Hydroxylation of flavonoids in a cascade reaction using recombinant enzymes

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The search for new biocatalysts for efficient, tightly directed reactions is a leading concern in current biotechnology. This seems particularly relevant for types of reactions that are difficult to carry out using classical synthetic chemistry, such as the hydroxylation of aromatic compounds. Traditional screening method using collection of microorganisms with interesting catalytic properties are now in decline, replaced by *in silico* assays, that offers the possibility of quickly discovering new enzymes or expanding the knowledge of partially characterized biocatalysts [1]. Natural compounds remain an undiminished reservoir of new pharmaceuticals or their precursors. Furthermore, metabolic pathways for their synthesis or degradation represent an ideal source of potential enzymes with desirable catalytic capabilities [2].

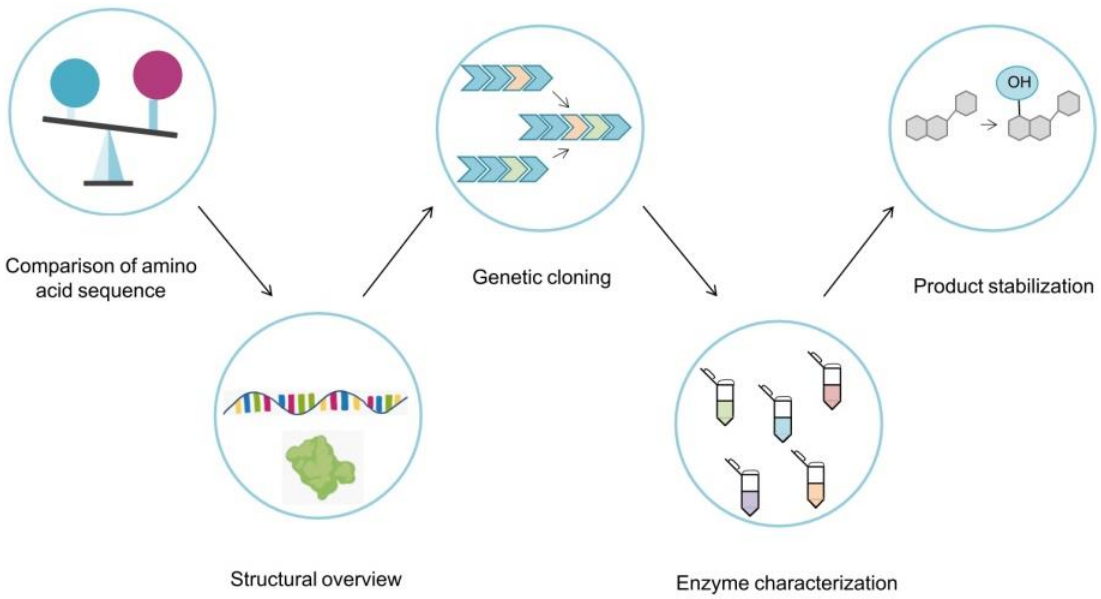

Figure 1. Flow scheme of the work.

This work demonstrate evidence for this approach. Based on preliminary bioinformatic analysis, a set of enzyme was selected and produced in *Escherichia coli* cells. This was followed by biochemical analyses and substrate specificity assays for each of the tested

enzymes. The final step was reaction engineering for stabilization of easily degradable hydroxyderivatives of flavonoids. The application of the above workflow (Fig. 1) allowed us to obtain products enriched with an additional hydroxyl group at the C6, C8, and C3' positions, using biocatalysts from different hosts, belonging to different enzymatic classes and requiring different cofactors.

References

[1] Höhne, M. *et al*., *Nature Chemical Biology*, 6, (2010), 807–813, DOI: 10.1038/nchembio.447.
[2] Heim, K. E., *et al.*,*The Journal of Nutritional Biochemistry* 13, (2002), 572–584, DOI: 10.1016/s0955-2863(02)00208-5.

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