TAILORED BIOSYNTHESIS OF PLANT-DERIVED GINSENOSIDE RH2 IN YEAST VIA REPURPOSING A KEY PROMISCUOUS MICROBIAL ENZYME

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With the development of synthetic biology, substantial progress has been made in the low-cost production of plant natural products in microbes. Ginsenoside Rh2 is a potential anticancer drug isolated from medicinal plant ginseng. Fermentative production of ginsenoside Rh2 in yeast has recently been investigated as an alternative strategy compared to extraction from plants. However, the titer was quite low due to low catalytic capability of the key ginseng glycosyltransferase in microorganisms. Herein, we have demonstrated high-level production of ginsenoside Rh2 in Saccharomyces cerevisiae via repurposing an inherently promiscuous glycosyltransferase, UGT51 (Fig. 1). The semi-rationally designed UGT51 presented a ~1800-fold enhanced catalytic efficiency (kcat/Km) for converting protopanaxadiol to ginsenoside Rh2 in vitro. Introducing the mutant glycosyltransferase gene into yeast increased Rh2 production from 0.0032 to 0.39 mg/g dry cell weight (DCW). Further metabolic engineering, including preventing Rh2 degradation and increasing UDP-glucose precursor supply, increased Rh2 production to 2.90 mg/g DCW, which was more than 900-fold higher than the starting strain. Finally, fed-batch fermentation in a 5-L bioreactor led to production of ~300 mg/L Rh2, which was the highest titer reported.In the present study, high production of ginsenoside Rh2 represented an excellent example of tailored biosynthesis of plant natural products in microbes via the use of an engineered promiscuous microbial enzyme.

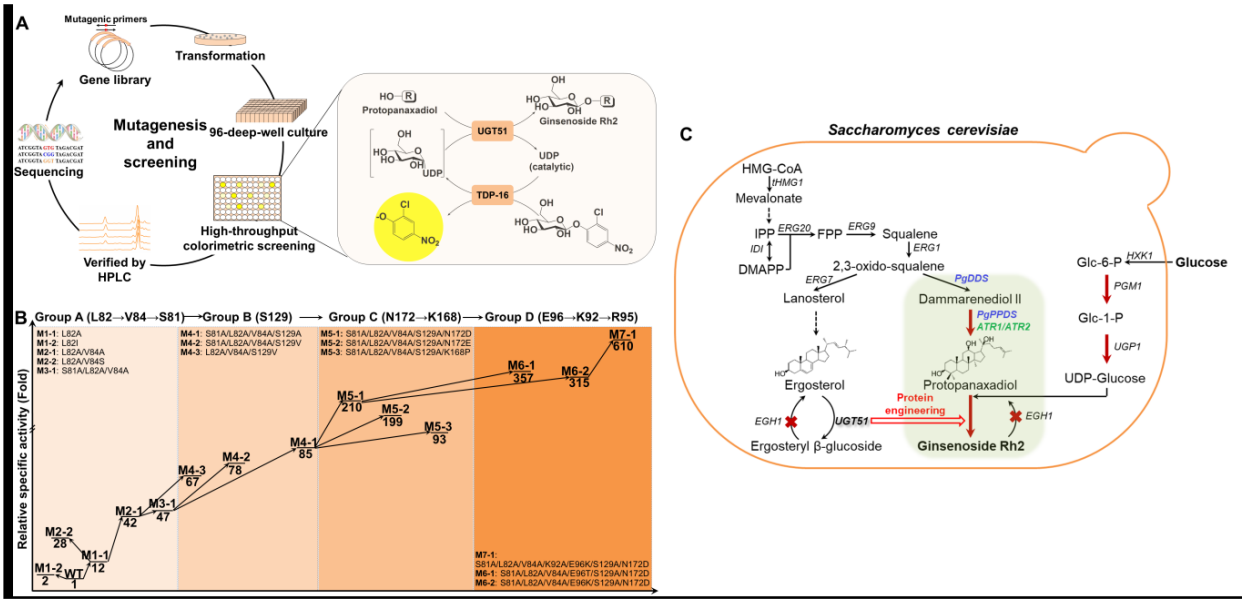

Fig. 1. Evolution of glycosyltransferase UGT51 towards an efficient Rh2-synthase (A-B) and biosynthetic pathway of ginsenoside Rh2 in the engineered yeasts (C).