Synthetic biology approaches and biochemical biodiversity towards Glucobrassicin production in *Saccharomyces cerevisiae*

Maestroni L.[1], Butti P.[1], Milanesi R.[1], Pagliari S.[1], Campone L.[1], Serra I.[1], Branduardi P.[1]

E-mail: letizia.maestroni@unimib.it

[1] Dipartimento di Biotecnologie e Bioscienze, Università degli studi di Milano Bicocca, 20126 Milano

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Abstract:
Glucosinolates (GLSs) are secondary metabolites naturally produced by members of cruciferous vegetables with cancer-preventive properties, mainly thanks to their hydrolysis products. GLSs extraction from natural producers still poses feasibility issues at industrial scale and their chemical synthesis is challenging due to the structures complexity. Microbial cells show several advantages compared to conventional chemical synthesis and the yeast *Saccharomyces cerevisiae* is an election chassis for these purposes.

Glucobrassicin (GLB), an indolyl-methyl glucosinolate, is the precursor of indole-3-carbinol, one of the most characterized bioactive compound. Nevertheless, the exact characterization of GLB biosynthetic pathway is still not completely defined as the sulfur-donating enzyme, the third step of the pathway, remains to be identified.

We developed a novel toolkit, a combination of synthetic biology approaches (Golden Gate Assembly and CRISPR-Cas9 system), to standardize and accelerate the construction of chassis carrying different variants of heterologous pathways.

To elucidate the pathway, we explored the potential of natural biodiversity by i) comparing the efficacy of the two different versions of GLB biosynthetic pathway and ii) the contribution of two different homologues of the first enzyme of the pathway, CYP79B2, by cloning them from two different plants. Here we show the obtained results, the evaluation of potential limiting steps and the final titer of the best producing strain, which represent a significant improvement in respect to what reported in literature.