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# Biocatalytic oxidative cleavage of alkenes using novel metal-dependent aromatic dioxygenases

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## PURPOSE OF THE ABSTRACT

Oxidative cleavage of alkenes can be used to obtain carbonyl compounds, valuable building blocks in various areas, including food, flavor, and the pharmaceutical industry. (1) While this can be done via chemical-synthetic approaches, like ozonolysis (2), we are interested in milder and safer enzymatic approaches using novel metal-dependent aromatic dioxygenases (ADOs). In the presence of oxygen, these enzymes enable the oxidative cleavage of substrates such as isoeugenol 1a to the corresponding aldehydes (Figure 1). In order to screen numerous substrates in a short time period, we are developing a pooling approach using the reported ABAO-assay (3), a photometric/ fluorometric assay for the detection of the produced aldehydes. The assay is based on the rapid reaction of aldehydes with 2-aminobenzamidoxime (ABAO), forming quinazolines 5 that exhibit UV absorption and fluorescence properties. Using this assay, we want to quickly analyze biotransformations with several pooled substrates by detecting positive/negative photometric/fluorometric responses. In the case of a positive response, GC/HPLC is used to identify the formed aldehydes/the accepted substrate. So far, substrates such as isoeugenol 1a, hydroxyanethole 1b , and 4-vinylguaiacol 1c were found to be successfully converted to the respective aldehydes in a whole-cell system reaction. Among the found VsADO from Valsa sordida, the PaADO from Podospora anserina, the TsADO from Talaromyces stipitatus, and the CpADO from Coniochaeta pulveracea, especially the MapADO from Moesziomyces aphidis showed promising properties and converted 10 mM isoeugenol to vanillin in a whole cell approach within 1 h.

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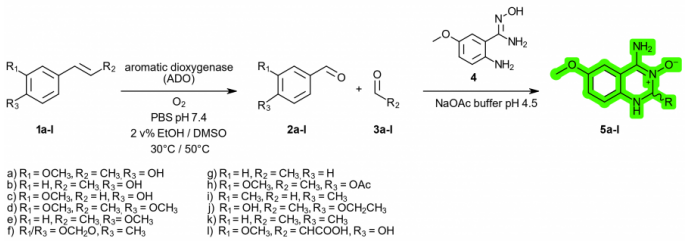
## FIGURES




### FIGURE 1

Overview of the enzymatic oxidative cleavage reaction and the detection strategy.

Oxidative cleavage of 1 by aromatic dioxygenases to the corresponding aldehydes (2, 3). Detection of the formed aldehydes via ABAO-assay using 2-aminobenzamidoxime derivate 4. The resulting quinazolines 5 exhibit UV absorption and fluorescence properties.

### FIGURE 2


## KEYWORDS

ABAO assay | aromatic dioxygenases | fluorometric/photometric aldehyde detection | oxidative carbon-carbon double bond cleavage


## BIBLIOGRAPHY


(1) Kazimirova, V.; Rebros, M. Production of Aldehydes by Biocatalysis. Int. J. Mol. Sci. 2021, 22 (9), 4949.

(2) Fisher, T. J.; Dussault, P. H. Alkene ozonolysis. Tetrahedron 2017, 73 (30), 4233.

(3) Ressmann, A. K.; Schwendenwein, D.; Leonhartsberger, S.; Mihovilovic, M. D.; Bornscheuer, U. T.; Winkler, M.; Rudroff, F. Substrate-Independent High-Throughput Assay for the Quantification of Aldehydes. 2019, 361 (11), 2538.