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Opinion Article

Microbial Production of Raspberry Ketone (RK)

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DESCRIPTION

Plant natural products represent a large and chemically diverse collection of secondary metabolites, but this diversity is generated from a limited number of conserved metabolic pathways. One such class of plant substances is the phenylpropanoids, which, like flavonoids, stilbenes and lignans, are formed from the common metabolic precursor p-coumaric acid *via* the amino acids phenylalanine and tyrosine. Raspberry Phenylbutanoid Ketone 4-(4-hydroxyphenyl)butan-2-one (RK) is a natural flavor found in plants such as raspberry, grape, peach and rhubarb. RK's low odor threshold berry flavors are used as food additives to create a variety of flavors such as cherry, strawberry, kiwi and other fruits. Thus, the economic incentives to produce raspberry ketones and the extremely low yield from plant tissue make this compound an excellent target for production using synthetically modified microbial strains. Previous studies have shown that raspberry ketones can be produced from p-coumaric acid in heterologous systems such as *E. coli* (5 mg/L) and Saccharomyces cerevisiae (trace). However, de novo production of raspberry ketones that does not require the addition of precursors has not yet been demonstrated. Demand for "natural" raspberry ketones has increased significantly.However, the product is very expensive. Therefore, there remains a desire to better understand how raspberry ketones are synthesized *in vivo* and which genes and enzymes are involved.With this information, alternative production strategies such as microbial fermentation can be better developed.

Microbial RK is produced using genetically engineered microorganisms such as yeast, *E. coli*, and other bacteria. To establish a heterologous system for the production of raspberry ketones, a de novo biosynthetic pathway containing four distinct enzymatic activities was engineered in *S. cerevisiae*. Synthetic protein fusions have also been investigated to optimize the production of this valuable flavor compound and have been found to increase the final concentration of raspberry ketones by a factor of five. Finally, metabolic engineering was performed on a wine strain of *S. cerevisiae* such that this engineered strain produced raspberry ketones at concentrations nearly two orders of magnitude above the predicted sensory threshold for Chardonnay grape juice under standard winemaking conditions. indicates that it is possible to synthesize while retaining the ability to fully ferment. Through metabolic engineering and chemical stimulation, we engineered *E. coli* to produce abundant p-coumaric acid and malonyl-CoA. After genetic selection and stepwise culture optimization, recombinant *E. coli* produced RK biosynthetic enzymes and fermented glucose to produce 62 mg/L RK.

Although this approach allows rapid construction of recombinant *E. coli*, several issues, such as plasmid stability, antibiotic cost, and stable enzyme production, must be addressed before applying it to large-scale fermentation need to do it. Genomic integration of introduced genes should be a possible approach to solve these problems. The CRISPR/Cas9 or λ-Red recombination system enables intact genome engineering in *E. coli* and should be a powerful tool for this purpose. These approaches improve our RK fermentation system into a more stable and economical system.

In conclusion, this study has established a microbial system that produces useful amounts of RK at low cost and within a reasonable time frame. The current supply of plant-derived RK is limited and expensive to extract. Our facile batch fermentation system offers a cost-effective and efficient alternative for extracting RK from plants based on a simple carbon source that greatly contributes to the flavor and fragrance industry.

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