Functional foods, Industrial materials, Medical / Healthcare

Development of sustainable production of useful secondary metabolites by microorganisms and plants

Division of Biotechnology, Graduate School of Engineering
Associate Professor Hikaru Seki Researchmap https://researchmap.jp/read0152753?lang=en
Professor Toshiya Muranaka Researchmap https://researchmap.jp/bio?lang=en

Abstract

Glycyrrhizin, which is contained in the underground part of the medicinal plant "licorice", has many pharmacological properties, such as anti-inflammatory, anti-ulcer and hepatoprotective activities. And also use as a natural sweetener because it is about 150 times sweeter than sugar. Almost all licorice is imported from overseas, but the decrease in the number of high-quality products and the increase in prices have become a problem. Therefore, there has been a demand to develop a sustainable alternative production method. We have been working on the isolation and functional analysis of glycyrrhizin biosynthesis genes in licorice, including the glycosyltransferase of the second sugar in 2019, the glycosyltransferase of the first sugar in 2020. In 2021, we elucidated the molecular mechanism of glycyrrhizin-producing/non-producing licorice. Furthermore, by introducing a total of seven genes into budding yeast, we succeeded in production of glycyrrhizin in yeast.

Background & Results

Triterpene glycosides (generally called saponins) are a group of compounds in which multiple sugars are attached to the carbon skeleton of a triterpene, and are known to be the main active ingredients in many herbal medicines. Glycyrrhizin, a saponin extracted from licorice root, is used as a raw material for medicines such as liver disease remedies, and also as a natural sweetener. The process of saponin synthesis in plant cells involves cytochrome P450 monooxygenase (CYP), which oxidizes and modifies the triterpene carbon skeleton to produce aglycones with various structures, followed by several UDP sugar-dependent glycosyltransferases (UGT), which transfer sugars to the aglycones (glycosylation). We first isolated CYPs involved in glycyrrhizin biosynthesis, and then UGT73P12 as the UGT that transfers the second sugar, glucuronic acid, in the second step of glycosylation (Nomura, Seki et al. 2019). However, the enzyme that catalyzes the glucuronic acid transfer to the 3-position hydroxyl group, the first step of glycosylation, remained unknown. We used a gene co-expression analysis method to narrow down the list of genes to search for candidates for the uncharacterized glucuronosyltransferase. As a result, we found that a protein with unknown function (named CSyGT), unlike UGT, which is highly similar to cellulose synthase, is an enzyme that catalyzes the glucuronyl transfer reaction to the hydroxyl group at position 3 (Chung, Seki et al. 2020). We also elucidated the molecular mechanism of glycyrrhizin-producing/non-glycyrrhizin-producing licorice (Fanani et al 2021). Furthermore, by introducing a total of seven plant enzyme genes, including CSyGT, into yeast, we succeeded in production of glycyrrhizin in yeast for the first time in the world.

Significance of the research and Future perspective

This research is expected to pave the way for the industrial production of glycyrrhizin using yeast and plants that have been genetically modified with the genes discovered in this study, which in turn will help prevent the overharvesting of natural resources and preserve the ecosystem. Plants produce a wide variety of specialized (secondary) metabolites, as many as one million different types. By applying new technologies, such as synthetic biology and genome editing, to many specialized metabolites other than glycyrrhizin, we can expect to build an environmentally friendly and sustainable material production system that can be used as raw materials for pharmaceuticals, functional foods, and industrial materials.

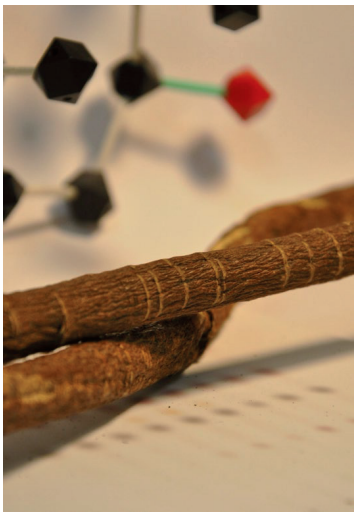

Fig. 1 Licorice root

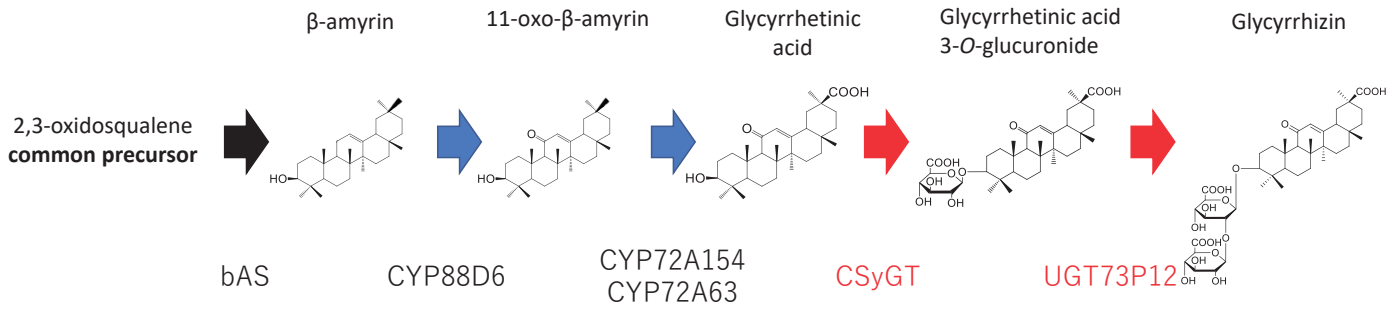

Fig. 2 Biosynthetic pathway of Glycyrrhizin

Patent JP6344774, PCT/JP2020/39175

Treatise Fanani, M.Z; Sawai, S; Seki, H. et al. Allylic hydroxylation activity is a source of saponin chemodiversity in the genus Glycyrrhiza . Plant Cell Physiol. 2021; 62(2): 262-271. doi: 10.1093/pcp/pcaa173
Chung, S.Y; Seki, H. et al. A cellulose synthase-derived enzyme catalyses 3-O -glucuronosylation in saponin biosynthesis. Nat. Commun. 2020; 11(1): 5664. doi: 10.1038/s41467-020-19399-0.
Nomura, Y; Seki, H. et al. Functional specialization of UDP-glycosyltransferase 73P12 in licorice to produce a sweet triterpenoid saponin, glycyrrhizin. Plant J. 2019; 99(6): 1127-1143. doi: 10.1111/tpj.14409.

URL http://www.bio.eng.osaka-u.ac.jp/pl/index_e.html

Keyword genome editing, medicinal plants, metabolic engineering, plant specialized metabolism, synthetic biology