Leveraging biodiversity for the biocatalytic production of esters *via* hemiacetal oxidation

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Abstract:
Esters, like methyl formate, ethyl acetate and butyl acetate are widespread commodity chemicals that find application in many industries, like food & beverages, pharma, and textiles. At the current time, they are produced in large quantities exploiting energy intensive chemical processes, starting from fossil feedstocks, with a high environmental impact. Biotechnologies constitute a valid alternative to the well established chemical methodologies. Enzymatic biocatalysis has severalfold advantages, such as mild reaction conditions, no harsh chemical agents requirements, and being safer for both humans and the environment. We then selected promising enzymes among alcohol dehydrogenases (ADHs), a class which is still poorly represented in the patent landscape and thus constitute an intriguing category for this research field. Some ADHs have been observed to oxidise hemiacetals, formed by the spontaneous reaction between an alcohol and an aldehyde, to esters, in presence of $NAD(P)^+$ as cofactor. Starting from the commercial Adh1 of *Saccharomyces cerevisiae*, in this work other ADHs from *Homo sapiens, Neurospora crassa, Clostridium beijerinckii and Helicobacter pylori* have been selected, heterologously produced and tested for the synthesis of methyl formate, methyl acetate, ethyl formate and ethyl acetate. Except for ADH from *H. pylori*, all the enzymes displayed the hemiacetal dehydrogenation activity, and showed the capability to regenerate autonomously the cofactors $NAD(P)^+$ allowing the production of high esters titers exceeding stoichiometric limits. This feature, which has never been observed before, is now patent pending. The hemiacetal oxidation route has been explored also for the production of other specialties esters: it has been successfully used for cellulose functionalization with *S. cerevisiae* Adh1 and ADH of *C. beijerinckii*, demonstrating its applicability also to large molecules like polymers in solid state and thus expanding the potential of this technology.