Towards the production of new-to-nature sweeteners using hernandulcin as starting skeleton

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Abstract

Hernandulcin is a naturally occurring intense sweetener that is present in the Mexican plant *Lippia dulcis*. However, its use is limited by the low synthetic yield *in planta* and its poor water solubility. Synthetic biology and enzyme engineering can circumvent these limitations and also permit the conception of new classes of molecules with improved physico-chemical properties and controlled stereoselectivity. In this context, we envision strain and enzyme engineering for the construction of a châssis strain derived from *Saccharomyces cerevisiae* dedicated to the production and diversification of bisabolol and hernandulcin.
The first step of our approach relies on i) the rerouting of the yeast sterol pathway to accumulate farnesyl pyrophosphate and ii) the introduction of the bisabolol synthase encoding gene in the yeast genome. This was achieved and a strain was constructed that produces milligrams of bisabolol per liter of culture. This chassis is suitable for *in vivo* screening of enzymes of interest to obtain a large panel of derivatives including ones that do not exist in nature.

For this purpose, two target modifications were selected:

- oxidation through the action of cytochrome P450 enzymes isolated from a collection of native enzymes covering a broad substrate specificity in order to obtain hernandulcin and/or oxidized bisabolol harbouring different patterns of oxidation. The screening assay has been designed in the engineered strain that produces bisabolol and also overexpresses the redox partner of cytochrome P450, namely the cytochrome P450 reductase.

- glycosylation by GH13 or GH70 transglucosylases to improve the water solubility of the new molecules. Various libraries of transglucosylases with different linkage and substrate

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specificities will be tested. To this end, an *in vitro* assay using *E. coli* cell lysates that overproduce GH enzymes was set up.
Finally, the "new to nature" molecules will be evaluated for their sweetness potency using an *in vitro* based assay relying on human sweetener receptor.

Keywords: Synthetic biology, yeast (Saccharomyces cerevisiae), sweetener, metabolic engineering