Adaptive Laboratory Evolution to Enhance Organic Acid Tolerance in *Kluyveromyces marxianus* on Residual Biomass

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The concept of biorefineries is revolutionising the production industry in order to sustainably meet the basic requirement of growing population with limited resources. In biorefineries one of the key instruments in the biochemical conversion of lignocellulosic feedstocks (the most abundant renewable biomass present on the planet) to advanced biofuels and other commodities is the development and usage of efficient, robust, versatile microbial cell factories with innate or engineered traits. Regrettably, the pretreatment of lignocellulosic biomass, necessary to make the sugars accessible, releases organic acids that are inhibitory to the production microorganisms and *Kluyveromyces marxianus, a* non- Saccharomyces yeast, is not an exception despite several advantages that it possesses. It has unique ability to grow at high temperatures (up to 45^{o}C) and to utilize broad range of substrates, including the C5 sugars that are present in lignocellulosic biomass.

In this work, we aim to enhance the organic acid tolerance of *K. marxianus* on sugar beet pulp (SBP), a residual lignocellulosic biomass composed of C5 and C6 sugars along with acetic and lactic acid that inhibit growth at low pH. Low pH is an important parameter in industrial production to minimize bacterial contamination and to ease the purification of organic acids when these are the desired end products. Adaptive Laboratory Evolution (ALE) was used to generate and select *K. marxianus* variants with improved tolerance to weak organic acids at low pH in SBP at two different temperatures. ALE was performed by sequential serial passages in shake flasks every 24 hours. \ALE was performed for up to 1805 and 1522 generations at 30^{o}C and 40^{o}C respectively over a period of 105 days. The performances of wild type strain and evolved isolates were tested by pH-gradient acetic acid plate assays and growth kinetics in liquid media. By comparing the specific growth rate and the duration of the lag phase, *K. marxianus* variants with improved tolerance were selected.

This work describes a strategy to obtain *K. marxianus* yeast strains with improved tolerance to organic acids present in lignocellulosic feedstocks for use in biorefineries. Genome sequencing will help in identifying the mutations involved; thereby better describing how their desirable phenotypic traits can be linked to their genotype. The best- evolved cell factory will be engineered for production of organic acid such as lactic acid, which has wide range of applications in various industrial sectors.