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IIBM Seminar
"Protein engineering for the biosynthesis of nutraceutical compounds"

Daniela Herrera

Postdoc RWTH Aachen University, Germany
(starting on October 2022)

Wednesday 14th September 2022 - 13:00 Hrs - Lunch included
Hybrid Seminar – Sala C-306 Const. Civil
Zoom link: Contact **secretariaiibm@uc.cl**

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"Protein engineering for the biosynthesis of nutraceutical compounds ”

Stilbenes are phenolic compounds derived from plants in response to different types of stress.

Resveratrol is one of the most studied stilbenoids due to its health promoting-properties but undergoes rapid metabolization and has low bioavailability. It has been reported that methylated derivates have an improved pharmacokinetic profile and, therefore, a higher industrial attractiveness.

Few studies have addressed the biosynthesis of pinostilbene, a valuable mono-methylated resveratrol derivative, because most of the characterized enzymes (O-methyltransferases, OMTs) di-methylate resveratrol, yielding pterostilbene as a principal product (Figure 1).

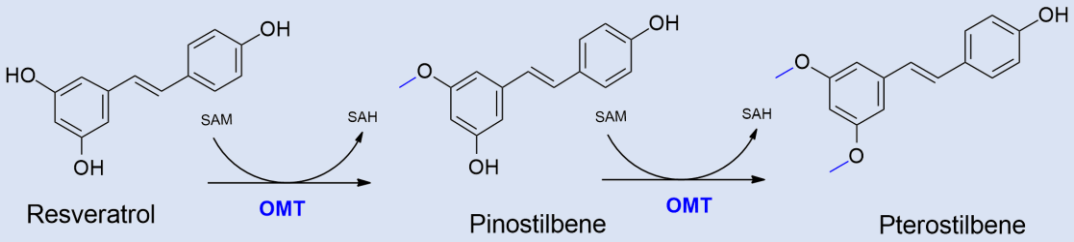

Figure 1. Representation of the resveratrol sequential di-methylation reaction by OMTs.

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Tailoring enzymes properties for biotechnological applications can be achieved by different protein engineering approaches. Here, we present the rational design of the resveratrol OMT from *Vitis vinifera* (VvROMT) to modify its substrate preference and develop an alternative pathway to obtain pinostilbene.

In the absence of a crystal structure of VvROMT, we constructed a three-dimensional protein model in a closed and catalytically competent conformation. Then, by applying different *in silico* tools, we identified four critical binding site residues. We performed site directed mutagenesis in these positions generating W20A, F24A, F311A, and F318A variants, which greatly reduced resveratrol's enzymatic conversion, validating our structural model.

Finally, we rationally designed eight variants through a structure-based strategy and comparison of the binding site residues of our model with other stilbene OMTs. The variant L117F/F311W showed the highest conversion to pinostilbene, and variant L117F presented an overall increase in enzymatic activity.

These variants could be potentially integrated into a synthetic pathway for sustainable production of pinostilbene in an existing metabolically engineered system. Furthermore, our results suggest that VvROMT can be tailor-made to diversify methylated stilbenes and other related phenolic compounds.