CRISPR/Cas9 strategy for synthetic biology in *Saccharomyces cerevisiae*: The case study of L-Ascorbic Acid production

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Abstract:
L-Ascorbic acid (L-AA) or vitamin C is a water-soluble vitamin produced by plants and animals except for human and other mammals. Vitamin C acts as cofactor for several enzymatic activities such as peroxidation, oxidation and hydroxylation. Lack of Vitamin C impairs proline and lysine hydroxylation, affecting collagen biosynthesis and leading to blood vessels damage and scurvy. Besides, ascorbic acid also acts as antioxidant and anti-inflammatory compound regulating immunity and inflammatory responses. Because of its properties, L-AA is added in food and beverages, as well as, used as food supplements.

Nowadays production of L-AA is based on a mixed process composed of a traditional step followed by a bioconversion process catalysed by *Acetobacter oxidans.*

In the past years, our laboratory developed a one-step bio-based production of vitamin C engineering *Saccharomyces cerevisiae* with *Arabidopsis thaliana* genes required for L-AA biosynthesis.

In the present work, a second-generation L-AA producing strain has been developed using the EASY-MISE toolkit, a newly adapted tool exploiting Golden Gate assembly and CRISPR/Cas9 editing for genes integration in *S. cerevisiae*. This strategy allows for the precise and modular introduction of expression cassettes in loci characterised for their stability and expression level.

Considering this improvement, the performances of the second-generation strain will be compared with those of the first-generation one. This allowed for a direct comparison of the two engineering strategies, highlighting the importance and the efficaciousness of synthetic biology tools for accelerating and ameliorating microbial cell factories construction.