Biosynthesis of vanillin from clove oil: a new enzymatic cascade to reconvert a past synthetic process

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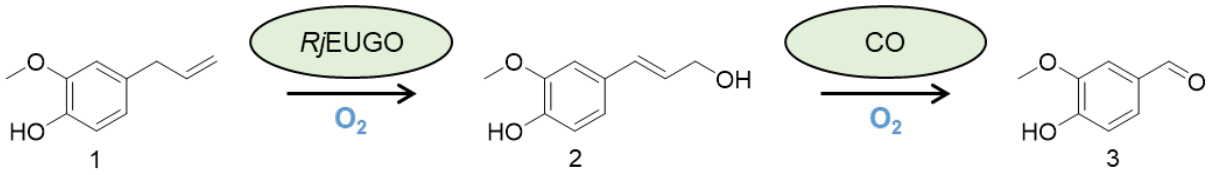
Vanillin is in high demand and is required for many applications. In the last decades its synthetic production largely relied on the petrol-chemical feedstock guaiacol. However, with the global call to exploit bio-based renewables, vanillin producers seek for novel and sustainable biosynthetic processes. We step 150 years back, when manufacturing of this already popular aroma was based on eugenol, the major component of clove oil. We revisited this simple – yet hazardous - synthetic route via isomerization and C=C cleavage to design a 2-step cascade requiring biocatalysts only. The double bond of eugenol was formally isomerized to get in conjugation to the aromatic ring, by oxidation to coniferyl alcohol with eugenol oxidase from *Rhodococcus jostii* (EUGO). Then, the C=C could be potentially cleaved in an oxidative fashion to obtain vanillin and glycolaldehyde as by-product. For this purpose, we screened putative and characterized alkene cleavage oxygenases (COs), 28 in total, among the carotenoid dioxygenase protein family for activity towards coniferyl alcohol. Finally, the combination of *Rj*EUGO and the alkene cleavage oxygenase from *Sphingomonadales bacterium* in a one-pot-one-step reaction led the bioproduction of vanillin from both eugenol as well as crude clove oil with comparable conversion. This new biocatalytic strategy enables to green synthetic processes, but also raises the value of the final product, which can be classified as natural when starting from natural eugenol.



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