RECENT RESEARCH ACTIVITIES

Yeast production of plant-derived bioactive prenylated phenolics

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Plant-derived metabolites have supported human life for a long time as medicines, nutrients, and so on. However, considering the assumption that plants produce 200,000–1,000,000 metabolites, much majority of plant metabolites that potentially show beneficial bioactivities for humans are remained to be exploited yet, which is mainly due to the generally low and variable quantity of metabolites in plants. For such problems, synthetic biology recently opens a way to highly and stably produce plant-derived bioactive compounds in microorganisms by introducing related biosynthetic genes.

Honeybees prepare propolis from resinous plant tissues to physically and chemically reinforce their hives. This honeybee product shows a variety of pharmaceutical bioactivities which are attributed to plant-derived metabolites. Among different propolis types by production sites, Brazilian green propolis is a major propolis type and globally commercialized. A main bioactive of this propolis type is artepillin C (di-prenylated *p*-coumaric acid) derived from an Asteraceae bush, *Baccharis dracunculifolia*. Artepillin C shows various bioactivities beneficial for human health such as anti-oxidation and anti-obesity, while the concentration of this metabolite in Brazilian green propolis is highly variable depending on natural factors such as climates and performance of honeybees, being a serious problem in quality control of propolis.

We tried synthetic biological production of artepillin C by introduction of its biosynthetic pathway in yeast. To reconstruct artepillin C pathway, we conducted identification of a gene encoding a prenyltransferase (PT) for *p*-coumaric acid which has not been reported so far. A promising gene source is *B. dracunculifolia*, the major habitat of which is restricted in southwestern part of Latin America, meaning that utilization of this species requires consideration of Nagoya protocol. Therefore, we selected *Artemisia capillaris* (kawara-yomogi in Japanese), which is native to Japan, as an experimental sample.

A transcriptome analysis of *A. capillaris* leaves that accumulate artepillin C found *A. capillaris PT1* (*AcPT1*) as a fine candidate. Biochemical characterization showed that its gene product solely catalyzes a sequential di-prenylation of *p*-coumaric acid to synthesize artepillin C via drupanin, the mono-prenyl intermediate (Fig. 1). Together with *p*-coumaric acid biosynthetic genes, *AcPT1* is introduced in budding yeast strain which is engineered to highly accumulate DMAPP, the prenyl donor substrate of AcPT1 [1]. The resulting yeast transformant was shown to produce artepillin C. Improvements of the artepillin C-producing strain toward higher productivity would provide a stable source of artepillin C alternative to propolis.

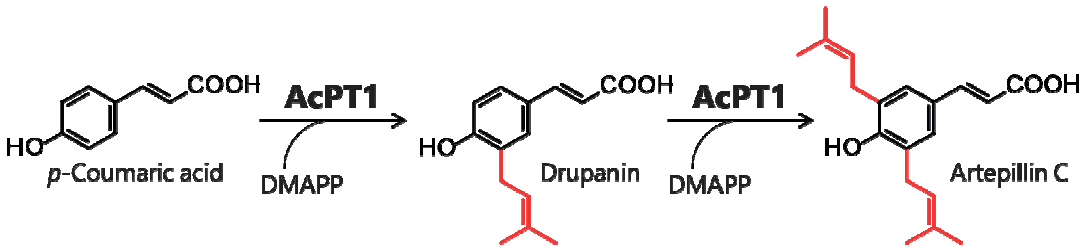

Fig. 1 The enzymatic function of AcPT1

Reference

[1] Ryosuke Munakata, Tomoya Takemura, Kanade Tatsumi, Eiko Moriyoshi, Koki Yanagihara, Akifumi Sugiyama, Hideyuki Suzuki, Hikaru Seki, Toshiya Muranaka, Noriaki Kawano, Kayo Yoshimatsu, Nobuo Kawahara, Takao Yamaura, Jérémy Grosjean, Frédéric Bourgaud, Alain Hehn, Kazufumi Yazaki, "Isolation of *Artemisia capillaris* membrane-bound di-prenyltransferase for phenylpropanoids and redesign of artepillin C in yeast", *Communications Biology*, 2, Article number: 384 (2019).