SYSU-CHINA 2022

ACCESS

Animalcule co-catalytic engineering for Salidroside synthesis

Background

Mountaineering, a breathtaking journey symbolizing human's courage of exploring the nature. With the rising elevation, the insufficient oxygen level may lead to high altitude illness. For many centuries, mountaineers from Asia take extract of *Rhodiola* for preventing the discomfort. However, unrestricted exploitation imperils the living of wild *Rhodiola*, which is currently listed as China national category-II protected plant.

Salidroside, the active ingredient of *Rhodiola*, has proven to be effective for preventing high altitude illness and its related symptoms. We wish to achieve de novo synthesis of salidroside in *E.coli* as an alternative to wild *Rhodiola* extracts for protecting this precious plant.

Design

The synthesis pathway from glucose as precursor to salidroside is considerably complicated (fig 1.). If put into just one strain, it would face enourmous metabolic burden. This does not benifit the proliferation of bacteria. Moreover intermediates (tyrosol, for example) are somewhat toxic and thus can accumulate to inhibit bacteria's growth. We broke down the pathway into 3 parts for 3 trains respectively:

1. Convertion from glucose to tyrosine.
2. From tyrosine to tyrosol by dehydrogenation and reduction.
3. From tyrosol to salidroside by transglycosulation.

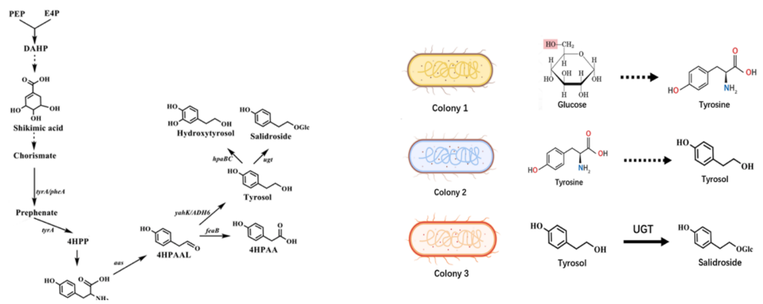

Fig 1. Synthetic pathway from glucose precursor to salidroside

Reference

[1] Yi, Xiao et al. "Plasmid hypermutation using a targeted artificial DNA replisome." Science advances vol. 7,29 eabg8712. 16 Jul. 2021

[2] Williams, Gavin J, and Jon S Thorson. "A high-throughput fluorescence-based glycosyltransferase screen and its application in directed evolution." Nature protocols vol. 3,3 (2008): 357-62

Co-catalysis

The concept of co-catalysis is different from co-culture. In a typical co-culture system, there should be metabolic relationship between different cells, and the bacteria are proliferating while fermenting. But in our co-catalytic system, there is no metabolic relationship between different chassis bacteria so that we can avoid the toxicity of metabolic intermediates.(fig 2.) Thus, different bacteria act as modularized separative parts. The only function of these parts is to catalyze the reaction they are responsible for. The changing and modification of one part will not affects others. In all, compared to co-culture system, co-catalytic system is more flexible, functional and versatile.

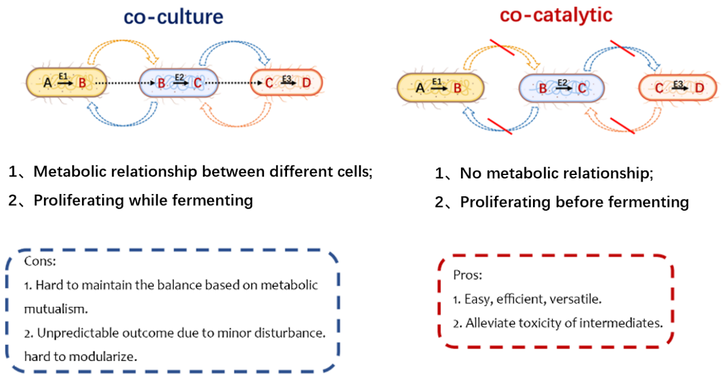

Fig2. The differences between co-culture and co-catalysis

Spytag - SpyCatcher

To increase the permeability of plasma membranes and promote transfusion of intermediate metabolites amongst different strains, we should heat the proliferated strains. To stablize the key enzymes during the process, we added Spytag and Spycatcher (fig 3.) on both ends of the enzyme to cyclize them.

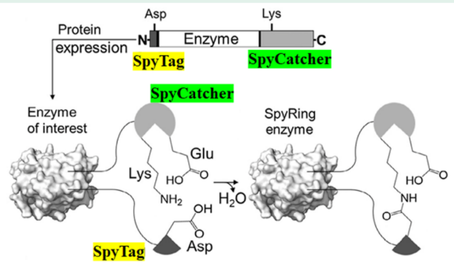

Fig 3. schematic repretation of Spytag and Spycatcher

Improvment of parts--Directed evolution of UGT

To increase salidroside yield, we are to use directed evolution to improve diphosphate dependent glycosyltransferase (UGT).

1. Use TADR system to accumulate mutation on UGT inside the bacteria.
2. Use glycosyltransferase based on fluorenscent signal to select the mutations.

TADR

Targeted artificial DNA replisome (TADR) accumulates mutation in vivo. This way we are able to screen more mutations without going through ligation and transformation.

Table 1. Introduction to TADR system

Component	Encoding position	Function
nickase	Cis gene on chromosome	Recognize specific sequences on target plasmid. Cleavage on both ends to create single strand breakage.
helicase- error -prone DNA polymerase complex	Helper plasmid	Unwind the target sequence. Introduce mutation while forming complementary strand.
target sequence of UGT	Target plasmid	Mutated while replicated.

Selection of UGTs

We will use 4-methylumbelliferone as acceptor of glycosyl group to assay the efficiency of glycosyltransferation. 4-methylumbelliferone is fluorescent, but when linked to a glycosyl group, the fluorescent signal is quenched. Therefore the rate of disappearance of fluorescent signal reflects the efficiency of UGT.