

Towards for the use of *Zygosaccharomyces parabailii* as synthetic biology chassis

<u>**Riccardo Milanesi**</u>, Pietro Butti¹, Camilla Magnani¹, Valeria Mapelli¹, Michela Clerici¹, Paola Branduadi¹ *E-mail: riccardo.milanesi@unimib.it* ¹ Department of Biosciences and Biotechnologies - University of Milano-Bicocca, Milan, Italy

Keywords: Non-Saccharomyces yeasts, Sustainable bioprocesses, Organic Acids Resistance, Synthetic Biology, Precision DNA editing, Metabolic Engineering

Abstract:

Zygosaccharomyces parabailii is a hybrid yeast originated with the mating of *Zygosaccharomyces bailii* and an unknown parental yeast. It presents a complex genome and heterosis (hybrid vigor) towards the tolerance of organic acids at low pH.

This phenotype can be of particular interest for the industrial production of organic acids in the protonated form, reducing the costs in buffering the growth media and product purification. Nevertheless, genomic complexity and a low efficiency in homologous recombination (HR) are hindering the valorisation and development of this yeast as chassis for industrial purposes.

Recently, a CRISPR/Cas9 tool for gene inactivation/deletion and directed chromosomal integration in *Z. parabailii* was established, despite its low efficiency. In the present work, novel approaches aimed to reduce the activity of non-homologous end joining (NHEJ) have been applied. Besides, integration cassettes and gRNA were re-design in order to improve mutant stability and integration precision.

Taken together, these solutions will facilitate the engineering of *Z. parabailii* and accelerate the construction of novel strains. Eventually, this will pave the way for the use of this yeast as synthetic biology chassis for industrial applications.

This study had the partial contribution of the MUSA – Multilayered Urban Sustainability Action – project, funded by the European Union – NextGenerationEU, under the National Recovery and Resilience Plan (NRRP) Mission 4 Component 2 Investment Line 1.5: Strengthening of research structures and creation of R&D "innovation ecosystems", set up of "territorial leaders in R&D". Contribution from Consorzio Interuniversitario Nazionale per la Scienza e Tecnologia dei Materiali (INSTM) is also acknowledged.