On the route of biocatalysis: *in vivo* selection in *Chlamydomonas* chloroplasts for an *in vitro* evolved bacterial formate dehydrogenase.

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The use of enzymes as biocatalysts in chemical synthesis is a growing technology. Its application in industrial processes requires stable, selective and productive enzymes that operate under the desired conditions and permit to minimize costs. Formate dehydrogenases (FDHs) are considered promising enzymes for the regeneration of NADH (or NADPH) when this expensive cofactor is required. The main advantages of FDHs are the irreversibility of the catalysed reaction, due to the gaseous nature of the CO_2 product, wide pH optimum and cheap substrate. However, their strict specificity for NAD^+ and the general low affinity for both substrate and cofactor are the major drawbacks for their otherwise wide applicability (Tishkov and Popov, 2006).
We have recently characterized a new FDH, named *Gra*FDH, identified by mining the genome of the extremophile prokaryote *Granulicella mallensis* MP5ACTX8. The new enzyme displays a valuable stability in the presence of many organic co-solvents as well as double cofactor specificity, with NADP+ preferred over NAD+ at acidic pH values, at which it also shows the highest stability (Fogal et al., 2015). The quite low affinities for both cofactors as well as for the substrate formate has prompted us to set experimental strategies for the optimization of the native enzyme, in view of its possible application as biocatalytic tool.
We opted for an *in vitro* directed evolution approach combined with an *in vivo* screening system performed in the chloroplast of the green alga *Chlamydomonas reinardhtii*. Indeed, it has long been known that formate inhibits photosynthesis and that this inhibition is reversed by bicarbonate (Xiong et al, 1998). Since formate is the substrate of FDH and bicarbonate the product, the inhibition by formate should be reversed in presence of a recombinant FDH in the chloroplast of *C. reinhardtii*. Moreover, the photosynthetic process consumes NADPH, which is regenerated by the recombinant FDH. Such a virtuous cycle could allow autotrophic growth in presence of formate to the cells expressing an efficient recombinant FDH. Therefore, the screening system is based on the expectation that the cells possessing more efficient variants of the enzyme would have different and more vigorous growth on formate compared to the other ones. We will report on a proof-of-concept experiment carried out to test and validate the designed system.

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