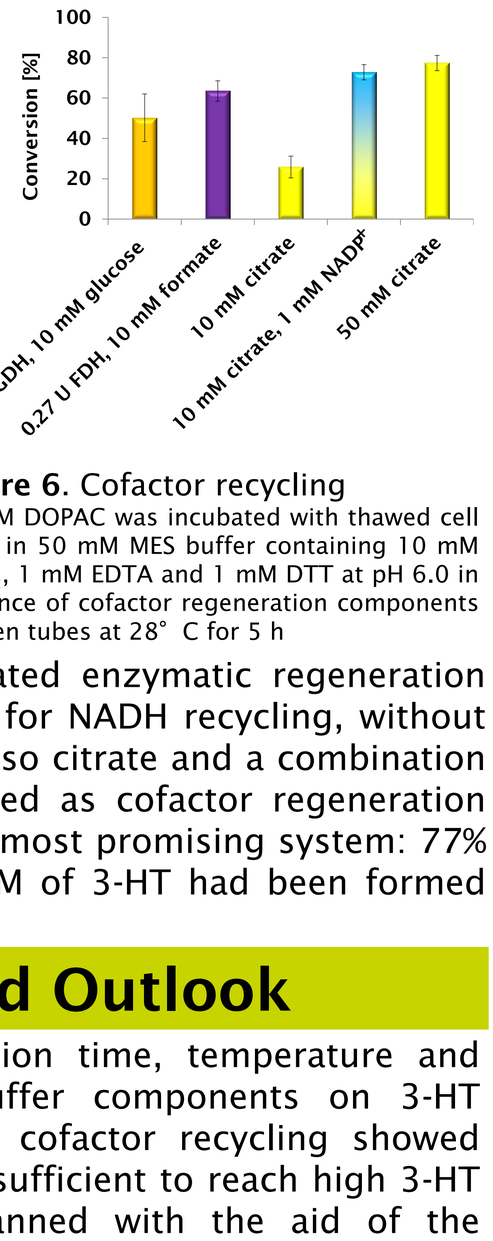

Figure 6. Cofactor recycling
10 mM DOPAC was incubated with thawed cell paste in 50 mM MES buffer containing 10 mM $MgCl_2$, 1 mM EDTA and 1 mM DTT at pH 6.0 in presence of cofactor regeneration components in open tubes at 28° C for 5 h

For cofactor recycling, we investigated enzymatic regeneration systems GDH for NAD(P)H and FDH for NADH recycling, without addition of nicotinamide cofactor. Also citrate and a combination of citrate and $NADP^+$ [5] was utilized as cofactor regeneration systems. Citrate appeared to be the most promising system: 77% conversion corresponding to 7.7 mM of 3-HT had been formed (Figure 6).

Conclusion and Outlook

We present the influence of reaction time, temperature and cofactors as well as different buffer components on 3-HT production. Initial experiments for cofactor recycling showed that the addition of citrate alone is sufficient to reach high 3-HT yields. Further optimization is planned with the aid of the 'design of experiments' concept.

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References

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