

Japan Academy Prize to:

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and

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for “Microbial Production of Plant Secondary Metabolites,
Isoquinoline Alkaloids, Based on Metabolic Engineering
Research” (Joint Research)

Outline of the work:

Plants produce a variety of secondary metabolites that have strong physiological activities, although their production entails serious problems such as low productivity, heterogeneous quality, and difficulty in raw material supply. On the contrary, microorganisms produce their primary and some secondary metabolites under a man-controlled environment, thus assuring high efficiency and uniform quality. This joint research by Drs. Fumihiko Sato and Hidehiko Kumagai aimed to overcome problems associated with plant secondary metabolite production by constructing a microbial platform for plant isoquinoline alkaloid (abbrev. IQA) production as a model, in which the microbial and plant metabolic pathways are unified into one system. The research consisted of the following three parts:

A. Clarification of the molecular basis of the IQA synthetic pathway in cultured plant cells and its alteration by applying metabolic engineering techniques

Because of low enzymatic activities in the IQA synthetic pathway of plants, its molecular basis had not been clarified for a long time. Dr. Sato selected a cultured cell line with high IQA productivity of a medicinal herb, *Coptis japonica*, from which he purified and characterized 11 enzymes and cloned and sequenced their genes, which are involved in IQA biosynthesis, thus contributing to complete elucidation of its molecular basis. Of the cloned genes of *C. japonica*, seven were used in later work for constructing the microbial platform for plant IQA production. Dr. Sato further developed an *Agrobacterium*-mediated transformation technique for cultured cells of another medicinal herb, *Eschscholzia californica*. Using this technique, he introduced two *C. japonica* genes, one for scoulerine 9-*O*-methyltransferase and the other for norcochlorine 6-*O*-methyltransferase, into the *E. californica* cultured cells, finding successful production of berberine and accumulation of sanguinarine, respectively, thus demonstrating the high flexibility of the IQA biosynthetic pathway to its genetic alteration.

B. Research on aromatic amino acid metabolisms in microorganisms

Dr. Kumagai contributed to the elucidation of aromatic amino acid metabolism in microorganisms, which information was indispensable in constructing the microbial IQA production platform. First, he screened amino acid decarboxylase in various microorganisms, discovering a new enzyme in *Micrococcus*, which he purified in crystalline form for the first time in all organisms. He demonstrated that this enzyme produced tyramine by decarboxylation of L-tyrosine. Afterward, he discovered an aromatic amino acid-specific decarboxylase in *Pseudomonas*, which converts L-DOPA to dopamine. Next, Dr. Kumagai screened and characterized amine oxidases of various microorganisms; based on the results, he proposed a new classification system for this group of enzymes using their reaction center, Cu or FAD, as the key. He discovered a new monoamine oxidase in *Micrococcus*, purifying it in crystalline form, and showed that this enzyme converts tyramine to 4-hydroxyphenylacetaldehyde. With this enzyme, he found non-enzymatic production of norlaudanosoline from its substrate (dopamine) and product (3, 4-dihydroxyphenylacetaldehyde, abbrev. 3, 4-DHPAA), noticing its identical basic chemical structure to that of norcoclaurine, the first product in the plant IQA biosynthetic pathway. From this observation, he derived the idea of plant IQA production by microorganisms. Genes of this enzyme and L-DOPA-specific amino acid decarboxylase were used later in constructing the microbial platform for plant IQA production.

C. Construction of the microbial platform for plant IQA production

Drs. Sato and Kumagai in close collaboration successfully constructed the microbial platform for plant IQA production for the first time in the world. The platform consisted of the following four modules: “universal aromatic amino acid synthetic module” prepared by genetic modifications of the *Escherichia coli* metabolic system to facilitate overproduction of L-tyrosine from glucose as the sole carbon source; “tailor-made biosynthetic module” optimized for production of two IQA precursors, dopamine and 3, 4-DHPAA, from L-tyrosine using three microbial genes, two of which were cloned by Dr. Kumagai; “fundamental IQA synthetic module” for production of (*S*)-reticline, the key substance in the IQA synthetic pathway, that consisted of four genes cloned from *C. japonica* by Dr. Sato; and “*ad hoc* IQA synthetic module” consisting of three *C. japonica* genes cloned by Dr. Sato, that produced different IQAs from (*S*)-reticline. The first three modules were installed in *E. coli* cells, whereas the last one was prepared with budding yeast. Co-cultures of those *E. coli* and yeast cells efficiently produced (*S*)-reticline and other IQAs, proving the usefulness of the constructed microbial platform for plant IQA production.

In summary, Drs. Sato and Kumagai opened a new avenue for the microbial production of plant secondary metabolites using IQAs as the model, and contributed to the development of a new area in both Synthetic Biology and Metabolic Engineering.

Selected articles relating to the research subject:

- (1) Sato, F. and Yamada, Y. (1984) High berberine-producing cultures of *Coptis japonica* cells. *Phytochem.* 23: 281-285.
- (2) Takeshita, N., Fujiwara, H., Mimura, H., Fitchen, J. H., Yamada, Y., and Sato, F. (1995) Molecular cloning and characterization of *S*-adenosyl-L-methionine: scoulerine-9-*O*-methyltransferase from cultured *Coptis japonica* cells. *Plant Cell Physiol.* 36: 29-36.
- (3) Sato, F., Hashimoto, T., Hachiya, A., Tamura, K., Choi, K.-B., Morishige, T., Fujimoto, H., and Yamada, Y. (2001) Metabolic engineering of plant alkaloid biosynthesis. *Proc. Natl. Acad. Sci. USA* 98: 367-372.
- (4) Minami, H., Dubouzet, E., Iwasa, K., and Sato, F. (2007) Functional analysis of norcoclaurine synthase in *Coptis japonica*. *J. Biol. Chem.* 282: 6274-6282.
- (5) Sato, F., Inai, K., and Hashimoto, T. (2007) Metabolic engineering in alkaloid biosynthesis: case studies in tyrosine- and putrescine-derived alkaloids. In: *Applications of Plant Metabolic Engineering* (eds. Verpoorte, R., Alfermann, A. W., and Johnson, T. S.), Springer, New York, pp. 145-173.

- (6) **Kumagai, H.**, Yamada, H., Suzuki, H., and Ogura, Y. (1971) Action mechanism of tyramine oxidase from *Sarcina lutea*. *J. Biochem.* 69: 137-144.
- (7) Yamada, H. and **Kumagai, H.** (1975) Synthesis of L-tyrosine-related amino acids by β -tyrosinase. In: *Advances in Applied Microbiology* (ed. Perlman, D.), Academic Press Inc., New York, 19, pp. 249-288.
- (8) Nakazawa, H., **Kumagai, H.**, and Yamada, H. (1981) Aromatic L-amino acid decarboxylase from *Micrococcus percitreus*. Purification, crystallization and properties. *Agric. Biol. Chem.* 45: 2543-2552.
- (9) **Kumagai, H.** and Yamada, H. (1985) Bacterial and fungal amine oxidases. In: *Structure and Function of Amine Oxidases* (ed. Mondovi, B.), CRC Press Inc., Boca Raton, Florida, pp. 37-43.
- (10) Roh, J.-H., Wouters, J., Depiereux, E., Yukawa, H., Inui, M., Minami, H., Suzuki, H., and **Kumagai, H.** (2000) Purification, cloning, and three-dimensional structure prediction of *Micrococcus luteus* FAD-containing tyramine oxidase. *Biochem. Biophys. Res. Commun.* 268: 293-297.
- (11) Minami, H., Kim, J.-S., Ikezawa, N., Takemura, T., Katayama, T., **Kumagai, H.**, and **Sato, F.** (2008) Microbial production of plant benzylisoquinoline alkaloids. *Proc. Natl. Acad. Sci. USA* 105: 7393-7398.
- (12) Nakagawa, A., Minami, H., Kim, J.-S., Koyanagi, T., Katayama, T., **Sato, F.**, and **Kumagai, H.** (2011) A bacterial platform for fermentative production of plant alkaloids. *Nature Commun.* 2, Article number: 326, doi:10.1038/ncomms1327.