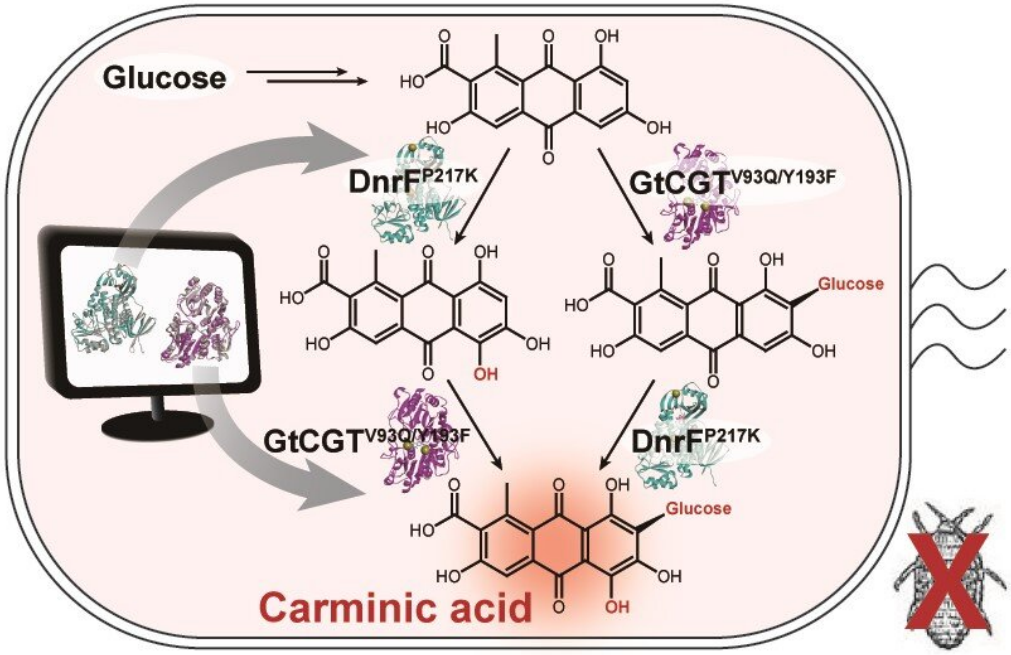
Microbial production of a natural red colorant carminic acid

April 6 2021



A schematic biosynthetic pathway for the production of carminic acid from glucose. Biochemical reaction analysis and computer simulation-assisted enzyme engineering was employed to identify and improve the enzymes (DnrFP217K and GtCGTV93Q/Y193F) responsible for the latter two reactions. Credit: The Korea Advanced Institute of Science and Technology (KAIST)

A research group at KAIST has engineered a bacterium capable of producing a natural red colorant, carminic acid, which is widely used for food and cosmetics. The research team reported the complete

biosynthesis of carminic acid from glucose in engineered Escherichia coli. The strategies will be useful for the design and construction of biosynthetic pathways involving unknown enzymes and consequently the production of diverse industrially important natural products for the food, pharmaceutical, and cosmetic industries.

Carminic acid is a natural red colorant widely being used for products such as strawberry milk and lipstick. However, carminic acid has been produced by farming cochineals, a scale insect which only grows in the region around Peru and the Canary Islands, followed by a complicated multi-step purification processes. Moreover, carminic acid often contains protein contaminants that cause allergies, so many people are unwilling to consume products made of insect-driven colorants. On that account, manufacturers around the world are using alternative red colorants despite the fact that carminic acid is one of the most stable natural red colorants.

These challenges inspired the metabolic engineering research group at KAIST to address this issue. Its members include postdoctoral researchers Dongsoo Yang and Woo Dae Jang, and Distinguished Professor Sang Yup Lee of the Department of Chemical and Biomolecular Engineering. This study entitled "Production of carminic acid by metabolically engineered Escherichia coli" was published online in the *Journal of the American Chemical Society* (*JACS*) on April 2.

This research reports for the first time the development of a bacterial strain capable of producing carminic acid from glucose via metabolic engineering and computer simulation-assisted enzyme engineering. The research group optimized the type II polyketide synthase machinery to efficiently produce the precursor of carminic acid, flavokermesic acid.

Since the enzymes responsible for the remaining two reactions were neither discovered nor functional, biochemical reaction analysis was

performed to identify enzymes that can convert flavokermesic acid into carminic acid. Then, homology modeling and docking simulations were performed to enhance the activities of the two identified enzymes. The team could confirm that the final engineered strain could produce carminic acid directly from glucose. The C-glucosyltransferase developed in this study was found to be generally applicable for other natural products as showcased by the successful production of an additional product, aloesin, which is found in aloe leaves.

"The most important part of this research is that unknown enzymes for the production of target natural products were identified and improved by biochemical reaction analyses and computer simulation-assisted enzyme engineering," says Dr. Dongsoo Yang. He explained the development of a generally applicable C-glucosyltransferase is also useful since C-glucosylation is a relatively unexplored reaction in bacteria including Escherichia coli. Using the C-glucosyltransferase developed in this study, both carminic acid and aloesin were successfully produced from glucose.

"A sustainable and insect-free method of producing carminic acid was achieved for the first time in this study. Unknown or inefficient enzymes have always been a major problem in natural product biosynthesis, and here we suggest one effective solution for solving this problem. As maintaining good health in the aging society is becoming increasingly important, we expect that the technology and strategies developed here will play pivotal roles in producing other valuable natural products of medical or nutritional importance," said Distinguished Professor Sang Yup Lee.

More information: Dongsoo Yang et al, Production of Carminic Acid by Metabolically Engineered Escherichia coli, *Journal of the American Chemical Society* (2021). DOI: 10.1021/jacs.0c12406