ABSTRACTS (MASTER THESIS)

RNA-Seq analysis of shikonin-producing *Lithospermum erythrorhizon*

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Shikonin, a red naphthoquinone pigment, is a plant specialized metabolite occurring only in some restricted species in Boraginaceae. A representative species is *Lithospermum erythrorhizon*, a medicinal plant growing in Japan, Korea and China. In this perennial herbal plant, shikonin deriveatives are specifically accumulated at the root bark. The name shikonin is derived from the Japanese name of the root 'shikon' that has been used as a crude drug in Asian countries for treatments of wounds, burns and hemorrhoids. *L. erythrorhizon* is, however, on the brink of extinction due to the environmental changes and indiscriminate harvesting.

On those backgrounds, cell suspension cultures of this plant capable of producing a large amount of shikonin derivatives were established in 1970s by the working group of the late Professor Mamoru Tabata, and the cell cultures were utilized for industrial production of shikonin by Mitsui Petrochemical Industries in 1980s [1]. The key point of this success is the establishment of shikonin production medium M9 [2], and the application of two-stage cultivation method with plant growth medium and pigment production medium, which enabled a large production rate of this plant specialized metabolite in cell suspension cultures. By this method almost the same composition of shikonin derivatives, which are esters of low molecular weight organic acids like acetate, could be produced.

We have been interested in understanding the biosynthetic rout of shikonin and its characteristic secretion mechanism, as these red lipophilic pigments are accumulated as a large number of red granules and oil droplets at the cell surface. In order to get an inventory of genes involved in the production of shikonin, we carried out transcriptome analysis using RNA-Seq of next generation sequencer. To narrow down the genes specifically expressed under shikonin production, we prepared three sets of RNA samples from shikonin-producing and non-producing tissues/cells: dark- and light-growing cell cultures, the same set of hairy root cultures, and root bark and peeled root, respectively.

As the results, we obtained ca. 254,000 reads, from which genes expressed more than three times higher under shikonin-producing tissues/cells than non-producing ones were picked up. Out of them, ca. 250 genes were up-regulated commonly under three independent tissues/cells, which were listed as triple positive genes. After cutting redundancy, 143 non-redundant genes were obtained, which were then classified according to the predicted functions, such as oxidoreductases, isomerases lyases, etc. Then, we will establish an evaluating system of those genes validating the relevance for shikonin biosynthesis and secretion.

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References

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