Characterization of a novel Pictet–Spenglerase toward the biosynthesis of new plant natural product scaffolds

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Abstract: Plants have long been a valuable source of secondary metabolites with pharmaceutical relevance, and the demand for these compounds is continuously increasing. Among these, alkaloids represent one of the most structurally diverse classes and include numerous families, such as the benzylisoquinoline alkaloids (BIAs). Often these groups of metabolites originate from a biosynthetic scaffold formed through the Pictet–Spengler condensation.

The integration of biocatalysis with synthetic biology has recently enabled the development of alternative production platforms for plant natural products (PNPs), overcoming typical limitations of conventional synthesis.. Specifically, Pictet–Spengler reactions have been reconstituted *in vitro* using characterized Pictet-Spenglerases such as norcoclaurine synthase (NCS) and derived truncated forms, strictosidine synthase (STR), and related homologs identified in diverse plant lineages [Masakatsu et al. (2014) https://doi.org/10.1080/09168451.2014.890039;Treimer et al. (1979). https://doi.org/10.1111/j.1432-1033.1979.tb04235.x]

Plant biodiversity, however, continues to offer a rich source of previously uncharacterized enzymatic enzymes. Within this context, the newly discovered enzyme, PSS (1-phenethylisoquinoline scaffold synthase), from *Gloriosa superba,* was proved to catalyse the condensation of 4-HDCA (4-hydroxydihydrocinnamaldehyde) and dopamine [Sun et al. (2025). https://doi.org/10.1007/s11033-025-10364-y] yielding 1-phenethylisoquinoline scaffold.

In silico analyses, carried out in the work here presented, revealed that its amino-acid sequence has structural motifs compatible with broader substrate scope, suggesting potential catalytic activity toward the synthesis of norcoclaurine and other alkaloids, including norbelladine.
Building on these predictions, the project aims to functionally characterize *Gs*PSS and evaluate its ability to generate PNPs scaffolds. These findings could provide a proof-of-concept for how evolutionary diversification can give rise to novel biocatalysts with significant potential for industrial biomanufacturing.