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Improving cellobiose utilization and fermentation by Saccharomyces cerevisiae strains via metabolic and evolutionary engineering

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Abstract

Engineered yeast chassis are frequently used for the industrial production of biofuels and chemicals. However, after some initial metabolic engineering steps, further optimization of the microbial host is usually required to achieve high product yields and productivities. In this project, we aim to improve the conversion of cellobiose into ethanol by Saccharomyces cerevisiae. For this purpose, we first metabolically engineered cellobiose transporters and intracellular cellobiases into yeast. Subsequently, the engineered strains were subjected to laboratory evolution, in order to improve their growth rates on cellobiose.

Key words: *Saccharomyces cerevisiae, cellobiose, evolutionary engineering. .*

Introduction

Cellobiose is a disaccharide present in the context of a second-generation biorefinery (2G), which employs lignocellulosic raw materials for the production of bio-based fuels and chemicals. The fuel ethanol industry currently uses the yeast *Saccharomyces cerevisiae* as the microbial platform for the production of ethanol via fermentation. However, *S. cerevisiae* is not able to naturally metabolize cellobiose, an intermediate product that accumulates during the enzymatic hydrolysis of cellulosic materials. In a previous work, some *S. cerevisiae* strains expressing different fungal genes involved in cellobiose utilization were engineered and subsequently evaluated for their ability to grow on cellobiose as the sole carbon and energy source. However, the strains displayed poor growth under this condition (μ_{max} << 0.1 h^{-1}). Thus, we decided to improve the kinetic performances of these strains by evolutionary engineering[1], and subsequently characterize the evolved phenotypes by physiological analysis.

Results and Discussion

Two of the engineered strains (namely PBY_09 and PBY_14) were subjected to evolutionary engineering via several serial transfers in synthetic medium with 20 g/L initial cellobiose. To evaluate the behavior of the maximum specific growth rates (μ_{max}) throughout the course of evolution, growth profiles of cells isolated from single-colonies at different stages of the experiments (the "fossil record") were obtained from microplate cultivations. After ~162 generations, single-isolates originated from PBY_14 reached μ_{max} between 0.14 and 0.16 h^{-1}, representing a 110-145% increase compared to the parental strain (figure 1). Regarding strain PBY_09, single-colonies isolated after only ~45 generations showed μ_{max} between 0.11 and 0.14 h^{-1}, while the parental strain displayed no growth on cellobiose (figure 1). In spite of the observed growth rate increases, when the evolved strains were characterized in shake-flask cultivations, no ethanol formation was observed (results not shown), indicating respiratory metabolism, probably as a result of slow sugar consumption.

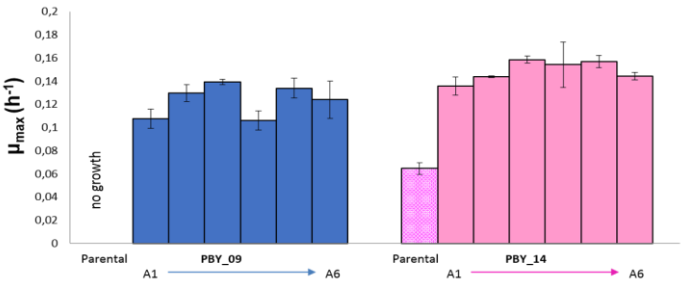

Figure 1. μ_{max} reached by single-colony isolates (A1-A6) originated from PBY_09 and PBY_14 after ~45 or 162 generations of evolution, respectively. Values are the mean of three replicates. Error bars show the standard deviation from the mean value.

Conclusions

- Evolutionary engineering is an efficient and simple methodology for the selection of phenotypes with higher growth rates on non-favored carbon sources;
- After several transfers in cellobiose-containing media, single-colonies originated from PBY_09 and PBY_14 reached μ_{max} between 0.11 and 0.14 h^{-1} and 0.14 and 0.16 h^{-1}, respectively, while the parental strains displayed poor or no growth;
- Physiological characterizations of the evolved phenotypes revealed no ethanol formation in shake-flask cultivations; suggesting that cellobiose metabolism is different from glucose metabolism; further evolution under anaerobic conditions is required;
- Due to the promising results achieved so far, the evolutionary engineering strategy will now proceed with the remaining strains.

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