RECENT RESEARCH ACTIVITIES

Shikonin is a new model for accumulation studies of lipophilic substances in plants

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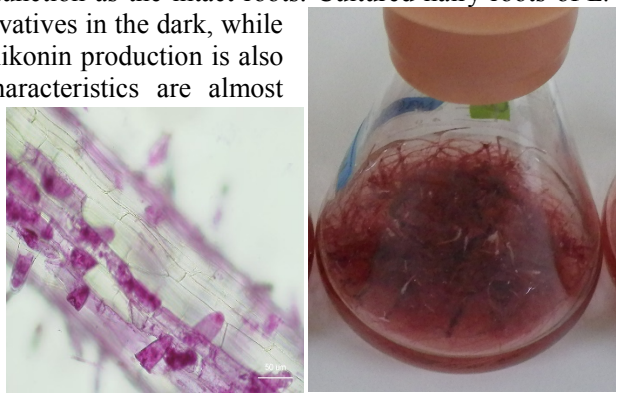
Bioactive substances often have hydrophobic properties. It makes actually sense as those substances should penetrate across the plasma membrane to exhibit their activities inside the cells, where hydrophobicity is beneficial in terms of the membrane permeability. In plants, lipophilic secondary metabolites are often secreted out of the cells and accumulated in apoplastic space, e.g. furanocoumarins are accumulated in oil gland after excretion form the epithelial cells, and monoerpenes are often accumulated in subcuticular cavities after biosynthesis in secretary cells in many plant species. However, the secretion mechanisms of those lipophilic metabolites are largely unknown.

Shikonin derivatives are medicinal compounds found only in a limited number of boraginaceaeous medicinal plants. *Lithospermum erythrorhizon* (purple gromwell) is a representative, which has been used as a medicinal plant over a thousand years for burns, wounds, frostbite, inflammation, and hemorrhoids as external use, and for internal use it is effective for leukemia and tumors. As these naphthoquinone compounds have bright red color, it has been used as a natural dye to stain cloths, especially silk, to obtain beautiful purple color, which was a representative color of highest public servant in Nara Era. These characteristics nicely fit to the purpose of Mission 5 “Quality of the Future Humanosphere”, especially Submission 5-1 “Harmonization of human health and the environment”. The wild plants are, however, facing to extinction, because the useful part is the roots and therefore harvest of *L. erythrorhizon* directly reflects the reduction of individual number. Also epidemic of plant viruses is another cause of the endangerment. To keep the sustainable supply of this plant, more fundamental studies are necessary.

Hairy roots are valuable materials for fundamental studies of root function. From *L. erythrorhizon*, we formerly showed that a virulent *Agrobacterium rhizogenes* strain ATCC15834 can be used to establish fast growing hairy roots of this plant [1]. We have been using this hairy root system to study biosynthetic regulation and secretion mechanism of shikonin derivatives, as it can be kept aseptically and stably subcultured, and also maintain the same metabolic function as the intact roots. Cultured hairy roots of *L. erythrorhizon* are capable of producing shikonin derivatives in the dark, while it is strongly inhibited by light irradiation and the shikonin production is also susceptible to ammonium ion as well. These characteristics are almost identical as dedifferentiated cultured cells of this plant. Moreover, the shikonin accumulation occurs only in the epidermal cells in hairy roots, which is the same as in the intact root. Using cultured hairy roots as a model of secretion system of lipophilic metabolites, we have been studying the secretion mechanism of lipids from plant cells [2]. Details are described by our PhD student, K. Tatsumi, in this issue.



Hairy roots of *L. erythrorhizon* producing shikonin

References

[1] Yazaki, K., Tanaka, S., Matsuoka, H., and Sato, F. (1998). Stable transformation of *Lithospermum erythrorhizon* with *Agrobacterium rhizogenes* and shikonin production of the transformants. Plant Cell Rep., 18, 214-219.

[2] Tatsumi, K., Yano, M., Sugiyama, A., Sato, M., Toyooka, K., Aoyama, T., Sato, F., Yazaki, K. (2016) Characterization of shikonin derivative secretion in *Lithospermum erythrorhizon* hairy roots as a model of lipid-soluble metabolite secretion from plants, Frontiers Plant Sci. 7, 1066.