

Genetic and molecular approaches for increasing the isoprenoid pathway flux in *Saccharomyces cerevisiae*

Abstract:

Saccharomyces cerevisiae is one of the preferred hosts for terpenoid production through the synthetic biology route and various metabolic engineering strategies have been developed to re-engineer pathways and networks to increase the yields of terpenoids. Most of metabolic engineering efforts have focused on the core isoprenoid pathway and their cofactor requirements. Therefore, in this study we were interested in taking an unbiased approach for the identification of novel genes/players affecting isoprenoid pathway flux in *S. cerevisiae*. Towards the goal a carotenoid- based visual genetic screen for increased isoprenoid flux was developed. The visual screen was developed by exploiting the carotenogenic genes from the highest β -carotene producing red yeast, *Rhodospiridium toruloides*. The metabolic bottleneck at the rate limiting step phytoene dehydrogenase was an impediment to the development and was overcome by isolating variants of phytoene dehydrogenase with enhanced catalytic efficiency through directed evolution and by limiting the precursor pools (GGPP). The screen was also validated by *tHMG1*- a known flux enhancer in isoprenoid pathway of *S. cerevisiae*.

In the second part of study, attempts were made to isolate the novel mutants of global TATA binding protein, SPT15 for enhanced isoprenoid pathway flux using the carotenoid based visual screen. Three *spt15* variants were isolated that were capable of enhancing the overall isoprenoid pathway flux in *S. cerevisiae*. To investigate the mechanism for enhanced isoprenoid pathway flux in one of these mutants, *spt15_A101T* constraint based metabolic flux analysis was exploited. It was observed that the carbon rerouting for enhancing precursors, acetate and acetyl CoA and NADPH pools was important for enhancing flux. Transcriptomics and FBA suggest the critical role of pyruvate decarboxylase, *PDC6* and phosphate limitation for enhancing isoprenoid pathway flux in *spt15_A101T* mutant. In the last part of study, attempts were made to decipher the pathway for Torularhodin biosynthesis, a carotenoid found in red yeast *R. toruloides*. The identification of putative/probable enzymes in the predicted pathway for Torularhodin synthase was carried out and attempted to validate these predictions through multiple approaches.

