Enzymatic generation of odor active aldehydes

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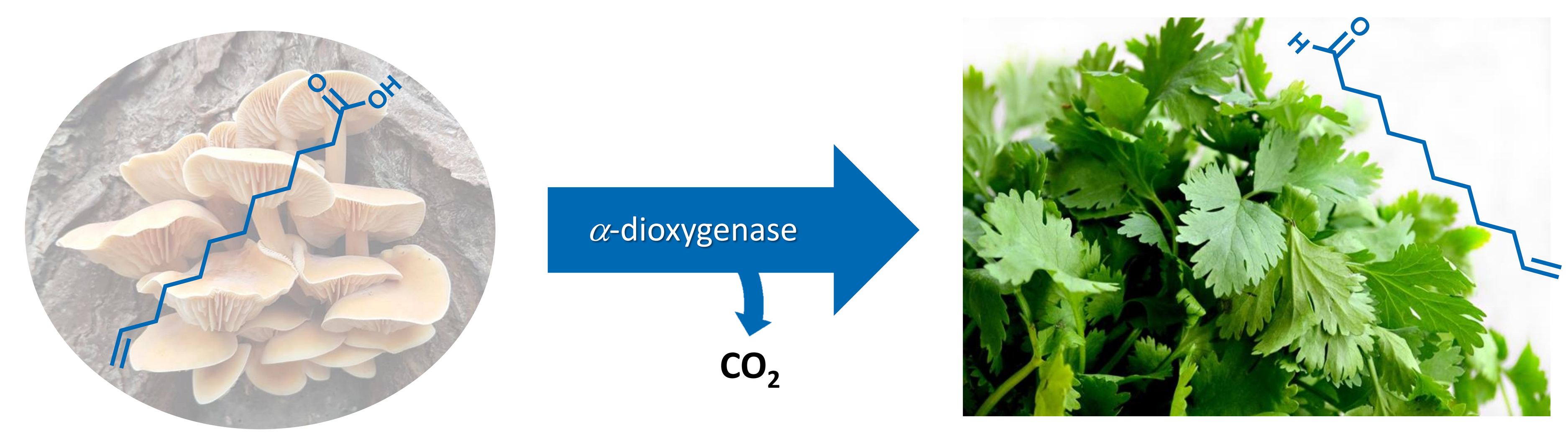
Introduction

Various aldehydes are shaping the aroma of flowers and leaves, fruits, vegetables, and meat [1]. The perceived odors greatly differ with the chemical structure (e.g. degree of unsaturation, chain branches), ranging from cucumber- ((2*E*,6*Z*)-nona-2,6-dienal) to stewed beef-juice-like (12-methyltridecanal) impressions [1,2]. Therefore, aldehydes represent promising target compounds for the development of flavors and perfume oils. To overcome current problems, like extensive downstream processing during production, fatty acids may be transformed to aldehydes by more modern and "greener" techniques such as the application of powerful enzymes.



Graphical abstract: Enzymatic generation of undec-10-enal (coriander-like odor) from fungal dodec-11-enoic acid.

Methods and results

Because fungal lipids contain of an enormous diversity of fatty acids, fungi grown in submerged culture were screened for precursors of aldehydes with interesting flavor properties. Mycelial lipids were extracted with *n*-hexane via a soxtherm apparatus. Fatty acid compositions were determined after forming the corresponding fatty acid methyl esters (FAMEs) by means of GC-MS (fig. 1A). Numerous uncommon fatty acids, including e.g. branched chain fatty acids and mono-, di-, and polyunsaturated fatty acids with double bonds at unusual positions were identified: *Conidiobolus heterosporus* provided 12-methyltridecanoic acid (an utilizable precursor for production of 12-methyltridecanal [2]), *Pleurotus eryngii* (9*Z*,12*Z*)-hexadeca-9,12-dienoic acid, and *Flammulina velutipes* dodec-11-enoic acid (fig. 2).

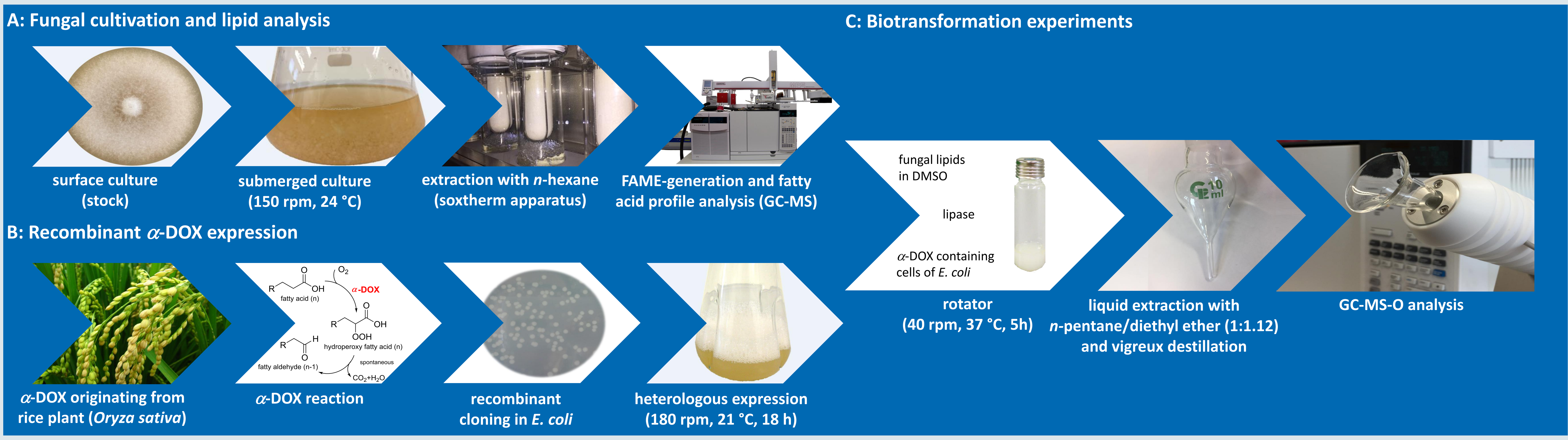
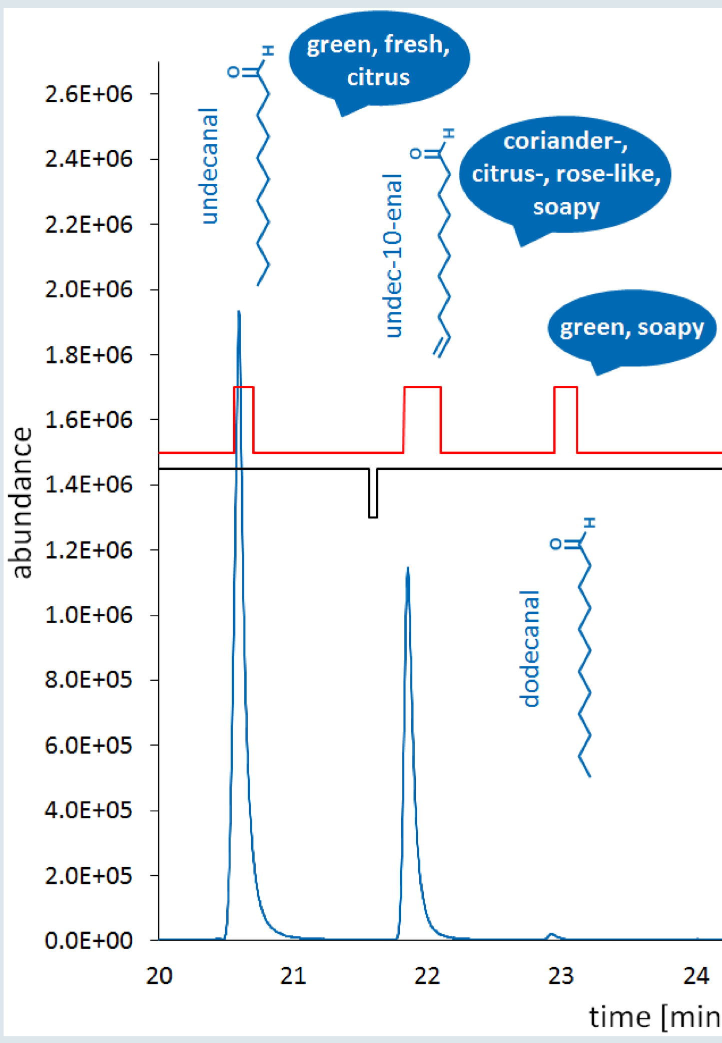


Figure 1: Experimental workflow.



fatty acid	content
lauric acid	39%
dodec-11-enoic acid	16%
tridecanoic acid	0.2%
myristic acid	7%

Figure 2: GC-MS-O analysis of biotransformed lipids of *F. velutipes*.

α-Dioxygenase (α-DOX) originating from *Oryza sativa* was recombinantly expressed in *E. coli* W3110(DE3) according to Kaehne *et al.* (2011) [3] (fig. 1B). Biotransformation experiments for generation of aldehydes were performed as one-pot-reactions in 20-mL-vials (fig. 1C). The reaction mixture was extracted three times with 4 mL *n*-pentane/diethyl ether (1:1.12, v/v), and concentrated via a vigreux column (43 °C) to a final volume of approximately 1 mL. The extract was analyzed by means of GC-MS-O. During olfactory analysis of biotransformed lipids of *F. velutipes* the coriander-like odor of undec-10-enal, derived from dodec-11-enoic acid, was clearly perceived (fig. 2).

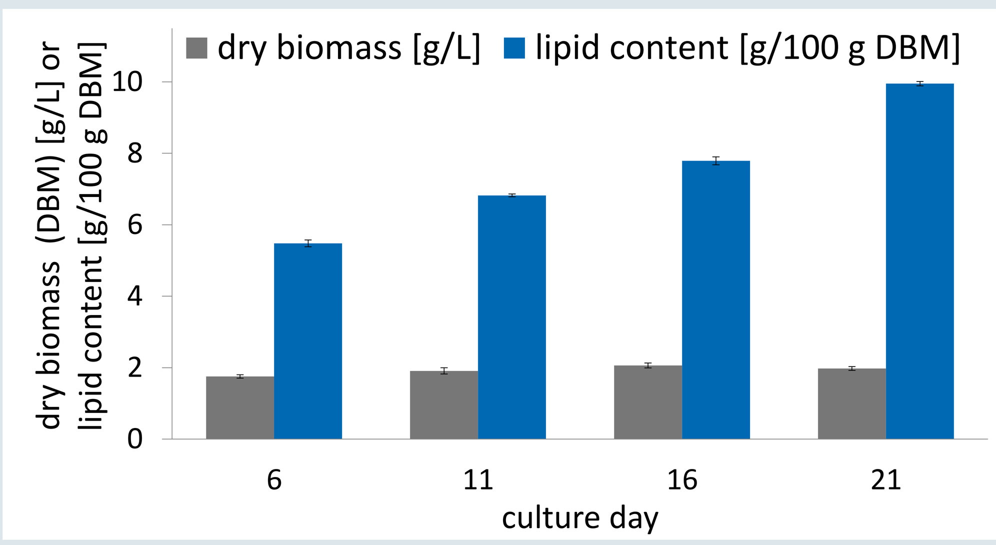


Figure 3: Lipid yields of nitrogen-limitated grown *F. velutipes*.

Naturally, low lipid contents of fungi are a bottleneck for their industrial application as sustainable lipid source. By establishing a culture medium containing malt extract, peptone, and glucose with high C/N-ratio, *F. velutipes* entered a nitrogen-limitated growth phase. It was characterized by a stagnation of biomass-production with simultaneous accumulation of storage lipids, leading to relatively high yields of lipids during prolonged culture time (fig. 3).

Conclusions and outlook

α-DOX was heterologously expressed in *E. coli* and enabled the generation of aldehydes as potent flavoring substances. Fungal lipids were used as raw material providing fatty acids, scarce in nature, which led to aldehydes with interesting odorant properties. A complex nutrient medium with high C/N-ratio allowed for an increased production of fungal lipids. Upscaling of the process will be the next step for future industrial applications.

Carboxylic acid reductases (CAR) represent another enzyme class which directly reduces the carboxyl group of fatty acids to aldehydes without shortening the carbon chain [4]. The application of these enzymes allows the generation of a second product from a single substrate. This fact makes further specimen of fungi attractive producers of precursors.

References

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