Glucobrassicin production in yeast using a synthetic biology approach

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Abstract:
Plants can produce a wide range of secondary metabolites, many of which are valuable pharmaceutical and nutraceutical compounds that when in human organism can interact also with the gut microbiota to produce a wide range of compounds with different effects on our health. For instance, glucosinolates are naturally produced by members of cruciferous vegetables and possess cancer-preventive properties mainly thanks to their hydrolysis products.

We focused our attention on the microbial production glucobrassicin, an indolyl-methyl glucosinolate contained mainly in *Brassica* and *Raphanus* species. We started designing a recombinant strain of *Saccharomyces cerevisiae*, using a synthetic biology approach. Since this yeast does not naturally produce glucosinolates, the introduction of a heterologous pathway is necessary to allow the production of glucobrassicin. The pathway is composed by five enzymes from a cauliflower cDNA library: two P450 cytochromes, a C-S lyase, an UDP-glucose transferase and a sulfotrasferase. The strategy involves the creation of a collection of more than 30 DNA parts, comprising promoters, terminators and the five genes of the heterologous pathway, as well as some extra parts which will simplify the pathway's engineering and monitoring.

These parts will be assembled in three devices using the Golden Gate Assembly, so to build up a library of expression cassettes with different promoters, that can be easily integrated in yeast's genome using CRISPR-Cas9 system. The obtained strains will be screened to identify the optimal combination that allows the best production. The systems, which is modular by design, will be further improved by targeting the five enzymes to a protein scaffold, to maintain them in proximity and enhance their efficiency.

At the same time, we will develop the analytical methods necessary to detect the final product and, when possible, the pathway intermediates, to allow iterative cycles of strains optimization.