Expanding *Kluyveromyces marxianus* synthetic biology toolbox for streamlining natural dyes cell factories construction

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Keywords: *Kluyveromyces marxianus*, chassis strains, metabolic engineering, natural dyes, tryptophan

Abstract:

Kluyveromyces marxianus is a promising yeast industrial host that has lately drawn attention as a chassis for bioprocessess also based on second generation feedstock due to its thermotolerance, fast growth rate and capability to use a wide range of substrates. Thanks to these characteristics, *K. marxianus* was used to successfully generate chassis strains for the production of tryptophan-derived, violacein-related pigments to be used as natural dyes. Nevertheless, some synthetic biology imitations blocked the strain optimization and, more generally, represented an important limit to *K. marxianus* chassis use. Specifically, the shortage of integration sites at chromosome level and selection markers is a key limitation. We developed a bioinformatic routine to ease the search for new integration sites and designed new parts for marker-free cloning with the final aim of streamlining *K. marxianus* use and accelerating the genetic manipulation process. The systems developed are being implemented on violacein-related producing chassis to assess their effectiveness.