Glucosinolates as nutraceuticals: Process optimization and scale-up of Bio-based microbial production of glucobrassicin

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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 with the gut microbiota with different effects on our health.

We focused the attention on microbial based production of glucosinolates (GLSs), which are naturally produced by members of cruciferous vegetables and possess 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 complexity of the structures.

Glucobrassicin, an indolyl-methyl glucosinolate contained mainly in *Brassica* and *Raphanus* species, is the precursor of indole-3-carbinol (I3C), one of the most characterized bioactive compound. We started working on a recombinant strain of *Saccharomyces cerevisiae* which expresses all enzymes involved in glucobrassicin biosynthetic pathway, constructed in a previous work. The first aim is to improve the titer thanks to further engineering and at the same time to develop analytical methods to evaluate the best glucobrassicin producers and the best production process. 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. 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 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.

In parallel, we are working on the development of an *in vitro* test on Intestinal epithelial cell line Caco-2 to verify if GLSs can exhibit a protective effect on the intestinal barrier.

As future perspective, to make the process economically feasible, we will work on the selection of a residual biomass and the development of a bio-based process.