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Diversification of carotenoid cleaving enzymes serving as ‘ozonylases’ for generation of aldehydes from a lignin-based feedstock

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PURPOSE OF THE ABSTRACT

Carotenoid cleaving enzymes (CCOs or CCDs) are non-heme iron-dependent oxygenases that can be used as an alternative to ozonolysis. Unlike ozonolysis, they use molecular oxygen instead of ozone to cleave alkenes to form two equivalents of carbonyl compound (aldehyde or ketone, depending on the substitution pattern of the alkene). The catalytic center is an Fe^{2+} that is bond by 4 histidine residues. Based on the known enzymes Aromatic DiOxygenase (ADO) from Thermothelomyces thermophila[1] we conducted a sequence similarity search and found several promising enzymes.

A selected set of enzymes was tested in a mono-phasic whole cell reaction to convert several substrates, including 4-vinyl-guaiacol and isoeugenol, to vanillin. Among the tested enzymes, AspADO (Altererythrobacter sp.), TsADO (Talaromyces stipitatus), PaADO (Podospora anserina), VsADO (Valsa sordida), and CpADO from (Coniochaeta pulveracea) showed significant yields of product. However, MapADO from the marine fungus Moesziomyces aphidis was found to be the most effective in converting isoeugenol to vanillin. Using whole cells containing MapADO, we achieved quantitative conversion of 60 mM isoeugenol to the corresponding aldehyde overnight. Furthermore, 1 mg/mL of purified MapADO converted 20 mM of the same substrate completely in less than 30 minutes.

In conclusion, the novel ‘ozonylases' produced up to more than 75 times more product compared to the already described ADO.

FIGURES

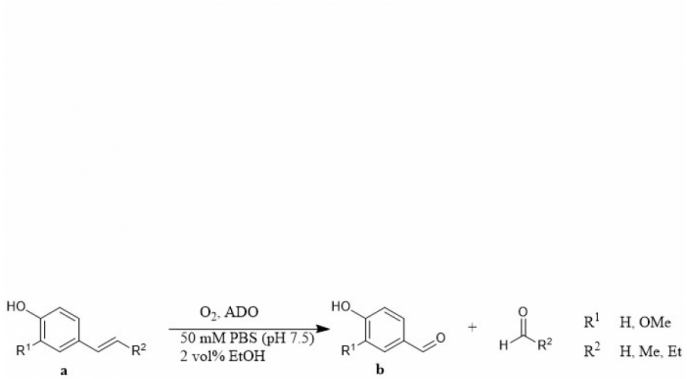

FIGURE 1

Reaction scheme:

Enzymatic oxidative alkene cleavage

FIGURE 2

KEYWORDS

Oxidative alkene cleavage | Ozonolysis | AromaticDiOxygenase

BIBLIOGRAPHY

[1] J. Ni, Y. T. Wu, F. Tao, Y. Peng, P. Xu, J. Am. Chem. Soc. 2018, 140, 16001-16005.