ABSTRACTS (MASTER THESIS)

Characterization of geranyl diphosphate synthase from *Lithospermum erythrorhizon*

**(Graduate School of Agriculture,
Laboratory of Plant Gene Expression, RISH, Kyoto University)**

Hayato Ueoka

Plants have evolved secondary (specialized) metabolisms, which appeared to be non-essential for their life, in addition to primary metabolism ubiquitously found in all organisms. It has been recently recognized that specialized metabolites have important functions in the adaptation to their environment, including pollinator attraction, pest repellent, and plant-plant interactions. Several compounds in specialized metabolism have been used for human life, such as natural dyes, flavors, fragrances, spices, and medicines as well. Based on the chemical structures and biosynthetic pathways, those metabolites are classified into three major classes, i.e. terpenoids, alkaloids, and phenols. Among them, terpenoids represents the largest class, with over 60,000 compounds described to date. According to the number of carbon atoms, terpenoids are subdivided into mono- (C10), sesqui- (C15), di- (C20), sester- (C25), tri- (C30), tetra- (C40), and polyterpenoid (>C40). All terpenoids are constructed from universal building blocks, isopentenyl diphosphate (C5) and its allylic isomer dimethylallyl diphosphate (C5), both of which are provided either *via* mevalonate (MVA) pathway in the cytosol or methylerythritol phosphate (MEP) pathway in plastids. In general, each terpenoid is produced in specific subcellular compartment, i.e. monoterpenoid and diterpenoid are formed in plastids *via* MEP pathway, while sesquiterpenoid is formed in the cytosol *via* MVA pathway. Besides these typical terpenoids, natural products with combined structures of terpenoid and other class compounds are called meroterpenoids.

A medicinal herbal plant, *Lithospermum erythrorhizon*, produces shikonin derivatives, red meroterpenoid pigments. Because of their anti-inflammatory and anti-microorganism effects and their beautiful purple color by co-pigmentation with aluminum, they have been used as ointment and natural dyes in Asian countries, including Japan, China, and Korea. For the mass production of shikonin derivatives, two-step culture system was established using cell suspension cultures of this plant, leading to the stable and rapid production of those valuable compounds (approximately 2 g/l shikonin in culture tank at two weeks) [1]. The development of cell culture system also advanced the study of shikonin biosynthetic pathway at molecular level. One of the rate-limiting steps of shikonin biosynthesis is the formation of *m*-geranyl-*p*-hydroxybenzoic acid (GBA), in which geranyl diphosphate (GPP, C10), a common precursor of monoterpenoid, is attached to *p*-hydroxybenzoic acid.

With respect to the GPP formation, Sommer et al. (1995) reported that GPP synthase activity was mainly detected in the cytosol fraction, not in plastids where GPP and monoterpenoids are commonly formed [2]. Li et al. (1998) also reported that the geranyl moiety of shikonin is derived from the MVA pathway in the cytosol [3]. These reports suggest that GPP synthase of *L. erythrorhizon* (LeGPPS) is exceptionally localized in the cytosol. Toward the elucidation of shikonin biosynthetic pathway, we aimed to identify *LeGPPS* gene through EST- and BLAST-based approaches. Through a series of analyses, we could identify a putative *GPPS* gene. Focusing on this candidate, further biochemical analyses will be performed to uncover the novel GPPS in plants.

References

[1] Yazaki K (2017) *Lithospermum erythrorhizon* cell cultures: Present and future aspects. Plant Biotechnol 34: 131-142.

[2] Sommer S, Severin K, Camara B, Heide L (1995) Intracellular localization of geranylpyrophosphate synthase from cell cultures of *Lithospermum erythrorhizon*. Phytochemistry 38: 623-627.

[3] Li SM, Hennig S, Heide L (1998) Shikonin: A geranyl diphosphate-derived plant hemiterpenoid formed via the mevalonate pathway. Tetrahedron Lett 39: 2721-2724.